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# Unraveling the Mechanisms of Gene Transfer in Bacteria

Catherine Guynet

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MEMOIRE EN VUE DE L'OBTENTION DE L'HABILITATION A  
DIRIGER LES RECHERCHES

**Unraveling the Mechanisms of Gene Transfer in Bacteria**

**Catherine GUYNET**

Chargée de Recherche au CNRS

Laboratoire de Microbiologie et Génétique Moléculaires,

Centre de Biologie Intégrative de Toulouse

**Soutenance le 21 novembre 2024**

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MARRAINE

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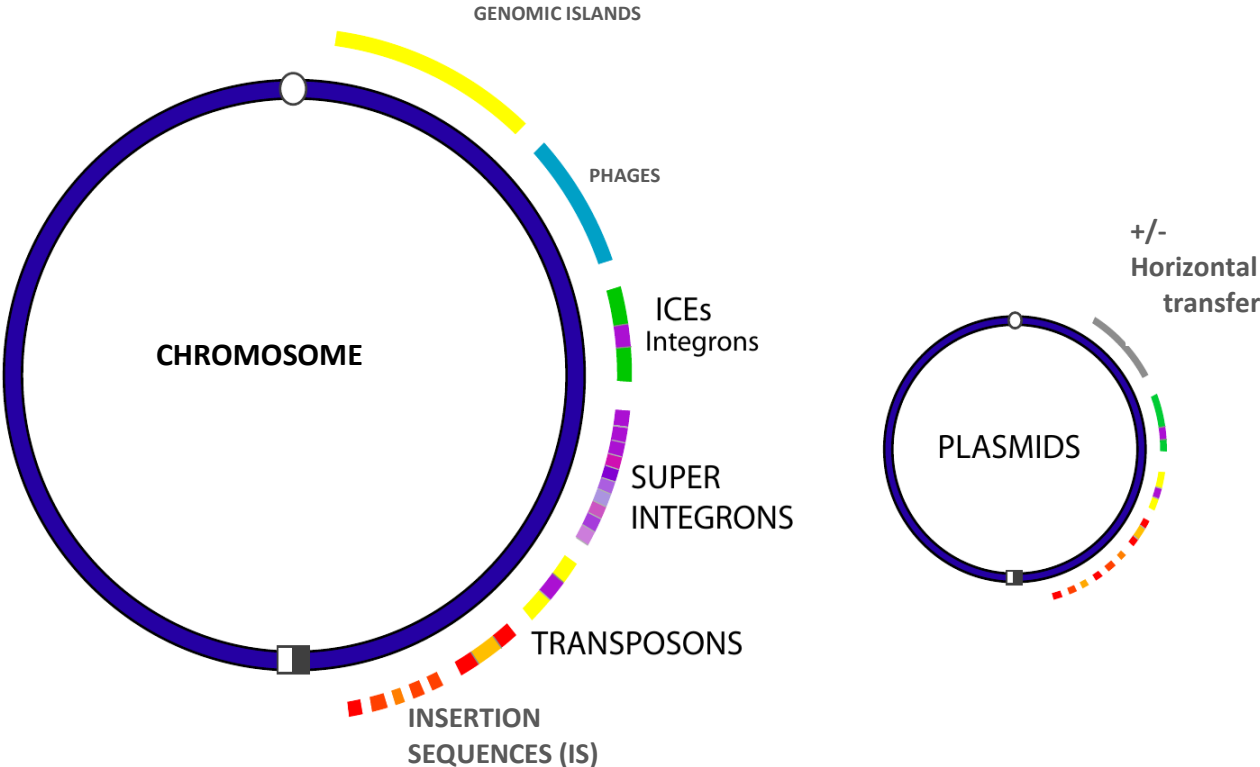
## 1. PREAMBLE

The main objective of my research is to understand the mechanisms involved in gene exchanges and genome plasticity in bacteria. In particular, I have been studying the processes by which mobile genetic elements (MGEs) propagate since my PhD.

Bacterial mobile genetic elements (MGEs), also referred as the mobilome, are segments of DNA that can move within and between genomes through various mechanisms. They include insertion sequences (ISs), transposons, integrons, integrative and conjugative elements (ICEs), plasmids and bacteriophages (**Figure 1**). MGEs, which are transferred horizontally between different bacterial cells through horizontal gene transfer, represent an important source of diversity and key players in the evolution of bacterial genomes. This mobility allows the rapid spread of advantageous traits, such as antimicrobial resistance (AMR) or metabolic capabilities, enabling bacteria to adapt rapidly to environmental changes and selective pressures. The ability of transposable elements to insert into various genomic locations allows them to influence bacterial gene expression and regulation. This can result in mutations, gene disruptions, creation of new gene combinations, alter regulatory networks, and facilitate horizontal gene transfer by mobilizing adjacent genes, thus influencing bacterial phenotypes. Conjugative plasmids are extrachromosomal DNA elements capable of autonomous replication and vertical transmission from mother to daughter bacteria as well as between bacterial cells via the process of conjugation. Acting as natural cargo ships of other MGEs, they are considered as the most active vectors of virulence and antibiotic resistance genes that are exchanged and combined, greatly contributing to the emergence of multidrug-resistant bacteria (Partridge et al., 2018). This phenomenon is a major and ever-increasing burden, not only because it is a leading cause of human mortality worldwide but also because it threatens the health of animals and plants, with effects on food safety and the environment. Numerous examples demonstrate their considerable impact on the spread of genes encoding AMRs, especially in well-studied human pathogens (Carattoli, 2013; von Wintersdorff et al., 2016).

More generally, MGEs are not only fascinating elements of genetic exchange but also critical players in the broader context of adaptation of bacteria to new environments

potentiating the fitness of species and shaping microbial communities. All these reasons motivate my research into their biology and the mechanisms underlying their transfer.



**Figure 1.** The bacterial mobilome.

## 2. DOCTORAL RESEARCH

### Study of the transposition mechanism of the bacterial Insertion Sequence IS608

*This work was carried out at the Laboratoire de Microbiologie et de Génétique Moléculaires (LMGM-UMR5100, Toulouse, France), in Dr. M. Chandler's group, under the supervision of Drs. M. Chandler and B. Ton-Hoang.*

#### **Funding :**

Oct. 2004 – Oct. 2007 : M.E.N.R.T. fellowship

Nov.2007 – Aug. 2008 : CNRS research engineer contract

#### **Publications :**

**Ton-Hoang B., Pasternak C., Siguier P., Guynet C., Hickman A.B., Dyda F., Sommer S., Chandler M. (2010)** Single-stranded DNA transposition is coupled to host replication. *Cell* 142(3):398-408.

**Guynet C., Achard A., Ton-Hoang B., Barabas O., Hickman A.B., Dyda F., Chandler M. (2009)** Resetting the site: redirecting integration of an insertion sequence in a predictable way. *Mol Cell* 34(5):612-9.

**Guynet C., Hickman A.B., Barabas O., Dyda F., Chandler M. and Ton-Hoang B. (2008)** *In Vitro* Reconstitution of a Single-Stranded Transposition Mechanism of IS608. *Mol Cell* 29:1-11.

**Barabas O., Ronning D.R.<sup>+</sup>, Guynet C.<sup>+</sup>, Hickman A.B., Ton-Hoang B., Chandler M. and Dyda F. (2008)** Mechanism of IS200/IS605 Family DNA Transposases: Activation and Transposon-Directed Target Site Selection. *Cell* 132, 208-220. <sup>+</sup> contributed equally to the work.

**Ronning D.R., Guynet,C., Ton-Hoang, B., Perez, Z., Ghirlando, G., Chandler, M. and Dyda, F. (2005)** Active site sharing and synaptic assembly in a new class of DNA transposases. *Mol Cell.*: 20(1):143-54.

**Ton-Hoang, B., Guynet, C., Ronning, D.R., Cointin-Marty, B., Dyda, F., and Chandler, M. (2005)** Transposition of ISHp608, member of a novel family of bacterial insertion sequences. *EMBO J.*:24(18):3325-38.

#### **Thesis manuscript :**

**Guynet C.** (2008) Etude du mécanisme de transposition de la Séquence d'Insertion bactérienne IS608. Thesis from Paul Sabatier University (<http://theseups.ups-tlse.fr/415/>)



Transposition is defined as the movement of a discrete segment of DNA from one locus to another, between non-homologous fragments, within the genome that harbors them. Transposons encode specific enzymes, called transposases, whose function is to recognize specifically the element's ends and catalyze their endonucleolytic cutting and their transfer to a target DNA molecule (for a general and recent review, see (Guynet et al., 2020b)).

Insertion sequences (ISs) are the simplest form of transposable elements, capable of moving from one location to another within a genome. Typically, ISs range from 700 to 2,500 base pairs in length and consist of one or two genes, usually including a transposase gene that is crucial for their movement, flanked by inverted repeat sequences. IS608, isolated from *Helicobacter pylori* belongs to a family of atypical transposons, the IS200/IS605 family, whose members are strongly represented in eubacteria and archaea (He et al., 2015; Kersulyte et al., 2002). The ISs of this family have two open reading frames, in various combinations and orientations: *tnpA* and *tnpB*. The product of *tnpA*, the TnpA transposase, is essential for the transposition of these elements and is homologous to IS200 transposases (Kersulyte et al., 2002).

The product of *tnpB*, TnpB, is not required for transposition in *Escherichia coli*. By the time I finished my PhD, *tnpB* was described as encoding a protein of unknown function but showing homologies to the *gipA* virulence gene of the Salmonella Gifsy phage (Stanley et al., 2000). A role of TnpB in the regulation of transposition through the inhibition of the excision and the insertion steps of IS*dra2* has been shown, but no underlying mechanism has been proposed (Pasternak et al., 2013). Spectacular advances have been made in recent years to understand the role of this intriguing protein. These are summarized in section 3.2.1.2.

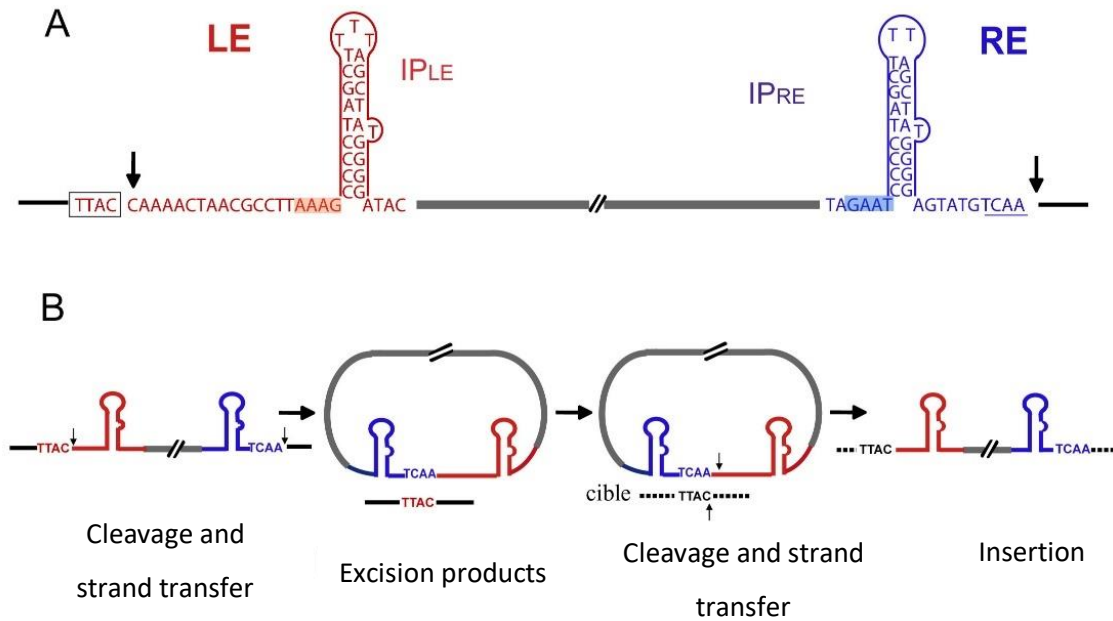
Unlike most ISs, IS200/IS605 family members do not carry inverted repeat sequences (IRs) at their ends, but contain imperfect palindromic sequences forming potential secondary structures. In addition, in contrast to most ISs, which integrate essentially randomly or with low sequence or structure specificity, they have the particularity of integrating into the 3' of a conserved AT-rich penta- or tetra-nucleotide sequence (Kersulyte et al., 2002, 2000, 1998).

## 1.1 IS608 transposition uses a single strand of DNA

IS608 is able to transpose in *Escherichia coli* and inserts 3' to the conserved tetranucleotide 5' TTAC (Kersulyte et al., 2002). We have shown that excision of IS608 *in vivo* releases the donor plasmid devoid of the element precisely recircularized at the 5' TTAC tetranucleotide, which is required for both excision and integration of the element (Ton-Hoang et al., 2005). The ends of IS608, LE (Left End) and RE (Right End) contain conserved imperfect palindromic sequences of 22 or 23 base pairs that can form secondary hairpin structures (IP<sub>LE</sub> and IP<sub>RE</sub>, Inverted Palindromes, **Figure 2A**) that I have shown to be important for IS608 transposition (Ton-Hoang et al., 2005).

*In vitro*, TnpA binds to both DNA ends and introduces a cut on the top strand at each terminal of the element (Ton-Hoang et al., 2005). I have developed a system that reconstitutes all the cleavage and strand transfer reactions required for IS608 transposition *in vitro*. This work has revealed a novel transposition mechanism that requires single-stranded (ss) DNA for all its steps (**Figure 2B**; (Guynet et al., 2008)).

The TnpA transposase is indeed capable of catalyzing a recombination reaction between its ssLE and ssRE ends *in vitro* in a “religature” assay. This reaction involves two oligonucleotides representing the top strands of both ends, and TnpA. Two single-stranded recombinant products are formed: the ssRE-LE junction formed by ligation of the cleaved ssLE and ssRE ends, and the ligation product of the flanking sequences. When both ends are on the same ssDNA molecule (“minitransposon”), the reaction generates a ss circle bearing the ssRE-LE junction. This implies that cleavage and strand transfer reactions are coordinated, consistent with a transposition mechanism involving excision of a single-stranded circular intermediate. TnpA also catalyzes the transfer of the ssLE and ssRE ends into a “target” DNA molecule, strictly in single-stranded form and bearing the target tetranucleotide 5'TTAC, in a tripartite reaction *in vitro*. This set of experiments has enabled us to characterize the various transposition intermediates *in vitro*.

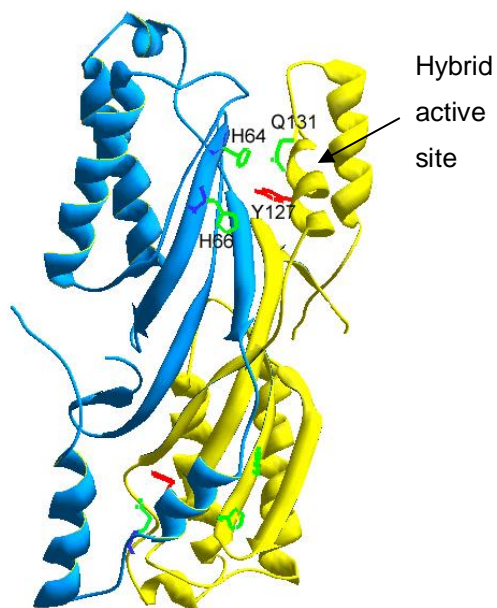


**Figure 2.** IS608 transposition involves single-stranded DNA  
 A: diagram showing IS608 ssDNA ends; B: schematic representation of the IS608 ss transposition cycle showing the formation of a circular ss intermediate. Cut sites are indicated by vertical arrows. LE: Left End in red, RE: Right End in blue, IP: Inverted Palindrome. The ssDNA sequences of the element, flanking element and target are shown as grey, black and dotted lines, respectively. This set of experiments has enabled us to characterize the various transposition intermediates *in vitro*, and today constitutes a tool for the functional study of IS200/IS605 family elements.

## 1.2 Identification and characterization of the new class of Y1 transposases

Transposase is the enzyme that catalyzes endonucleolytic cuts at the ends of transposable elements and their transfer into a target DNA molecule. Based on the biochemical properties of the reactions catalyzed by transposase, several groups of transposable elements, both eukaryotic and prokaryotic, have been defined: transposases with a DDE catalytic motif, belonging to the superfamily of polynucleotidyl transferases, are the most abundant and the most studied; tyrosine (Y) and serine (S) transposases, related to site-specific recombinases; and Y2 transposases, which exhibit characteristics of rolling circle replication initiator proteins. TnpA is a small transposase (155 residues) and its sequence does not contain a DDE catalytic motif, but several residues are strictly conserved, notably a tyrosine residue (Y127) and two histidine residues (H64 and H66).

A collaboration with Dr. Fred Dyda's group (NIH, Bethesda, USA) allowed us to conduct very informative structural studies from the beginning of the project. The structure of TnpA, either alone or complexed with a minimal ssDNA substrate (representing  $IP_{LE}$  or  $IP_{RE}$ ), was resolved by X-ray diffraction, with a resolution of around 2Å (**Figure 3**, (Ronning et al., 2005)). Structurally, TnpA does not resemble any other known transposase but is similar to proteins in the HUH endonuclease superfamily (Koonin and Ilyina, 1993; Ronning et al., 2005). Some closely related proteins to TnpA are the relaxases TrwC (plasmid R388) and TraI (plasmid F), and the replication initiator proteins of certain ssDNA viruses (AAV, Adeno-Associated Virus and TYLCV, Tomato Yellow Leaf Curl Virus). All these enzymes have the ability to catalyze site-specific DNA cuts by forming a 5'-phosphotyrosine intermediate and possess an HUH motif (Histidine-Hydrophobic residue-Histidine), which is involved in coordinating the bivalent cations required for catalysis.



**Figure 3.** Crystal structure of TnpA dimer in active conformation (DNA is not shown for the sake of clarity).

I performed site-directed mutagenesis analyses which confirmed that the three conserved residues of TnpA, H64, H66, and Y127, are required for the protein's activities in vitro (cleavage, formation of a covalent 5'-phosphotyrosine intermediate between TnpA and ssDNA substrates representing the element ends) and in vivo (Ronning et al., 2005). HUH family proteins use the two histidine residues of the HUH motif and a third residue to coordinate the metal cofactor. Site-directed mutagenesis analyses, based on residue

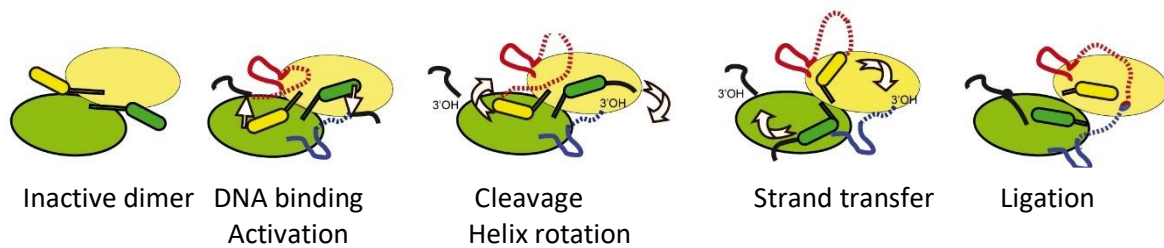
conservation, the nature of potential residues, and structural data, allowed me to identify this third residue: Q131. The Q131A mutation results in a loss of protein activity in vivo and in vitro, and an inability of the complex formed by TnpA and its ssDNA substrate to bind the bivalent cation (Barabas et al., 2008).

These results led us to propose a new category of transposition-catalyzing enzymes: Y1 transposases, which, unlike Y2 transposases, possess only a single catalytic tyrosine residue (Ronning et al., 2005).

### 1.3 A Dynamic Model for Catalysis

In its crystalline structure, two TnpA monomers are positioned "head-to-tail," assembling two hybrid active sites composed of H64 and H66 residues of the HUH motif from one monomer and the Y127 residue from the other monomer (**Figure 3**; (Ronning et al., 2005)). However, the catalytic site thus formed (in the absence of a DNA substrate, or in the presence of IPs) is in an inactive conformation. We showed that binding an appropriate substrate containing 4 additional bases at the 5' end of IPLE or IPRE activates the protein (**Figure 5A**: the 4 bases are highlighted in red (ssLE) or blue (ssRE); (Barabas et al., 2008)).

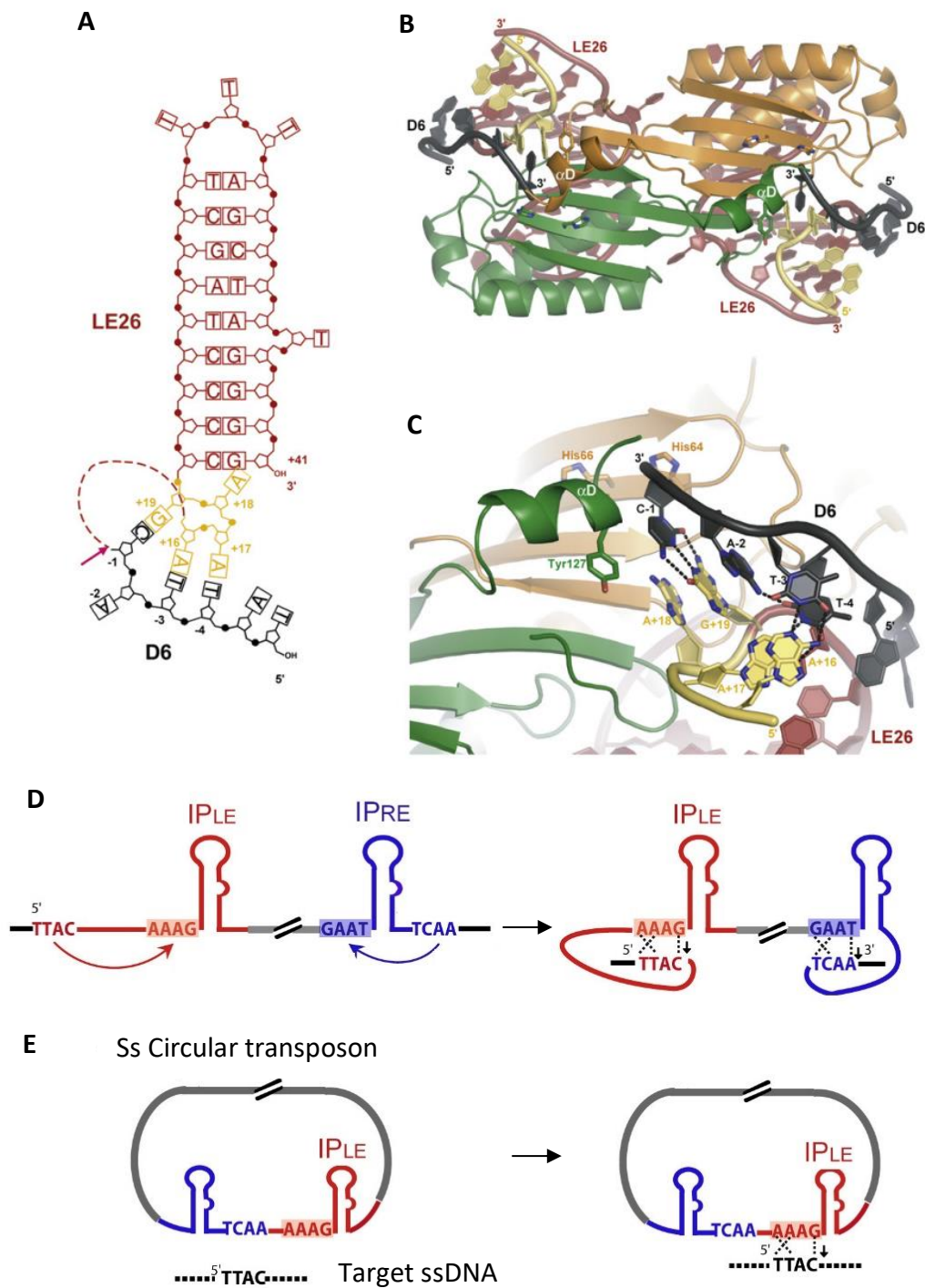
All these results allowed us to propose a model for the IS608 transposition mechanism involving a large conformational change of the protein between the cleavage reaction and strand transfer (**Figure 4**). Cleavages at the IS ends generate a 5'phosphotyrosine link between the transposase and the 5' end of the transposon at LE, and the 5' end of the sequence flanking the transposon at RE. Our model proposes that strand transfer occurs through a rotation of the two  $\alpha$ -helices carrying the catalytic residue Y127. Thus, phosphotyrosine links can be resolved by reciprocal nucleophilic attacks of the 3'OH ends released during cleavage reactions, generating the excision products. This large conformational change is suggested notably by the presence of a poorly structured and flexible region containing two glycine residues, as well as the different conformations observed in the TnpA structure depending on the substrate used.



**Figure 4.** Dynamic strand transfer model. The two TnpA monomers are shown in green and yellow.  $\alpha$ -helices carrying the Y127 residues are represented by dark cylinders. The ss ends of IS608 are shown in red (ssLE) and blue (ssRE), and the sequences flanking the element in black. Upon LE and RE binding, Y127 of each monomer cleaves the transposon ends and becomes covalently attached to the 5' side of the gap. Movement of  $\alpha$ D helices from *trans* to *cis* and resolution of the phosphotyrosine intermediates results in a transposon junction and a sealed donor backbone.

#### 1.4 Recognition of Cleavage Sites and Target Sequence by DNA-DNA Interactions

Our structural analyses also demonstrated that the cleavage sites, at the two ssLE and ssRE ends, are recognized by DNA-DNA interactions occurring near the transposase active site, and not by protein-DNA interactions. These interactions consist of pairings between three bases of the cleavage site and three of the four bases located 5' of the hairpin (Barabas et al., 2008); **Figure 5**). We determined the 2.9 Å resolution structure of TnpA cocrystallized with a RE 35-mer that contains the RE hairpin and the 10 nt in the 3' direction reaching the end of the transposon. Attempts to co-crystallize TnpA with longer LE sequences that extend 5' from the hairpin to the LE cleavage site have been unsuccessful but crystals of TnpA bound to a LE 26-mer and a 6-mer TATTAC (D6) were obtained (1.9 Å resolution, **figure 5 A-C**). In the ternary LE complex, the TTAC bases of D6 occupy the same position and orientation as the TCAA bases at the end of RE35 in the TnpA/RE35 complex. The TTAC tetranucleotide is located in the active site, stabilized by the 4 nt 5' extension of LE hairpin. The structural similarity between the LE ternary complex and the TnpA/RE35 complex is due to the conserved polarity of the cleavage reactions. The interactions are similar at both ends. We showed that the four bases located 5' of LE hairpin select the target tetranucleotide for integration by engaging the same type of interactions (**Figure 5E**). This mode of target sequence recognition is also used by group II introns, or targetrons (Perutka et al., 2004). These results allowed us to predict and redirect IS608 insertions *in vitro* and *in vivo* by introducing point mutations at the LE end (Guynet et al., 2009).



**Figure 5.** Recognition mode of cleavage sites. **A.** Schematic of the oligonucleotides used, where the dashed line indicates the break between LE26 (in red, AAAG guide site in yellow) and the donor flank D6 (in black). The red arrow indicates the cleavage site of LE. **B.** Overall view of the TnpA/LE26/D6 structure. **C.** Close-up of the active site showing the base pairs between the four bases 5' of IP<sub>LE</sub> (AAAG), in yellow and the bases at the donor flank **D** and **E**. Schematics of the recognition mode of cleavage sites for excision (**D**) and target insertion site (**E**). The tetranucleotides involved and the DNA-DNA interactions involved are indicated.

## 1.5 IS608 transposition is coupled to host chromosome replication

The transposition mechanism of IS608 strictly involves single-stranded DNA (ssDNA). Such a mechanism implies that at least partially single-stranded DNA substrates are generated *in vivo*, which constitutes an important regulation point for the transposition of the element. DNA is predominantly double-stranded in the cell, but several mechanisms generating ssDNA in the cell can be exploited by IS608, such as replication, conjugation, transformation, or DNA supercoiling.

We have shown that the excision and insertion of IS608 preferentially occur on the lagging strand during *in vivo* replication. Indeed, the frequency of IS608 excision depends on the direction of replication: it is higher when the active strand (upper strand) of the IS is on the lagging strand than when it is on the leading strand (Ton-Hoang et al., 2010). Additionally, IS608 transposition is affected by modifications of the replisome: transient inactivation of replication proteins leads to an increased frequency of excision of the element from the lagging strand; replication fork stalling induces IS608 integration events on the lagging strand. These results indicate that IS608 utilizes the ssDNA formed on the lagging strand behind the replication fork to disperse within the host genome (Ton-Hoang et al., 2010).

## 1.6 Conclusions

During my thesis, I contributed to the functional characterization of IS608, which has become the primary study model of the IS200/IS605 group. We discovered a new transposition mechanism involving only single-stranded DNA at all stages of the cycle (Gynet et al., 2008; Ton-Hoang et al., 2005) associated with a new category of transposases, the Y1 transposases (Barabas et al., 2008; Ronning et al., 2005). The original properties of this transposition system allowed us to modify the insertion specificity of IS608 *in vitro* and *in vivo* (Gynet et al., 2009). A second element of the IS200/IS605 family, ISdra2, was characterized *in vitro* and *in vivo* (Pasternak et al., 2010). The results suggest that the IS608 transposition mechanism we identified can be extended to all members of the family. However, it seems that the mode of target sequence recognition is only partially conserved (Hickman et al., 2010). Furthermore, our work suggests that IS200/IS605 elements exploit the ssDNA formed



during various cellular processes to disseminate (Pasternak et al., 2010; Ton-Hoang et al., 2010).

The *IS200/IS605* group constitutes one of the most significant families of transposons in terms of distribution and number in the prokaryotic world. Along with integron cassettes and bacteriophages (Bouvier et al., 2005; Val et al., 2005), it is among the few known genetic elements functioning in a single-stranded DNA form, but it is likely that they are just examples of a much larger group.

### 3. POST-DOCTORAL AND CURRENT RESEARCH

#### 2.1 Carrier path

**Post-doctorate in F. de la Cruz's group**, Instituto de Biomedicina y Biotecnología de Cantabria, departamento de Biología Molecular, Universidad de Cantabria (IBBTEC, Santander, Spain)

**Time period :**

Sept. 2008 – Aug. 2011

**Post-doctoral fellowships :**

Sept. 2008 – Jan. 2009 : FRM post-doctoral fellowship

Feb.2009 – Jan. 2011 : EMBO long term fellowship

Feb.2011 – Aug. 2011 : post-doctoral contract, University of Cantabria

**Publications :**

**Guynet C. and de la Cruz F. (2011)** Plasmid segregation without partition, *Mob Genet Elements* 1(3):236-241.

**Guynet C., Cuevas A., Moncalián G., de la Cruz F. (2011)** The *stb* operon balances the requirements for vegetative stability and conjugative transfer of plasmid R388. *PLoS Genet* 7(5):e1002073.

**Permanent researcher (Chargée de recherche CNRS) in the group headed by M. Chandler and then by B. Ton-Hoang**, Laboratoire de Microbiologie et Génétique Moléculaires (LMGM, Toulouse, France)

**Time period :**

Sept. 2011 – Dec. 2015

**PhD student supervised :**

**Alix Corneloup** (Oct. 2012 – Sept 2016). Co-supervised with B. Ton-Hoang (50%) "REP sequences dissemination in bacterial genomes: characterization of TnpAREP proteins activities" (graduated 10-2016)

**Publications resulting from this work :**

**Corneloup A., Caumont-Sarcos A., Kamgoue A., Marty B., Le P.T.N., Siguier P., Guynet C.\* and Ton-Hoang B.\* (2021)** Tnp<sub>AREP</sub> and REP sequences dissemination in bacterial genomes: REP recognition determinants, *Nucleic Acids Research*, 49 (12): 6982–6995.

**Guynet C., Siguier P., Chandler M. and Ton-Hoang B. (2021) Nonhomologous Recombination: Bacterial Transposons.** In: Jez Joseph (eds.) Encyclopedia of Biological Chemistry, 3rd Edition. vol. 4, pp. 303–312. Oxford: Elsevier. DOI: 10.1016/B978-0-12-819460-7.00122-5

**Guynet C.\*, Ton-Hoang B., Bouet, J.-Y. and Hallet, B.\* (2020)** First biochemical steps on bacterial transposition pathways. Horizontal gene transfer, *Methods in Molecular Biology*, 2075 pp.157–177.

**Guynet C.\*, Le P.T.N., Chandler M., Ton-Hoang B.\*(2020)** Detection and Characterization of Transposons in Bacteria. Horizontal Gene transfer, *Methods in Molecular Biology*, 2075 pp.81–90.

**Morero N.R., Zuliani C., Kumar B., Bebel A., Okamoto S., Guynet C., Hickman A.B., Chandler M., Dyda F., Barabas O. (2018)** Targeting IS608 transposon integration to highly specific sequences by structure-based transposon engineering *Nucleic Acids Res.* 46(8):4152-4163. doi: 10.1093/nar/gky235.

**Lavatine L., He S., Caumont-Sarcos A., Guynet C., Marty B., Chandler M., Ton-Hoang B. (2016)** Single strand transposition at the host replication fork. *Nucleic Acids Res.* 44(16):7866-83.

**He S., Corneloup A., Guynet C., Lavatine L., Caumont-Sarcos A., Siguier P., Marty B., Dyda F., Chandler M., Ton-Hoang B. (2015)** The IS200/IS605 family and “peel and paste” single-strand transposition mechanism. *Microbiol Spectrum* 3(4):MDNA3-0039-2014.

**He S., Guynet C., Siguier P., Hickman A.B., Dyda F., Chandler M., Ton-Hoang B. (2013)** IS200/IS605 family single-strand transposition: mechanism of IS608 strand transfer. *Nucleic Acids Res.* 41(5):3302-13.

**Permanent researcher (Chargée de recherche CNRS) in the group headed by J.-Y. Bouet and then co-headed with F. Cornet**, Laboratoire de Microbiologie et Génétique Moléculaires - Centre de Biologie Intégrative de Toulouse (LMGM-CBI, Toulouse, France)

**Time period :**

Jan. 2016 – now

**PhD students supervised :**

- **Valentin Quèbre** (Oct. 2012 – Sept 2016). Co-supervised with J.-Y. Bouet (50%) "Bacterial DNA segregation: control of the assembly of the partition complex" (graduated March 2022)
- **Romane Dusfour-Castan** (Oct. 2021 – now). Co-supervised with B. Ton-Hoang (50%) "Bacterial gene exchanges in a model gut microbiota"
- **Charlotte Hall** (Dec. 2023 – now). Co-supervised with F. Cornet (50%) "Role of the Stb System in the propagation dynamics of bacterial plasmids"

#### **Research Grants :**

2023-2027	Principal coordinator of the GeTBac project (ANR, 48 months)
2019-2023	Co-coordination of the PlasMED project for partner #3 with J.-Y. Bouet (ANR, 48 months)
2023-2024	Co-coordination of a funding from the LMGM with E. Brendon (10 k€)
2022	Co-coordination of a funding from the CBI with A. Mattout and A. Pérez-Escudero (10k€)
2019	Co-coordination of a funding from the CBI with A. Pérez-Escudero (8k€)
2015	Funding from the University of Toulouse (13 k€)

#### **Publications resulting from this work :**

**Ben Meriem Z., Mateo T., Faccini J., Denais C., Dusfour-Castan R., Guynet C., Merle T., Suzanne M., Di-Luoffo M., Guillermet-Guibert J., Alric B., Landiech S., Malaquin L., Mesnilgrete F., Laborde A., Mazenq L., Courson R., Delarue M.** An easy-to-use microfluidic mechano-chemostat for tissues and organisms reveals that confined growth is accompanied with increased macromolecular crowding bioRxiv 2023.03.29.534752; Lab Chip. 2023 Oct 10;23(20):4445-4455. doi: 10.1039/d3lc00313b. PMID: 37740366.

**Siguiet P., Campos M., Cornet F., Bouet J.Y., Guynet C.\*** (2023) Atypical low-copy number plasmid segregation systems, all in one? Plasmid. 127:102694. doi: 10.1016/j.plasmid.2023.102694. Epub 2023 Jun 8. PMID: 37301314.

**Cornet F., Blanchais C., Dusfour-Castan R., Meunier A., Quebre V., Sekkouri Alaoui H., Boudsoq F., Campos M., Crozat E., Guynet C., Pasta F., Rousseau P., Ton Hoang B., Bouet JY.** (2023) DNA Segregation in Enterobacteria. EcoSal Plus. eesp00382020. PMID: 37220081.

**Quèbre V., Del Campo I., Cuevas A., Siguiet P., Rech J., Le P.T.N., Ton-Hoang B., Cornet F., Bouet J.-Y., Moncalian G.\*, de la Cruz F.\* and Guynet C.\*** (2022) Characterization of the DNA Binding Domain of StbA, A Key Protein of A New Type of DNA Segregation System. *Journal of Molecular Biology*, 434(19):167752. doi: 10.1016/j.jmb.2022.167752. Epub ahead of print. PMID: 35868361. BioRxiv 2022.04.29.490116; doi:https://doi.org/10.1101/2022.04.29.490116.

Planchenault C., Pons M., Schiavon C., Siguier P., Guynet C., Dauverd-Girault J., Cury J., Rocha E.P., Junier I., Cornet F., Espéli O. (2020) Intracellular positioning systems limit the entropic eviction of 1 secondary replicons toward the nucleoid edges in bacterial cells. *Journal of Molecular Biology*, 432(3):745-761. doi: 10.1016/j.jmb.2019.11.027.

## 2.2 Research work

### 2.2.1 Transposition mechanism of IS608

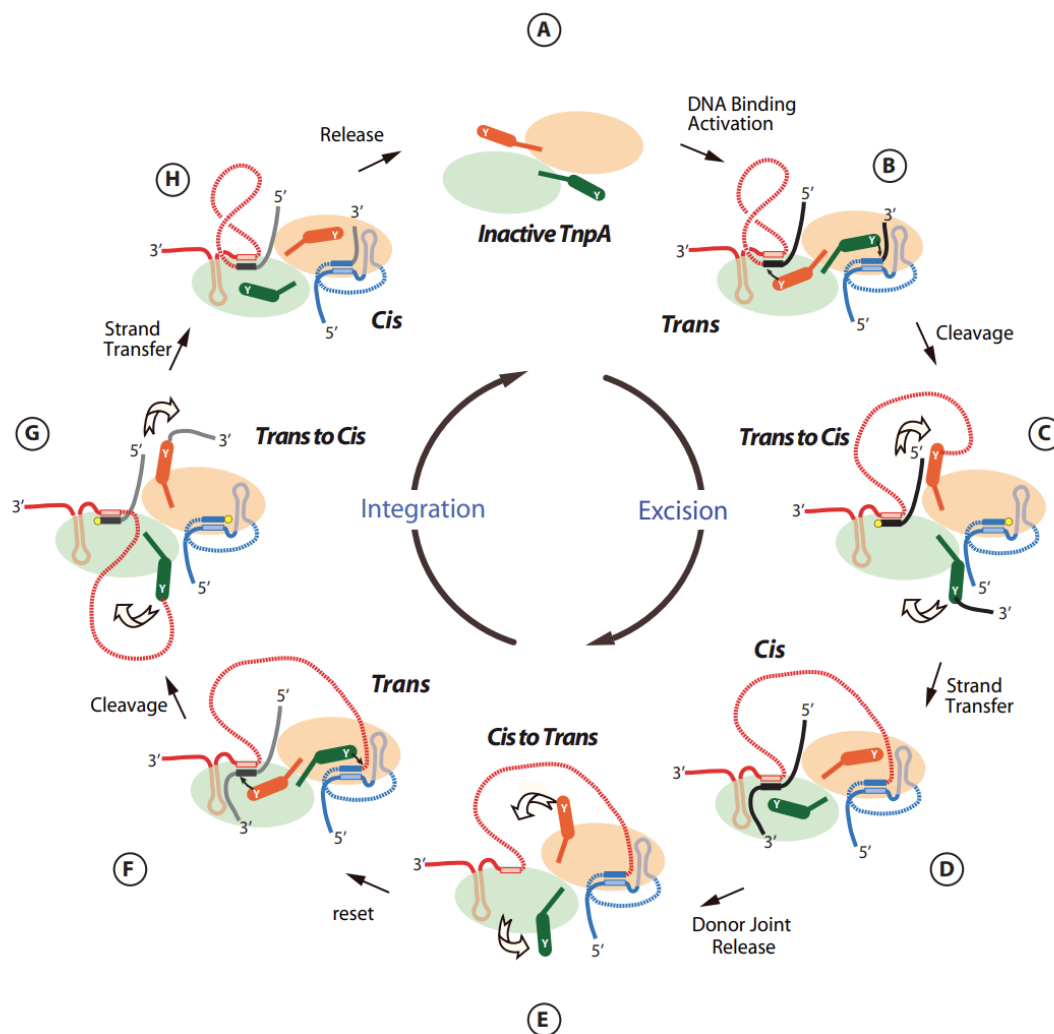
#### *Model for the IS608 transposition mechanism involving large conformational changes in TnpA*

In the period following my recruitment at CNRS, I participated in one of the ongoing projects in the team, which involves the study of the transposition of the IS608 element (see also **section 2**). IS608, isolated from *Helicobacter pylori* (Kersulyte et al., 2002), belongs to an atypical family of transposons, the IS200/IS605 family, whose members are highly represented in eubacteria and archaea (Ton-Hoang et al., 2005). These elements have the unique feature of using single-stranded DNA for all steps of the transposition cycle (Guynet et al., 2008). This cycle consists of two steps: the excision of IS608 as a single-stranded circular form and its integration into a single-stranded target DNA. Unlike most IS elements, IS elements from the IS200/IS605 family do not have inverted repeat sequences at their ends; instead, they contain imperfect palindromic sequences that form secondary structures. Unlike most IS elements, which integrate primarily randomly or with low sequence or structural specificity, they integrate specifically at the 3' end of a conserved AT-rich penta- or tetranucleotide sequence.

The transposase of IS608, TnpA, is part of the HUH endonuclease family, which catalyzes single-stranded DNA cuts and re-ligations by forming a 5'-phosphotyrosine intermediate and possesses an HUH motif (Histidine-hydrophobic residue-Histidine) involved in coordinating bivalent cations required for catalysis. The IS608 transposase thus belongs to the Y1 transposases since it uses a single catalytic tyrosine residue (Y127) to perform nucleophilic attacks on the phosphodiester bonds at the cleavage sites. Team studies have shown that this dimeric enzyme has two hybrid catalytic sites, consisting of an HUH motif from one monomer and a catalytic tyrosine residue (Y127) from the other monomer (*trans* configuration) (Barabas et al., 2008; Ronning et al., 2005). The Y127 residue is located on an

$\alpha$ D helix attached to the transposase by a flexible region, suggesting the possibility for the protein to adopt a *cis* configuration, where the HUH motif and the Y127 residue belong to the same monomer.

These properties led us to propose a model for the IS608 transposition mechanism involving large conformational changes in TnpA between the cleavage and strand transfer reactions (**Figure 6**; (He et al., 2015, 2013) ).



**Figure 6:** Strand transfer and reset model of IS608 transpososome.

Cleavage at the ends of the IS generates a 5'-phosphotyrosine linkage between the transposase and the 5' end of the transposon at LE, and the 5' end of the sequence flanking the transposon at RE. Our model proposes that strand transfer occurs through the rotation of the two  $\alpha$ D helices carrying the catalytic Y127 residue. Thus, the phosphotyrosine linkages can be resolved by reciprocal nucleophilic attacks of the 3'OH ends released during the cleavage reactions to generate the excision products.

These large conformational changes are suggested by several experimental data sets:

**(i)** The first argument in favor of the model concerns the differences between the lengths of the “linkers” at LE and RE ends (i.e., the distance between the stem-loop and the cleavage site): 10 nt for RE and 19 nt for LE. The model suggests that the length of the LE linker is crucial for strand transfer: a longer LE linker is required to allow the rotation of the LE 5'-phosphotyrosine linkage to position it near the immobile RE 3'-OH. Our experimental data confirm this hypothesis, as mutations leading to successive decreases in LE linker length significantly reduce the frequency of transposition *in vivo*. *In vitro*, the effects of these mutations are more significant on strand transfer than on cleavage, suggesting the importance of the linker for the mechanical movement of the  $\alpha$ D helix. Our study of over 100 different IS elements from the three subgroups of the IS200/IS605 family (35 from the IS200 group, 47 from the IS605 group, and 24 from the IS1341 group) shows that this asymmetry of IS608 ends is conserved throughout the family: the LE linker is always longer than the RE linker (15-16 nt versus 8 nt) (He et al., 2013).

**(ii)** The second argument in favor of the model comes from the behavior of heterodimers of TnpA carrying mutations in the HUH motif or the catalytic tyrosine residue Y127. These were expressed and assembled *in vivo* and purified using two different C-terminal tags (one for each monomer) to distinguish between heterodimeric and homodimeric forms. A heterodimer containing a combination of mutations favoring a *trans* active site (where the wild-type HUH motif and Y127 residue belong to different TnpA monomers) is competent in cleavage but not in religation. Conversely, a heterodimer with a *cis* active site (where the wild-type HUH motif and Y127 residue belong to the same monomer) is competent in religation but not in cleavage. This implies that all the chemical reactions involved in cleavage occur in *trans* configuration, whereas those involved in strand transfer occur in *cis* configuration. These data strongly support the model.

**(iii)** The flexible “arm” linking the  $\alpha$ D helix to the protein body would act as a pivot during the rotation. This flexibility could be facilitated by the presence of two glycine residues (G117 and G118). Their mutation does not affect cleavage but abolishes strand transfer, suggesting they are required for the protein to adopt the *cis* configuration. The conservation of these residues in the IS200/IS605 family underscores their importance (He et al., 2013). Although no

crystallographic structure confirms this to date, the existence of the *cis* configuration is strongly suggested by experimental data, supporting the *trans/cis* rotation model.

### ***Structure-based approach to target IS608 into highly specific DNA sequences***

As described above, the insertion sequence IS608, which in contrast to most ISs, recognizes a specific tetranucleotide sequence through base pairing, allowing its target choice to be reprogrammed by modifying the transposon DNA (Guynet et al., 2009). However, this target sequence specificity of 4-nt long is still short and poses a significant challenge for site-directed applications. I took part in a study along the lines of the publication Guynet et al., 2009, and developed in Orsolya Barabas' group (now at Université de Genève), aiming at engineer IS608 variants with improved target specificity (Morero et al., 2018). We determined the crystal structure of the IS608 target capture complex in its active conformation, offering a detailed view of the molecular interactions between the transposon and target DNA prior to integration. The use of an intact target substrate together with Ca<sup>2+</sup> ions to mimic the divalent metal ion cofactor without supporting cleavage, allowed us to visualize the conformation of the complete IS608 TnpA active site precisely aligned for target cleavage and transposon integration. Leveraging this structural information, we engineered IS608 variants to integrate specifically into various 12/17-nt long target sites by extending the base pair interaction network between the transposon and the target DNA. Our *in vitro* experiments demonstrate that these engineered transposons efficiently select their intended target sites. Additionally, our findings reveal how the unique secondary structure of the ss transposon intermediate prevents extended target specificity in the wild-type transposon, enabling it to move between diverse genomic sites.

This strategy allows for the efficient targeting of unique DNA sequences with high specificity in an easily programmable manner, paving the way for the use of the IS608 system in genome editing and biotechnology. Overall, this work highlights the successful application of structural biology to enhance the specificity of transposon integration, offering new tools for genetic manipulation.



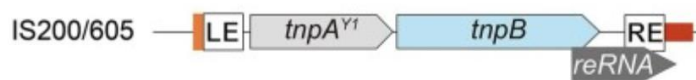
### *Recent advances in the understanding of the role of TnpB in IS608 transposition*

TnpB has been the subject of renewed interest in recent years, as it has been shown to act as a Cas RNA-guided DNA endonuclease, highlighting TnpB's potential evolutionary link to CRISPR-Cas systems, and suggesting a broader role in microbial genome dynamics beyond its initial characterization. The following is a brief summary of this new research, in which I was not involved, but which has implications for the IS608 transposition cycle, and notably sheds new light on the transposition cycle model, which was previously unable to explain the diffusion of IS200/IS605 elements. TnpB provides a crucial backup mechanism, ensuring the transposon is not lost during cell division, which can be critical for the survival and adaptability of the host organism.

CRISPR-Cas systems are adaptive immune mechanisms found in bacteria and archaea that protect against viral infections and other foreign genetic elements. "CRISPR" stands for "Clustered Regularly Interspaced Short Palindromic Repeats," which consist in segments of DNA containing repeated sequences interspersed with unique spacers derived from past infections by viruses or plasmids, and "Cas" refers to "CRISPR-associated" proteins. These are enzymes that perform various functions, including cutting DNA and RNA, and are essential for the CRISPR immune response. CRISPR-Cas systems provide a form of acquired immunity by storing fragments of viral DNA in the host genome and using them to recognize and combat future infections by the same virus (for a review: (Marraffini, 2015)). Briefly, when a bacterium is exposed to foreign DNA, specific sequences from this invader (protospacers) are captured and integrated into the CRISPR array within the host genome as new spacers that serve as a genetic memory of the invader. The CRISPR array, which consists of spacers interspersed with repeat sequences, is transcribed into a long precursor CRISPR RNA (pre-crRNA) and then processed into mature crRNAs. During the interference phase, this latter forms a complex with Cas proteins, creating an effector complex, in which the crRNA guides the complex to the complementary sequence in the invading DNA. This target recognition also requires the presence of a Protospacer Adjacent Motif (PAM) sequence near the protospacer for distinguishing self and non-self DNA. Once the target DNA is recognized, the effector complex induces cleavage. CRISPR-Cas systems are classified into two main classes, six types, and numerous subtypes based on their signature proteins and mechanisms of action, with diverse

functions and target specificities. Class 1 systems use multi-protein complexes for CRISPR interference, while Class 2 systems use a single effector protein, including types II Cas9 and type V Cas12, for interference (Koonin et al., 2017).

Cas9 and Cas12 proteins contain a RuvC endonuclease domain, which is responsible for cutting single-stranded DNA (ssDNA). Sequence analysis has indicated that Cas9 and Cas12 nucleases likely evolved from TnpB-like proteins (IscB and TnpB, respectively), which are found in bacterial IS200/IS605 elements. Surprisingly, the RuvC-like domains of Cas9 and Cas12 nucleases share greater sequence similarity with transposon-encoded TnpB proteins than with each other, suggesting that these proteins function as active nucleases. Supporting this hypothesis, biochemical experiments have shown that IscB and TnpB function as RNA-guided nucleases, similar to Cas9 and Cas12 effectors (Altae-Tran et al., 2021; Karvelis et al., 2021; Sasnauskas et al., 2023). The TnpB nuclease from *Deinococcus radiodurans* IS*Dra2* was shown to be directed by an RNA element derived from the right end of the transposon (**Figure 7**), enabling it to cut DNA at specific sites adjacent to the 5'-TTGAT motif associated with the transposon, called TAM for Transposon-Associated Motif, which plays similar functions as the PAM in CRISPR/Cas systems (Karvelis et al., 2021).



**Figure 7.** Loci architecture of IS200/IS605 transposons.

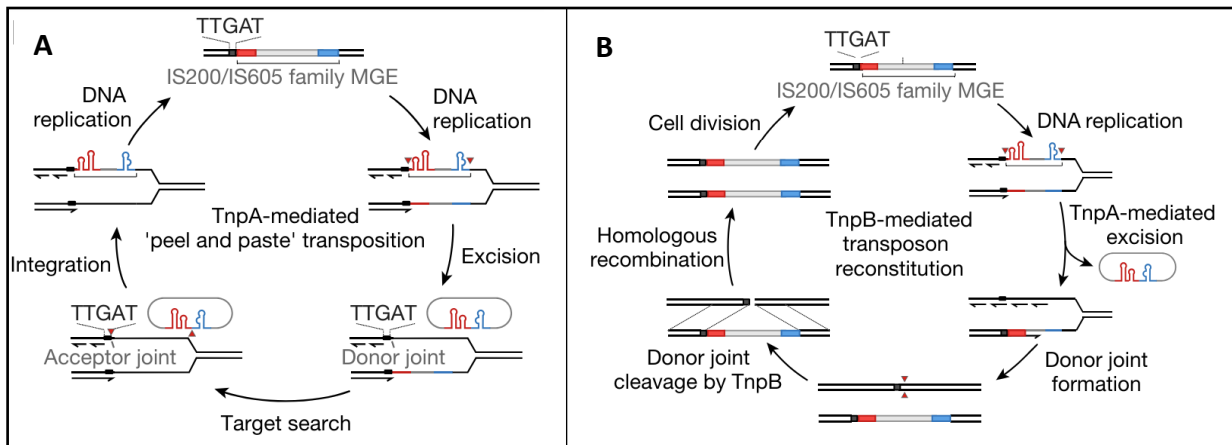
IS200/IS605 transposons encode proteins TnpA and TnpB, as well as a non-coding RNA called right-end RNA (reRNA). LE and RE correspond to left-end and right-end IS extremities. The transposon associated motif (TAM) and guide sequence are highlighted in orange and red, respectively. From (Yoon et al., 2023).

TnpB recognizes a specifically the TAM and is guided by a small RNA derived from the RE (reRNA), which helps in identifying and binding to the target DNA sequence. Once bound to the target site, TnpB introduces a double-strand break (DSB) at the site adjacent to the recognized TAM. The resulting DSB triggers a homology-directed repair process, which utilizes the donor DNA (original site of the transposon) as a template to repair the break, thus

reinserting the transposon back into its original location in a manner analogous to group I intron homing by intron-encoded endonucleases.

From these data, Karavelis and col. proposed that in elements containing both *tnpA* and *tnpB*, two types of transposition can thus occur: (1) excision and insertion of the transposon at a new site, catalyzed by TnpA, and (2) transposon 'homing,' where TnpB cuts DNA in a transposon-less allele, triggering recombination that copies the transposon into the same position. This ensures that both daughter cells acquire identical DNA copies, transforming the 'peel and paste' mechanism into a 'peel, paste, and copy' mechanism in the presence of TnpB (**Figure 8**).

The discovery of TnpB's role and its similarities to CRISPR-Cas systems also opens up potential biotechnological applications, such as genome editing, gene regulation, and possibly new strategies for combating antibiotic resistance by targeting mobile genetic elements. This evolutionary connection also underscores the adaptive reuse of genetic elements across different biological functions, from simple transposons to sophisticated immune systems in bacteria. This is yet another example of the potential use of data from basic research into the molecular mechanisms of EGMs mobility for biotechnological and medical purposes. It is also probably just the beginning of new discoveries on the involvement of RNA molecules in transposition, as shown by these very recent articles on *IS110*, whose smart transposition mechanism could offer an advantageous alternative to CRISPR systems for genome editing (Durrant et al., 2024; Hiraizumi et al., 2024).



**Figure 8 :** Model for the role of TnpB in *IS Dra2* transposition (From (Karvelis et al., 2021)). Transposon excision/insertion sites are marked by red triangles. LE is in red, RE is in blue.

**A.** TnpA-mediated 'peel and paste' mechanism. The TnpA dimer catalyses transposon excision from the lagging strand during DNA replication forming a circular single-stranded DNA intermediate and a donor joint. The excised transposon circle inserts at the acceptor joint into the lagging DNA strand 3' to the TTGAT sequence, completing the transposition cycle.

**B.** Model for the role of TnpB transposition: 'peel, paste, and copy' mechanism. The IS circle is excised from the lagging strand during DNA replication resulting in two DNA copies: one copy that originates from the leading strand and carries an intact transposon, and another copy that originates from the lagging strand and lacks the transposon at the original site due to the strand-specific transposon excision. However, the latter DNA copy still carries the transposon 'footprint' in the form of the donor joint, comprised of the 5'-TTGAT sequence and the 3'-flanking DNA sequence that becomes a target to the TnpB–reRNA complex. In this case, the 5'-TTGAT sequence serves as a TAM that initiates the binding of the reRNA sequence to the matching DNA sequence followed by dsDNA cleavage. TnpB-induced DSB could facilitate homology-directed repair to reinstate the transposon at the donor joint using its intact copy on the sister chromatid, ensuring that both DNA copies have a transposon-coding gene before cell division.

## 2.2.2 Single strand recombinases and REP/BIME dissemination in bacterial genomes: TnpAREP *in vivo* activity and mechanism

**My contribution to this work was directly linked to the supervision with B. Ton-Hoang of the thesis of A. Corneloup: “REP sequences dissemination in bacterial genomes: characterization of TnpAREP proteins activities”**

REP sequences (repeated extragenic palindromes) are found at high copy number in many bacterial genomes often clustered into structures called BIME (bacterial interspersed mosaic elements) consisting in two REP copies in inverted orientation separated by short

linkers of variable length. They have been attributed several important roles in cell physiology but their origin and dissemination mechanisms are poorly understood. These sequences are frequently clustered, separated by a variable linker and found as consecutive tandem copies over the chromosome. They might represent non-autonomous transposable elements mobilizable by TnpA<sub>REP</sub>, the first prokaryotic domesticated transposase associated with REP/BIME. TnpA<sub>REP</sub> are fundamentally different from classical transposases. They are, like the transposases of the IS200/IS605 family, members of the HuH superfamily of proteins. The HuH superfamily of proteins is widespread in all three domains of life and comprises a large number of proteins involved in various processes such as Rolling Circle plasmid and phage Replication (RCR Rep proteins) but also transfer of conjugative plasmids (Mob relaxases) and in transposition (transposases, Tpsases).

The underlying dissemination mechanism of REP/BIME remains to be elucidated. Previous work showed that *Escherichia coli* TnpA<sub>REP</sub> can cleave and join cognate single stranded REP *in vitro* and that this activity requires the integrity of the REP structure, in particular imperfect palindromes interrupted by a bulge and preceded by a conserved DNA motif (Messing et al., 2012; Ton-Hoang et al., 2012). A second group of TnpA<sub>REP</sub> are associated with REPs that rather carry perfect palindromes, raising questions about how the latter are recognized by their cognate TnpA<sub>REP</sub>. Recently, we reported for the first time TnpA<sub>REP</sub> *in vivo* activity. In addition, to go further in deciphering TnpA<sub>REP</sub> and get insight into the importance of REP structural and sequence determinants in these two groups, we developed a sensitive *in vitro* activity assay (CST, Cleavage and Strand Transfer) coupled to a mutational analysis for three different TnpA<sub>REP</sub>/REP duos via a SELEX approach. We showed that *in vitro* TnpA<sub>REP</sub> catalyzes excision and circularization of single stranded REP/BIME and their insertion into a single strand DNA target. We were able to detect/map REP/BIME cleavage and recombination/insertion on plasmids and bacterial chromosome DNA and to examine the importance of REP structural features. Our analysis demonstrated that TnpA<sub>REP</sub> of the two groups employ diverse strategies to recognize their REP substrates. Clearly both REP components, the GTAG tetranucleotide motif and the palindrome, are involved in TnpA<sub>REP</sub> activity but their respective impacts vary in each system. These results represent important progress in the comprehension of the distinct mechanism of TnpA<sub>REP</sub> mediated mobility and specificity of these expanding elements, which led us to discuss REPtrans

potential evolutionary routes, as well as a model of TnpA<sub>REP</sub> mediated REP/BIME dissemination/amplification (Corneloup et al., 2021).

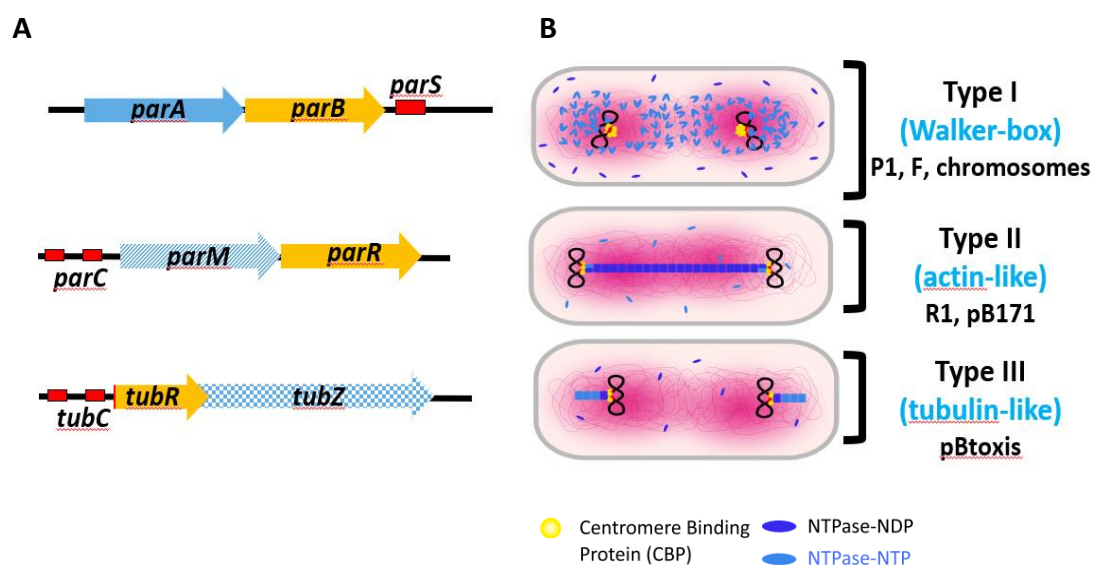
### 2.2.3 The Stb system of plasmid R388

***This work was initiated in F. de la Cruz's group during my post-doctorate. I reinitiated it few years after my recruitment and it is the basis of my current projects. Part of V. Quèbre thesis was devoted to this project (co-supervised 50% with J.-Y. Bouet).***

#### ***First characterization of the Stb system of plasmid R388***

Conjugative plasmids can be transmitted from a donor cell to a recipient cell *via* bacterial conjugation, sometimes between very distantly related species. They thus promote the dissemination of numerous genes, including virulence genes or antibiotic resistance genes, through horizontal transfer, making them major players in the diversity and evolution of bacterial genomes. The control of plasmid establishment in the new host cell after conjugation, which is very poorly studied, is a crucial aspect of the process, and this gives the region that first enters the recipient cell, called the leading region, a crucial role.

Conjugative plasmids are transmitted both vertically to ensure their stability and horizontally by conjugation. Vertical transmission, which requires the segregation of plasmid copies to daughter cells, is ensured by several processes, including copy number control or resolution of plasmid multimeric forms, and, in the case of most low-copy plasmids, an active partition system (Par) (for a review: (Bouet and Funnell, 2019)). These systems are composed of three elements: a centromere-like site functioning in *cis* containing a repeat of DNA sequences, a DNA-binding protein (ParB), and a motor protein (the ParA ATPase). Genetic organization and mechanisms of typical Par systems are illustrated in **Figure 9**.



**Figure 9 : Mechanisms of typical partition systems.**

**A.** Genetic organization of typical Par systems. Centromere-like sequences are shown in red, the gene encoding the NTPase in blue and the gene encoding the centromere binding protein (CBP) in yellow. **B.** As indicated, centromere binding proteins are represented as yellow circles, and NTPases as ovals (ATP bound, light blue; ADP-bound, dark blue). Type I partition systems use the Brownian ratchet mechanism, which relies on the ParA ATPase. ParA binds to the nucleoid in a nonspecific and ATP-dependent manner to DNA. The functional dynamics of the ParB-*parS* complex and its interactions with ParA are modulated by the CTP hydrolysis activity of ParB (Antar et al., 2021; Soh et al., 2019; Taylor et al., 2021). ParB/*parS* partition complexes associate with the nucleoid via ParA-ATP, and then stimulate the release of ParA from the DNA by ATP hydrolysis or conformational change. Due to the slow re-binding of ParA to the nucleoid, a void of ParA is created and serves as a barrier so that the partition complexes move toward opposite directions by following the ParA remaining bound to the nucleoid. The R1 paradigm plasmid type II partition systems uses ATP-dependent polymerization of the actin-like ParM ATPase to push plasmids poleward. ParR/*parC* partition complexes bind to the terminal ParM-ATP subunits at the growing end of the filament. Hydrolysis of ATP to ADP leads

to destabilization of the filaments, allowing entry of the ParM-ATP subunits. The filaments are polar and associate antiparallel, so that the plasmids are pushed in a bidirectional manner. In type III partition systems, the pulling mechanism, exemplified by pXO1, involves polymerization of the tubulin-like TubZ GTPase, which forms polar and dynamic filaments in a treadmilling-like pattern. These grow at the plus end by addition of TubZ-GTP and disassemble at the minus end, from which the TubR/tubC partition complexes are pulled to the pole.

Horizontal transmission, or conjugation, involves a DNA transfer machinery coupled to a type IV secretion system (T4SS, (Llosa et al., 2002)). The TrwC relaxase, which belongs to the HUH superfamily of enzymes including the TnpA transposase of IS608, catalyzes a cut at the *nic* site at the origin of transfer, *oriT*, and remains covalently linked to the DNA via a 5'phosphotyrosine bond (Llosa and de la Cruz 2005). Rolling-circle replication of the plasmid leads to displacement of the T strand, which is unidirectionally translocated (5'-3') into the recipient cell by the T4SS. The transferred strand is then ligated and its replication is initiated.

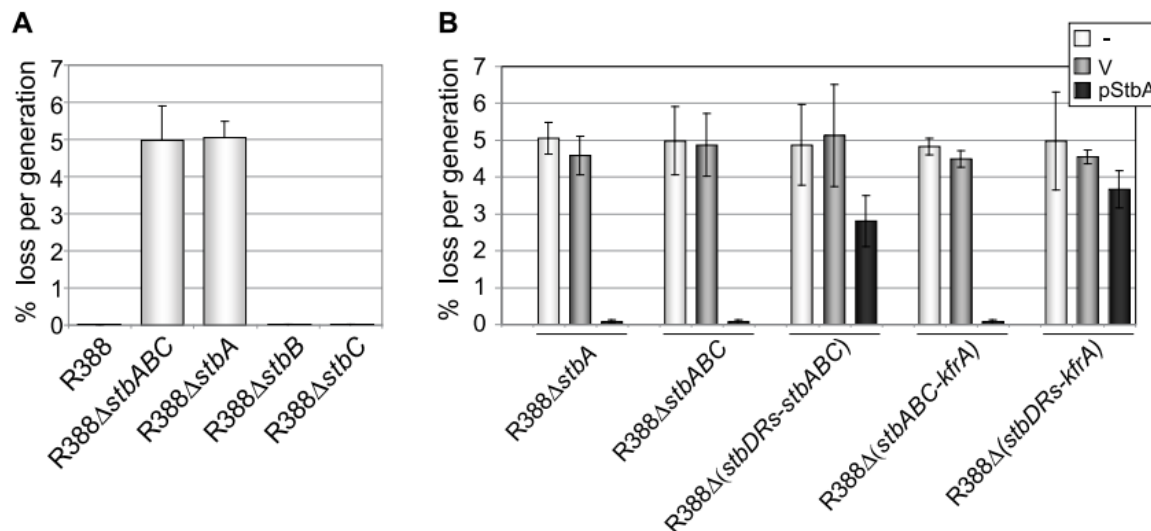
R388, isolated from *E. coli*, belongs to the broad host range IncW family. R388 is characterized by a compact genetic organization, representing a minimal conjugative plasmid genome divided into two main regions: one encoding general plasmid maintenance functions, and the other dedicated to conjugation (Fernández-López et al., 2006). R388 is one of the main models for studying conjugation systems (for a review: (de la Cruz et al., 2010)). R388 is maintained at 5-6 copies per cell, but no partition system had yet been identified (Fernández-López et al., 2006). R388, along with many other plasmids, carries an operon, the *stbABC* (*stb*) operon, always located in the leading region and adjacent to the plasmid region encoding the transfer machinery. This synteny conservation near *oriT* suggested an essential role of this operon in the conjugation process. I initiated the *in vivo* and *in vitro* characterization of this *stbABC* operon.

Our genomic analyses showed the synteny conservation of the three genes, with *stbB* being the most conserved, in the leading region of conjugative plasmids from several phylogenetic groups (MOBF11, MOBP11, MOBP6) (Guynet et al., 2011). This indicates that the *stb* operon is widespread in a significant portion of plasmids. Additionally, *stb* appears to be linked to type T secretion systems (MPFT T4SS). This observation could indicate that this type



of conjugation machinery requires the presence of an *stb*-like system for the plasmids that carry them.

Deletion of *stb* leads to instability of plasmid R388 (**Figure 10**; (Guynet et al., 2011)). Previous work has also shown that deletion of the *stb* operon leads to instability of plasmid R46 (IncN) (Paterson et al., 1999). Studying the stability of R388 derivatives with deletions of different components of the operon showed that only deletion of *stbA* leads to plasmid instability. This instability phenotype is not due to a decrease in plasmid copy number in the cell, as deletion of *stb* does not alter plasmid copy number (quantitative real-time PCR experiments), nor to the presence of multimeric plasmid forms, which can result from a partition defect. These results thus suggested a role for the StbA protein in active plasmid segregation.

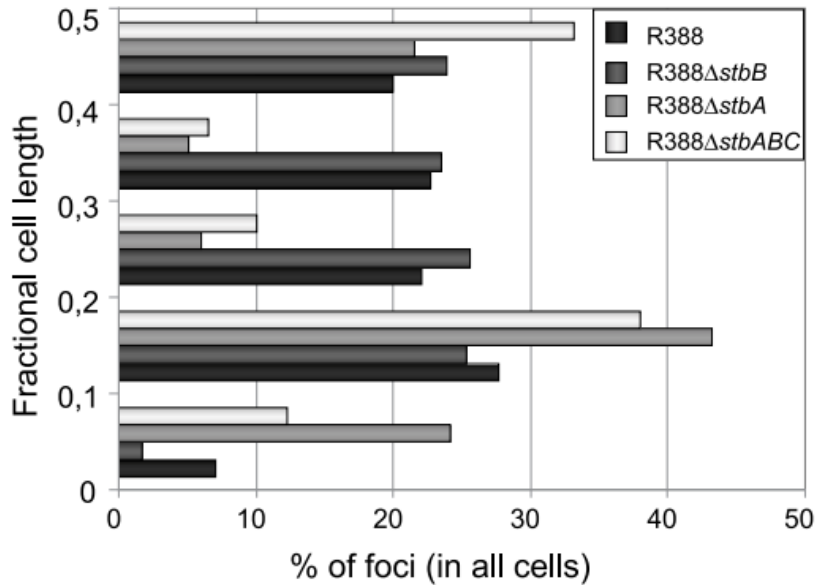


**Figure 10:** Stability of plasmid R388 derivatives. The stability of a series of derivatives of R388 was measured as the rate of loss per generation from strain LN2666 as described in Materials and Methods. Error bars show standard deviations calculated from at least four repeats of each assay. **A:** loss rates of R388 derivatives; **B:** loss rates of R388 derivatives in the presence of StbA. The loss rates of R388 derivative plasmids were measured when coresident with a second replicon (a p15A-derivative) as indicated in the top right panel (-, none; V, vector; pStbA, carrying *stbA*) (from (Guynet et al., 2011)).

Furthermore, the *stb* operon shows similarities with partition systems: StbB is a protein homologous to partition motor proteins, ParA ATPases; and its promoter region contains a region composed of two sets of five repeated 9 bp DNA sequences with the consensus sequence C/TTGCATCAT, called *stbDRs*, which could correspond to a "centromere." However,

I showed that deletion of *stbB* or *stbC* does not lead to R388 instability (**Figure 10A**) (Guynet et al., 2011). The results presented above indicated that the StbA protein is required for the stability of R388. It was shown that the TraK protein of plasmid RP4, homologous to StbA, specifically binds to a DNA sequence containing the region between *oriT* and the *traK* gene (Ziegelin et al., 1992). I showed that *stbDRs* are required for StbA activities. Furthermore, the stability of a plasmid deleted of *stb* (R388 $\Delta$ *stbABC*) as well as the entire plasmid maintenance region from *stb* to *kfrA* (R388  $\Delta$ *stbABC-kfrA*) is restored by expressing the StbA protein in *trans*, provided that the *stbDRs* are present (**Figure 10B**). These data indicated that *stbA* is the only gene required in the entire plasmid maintenance region for stable maintenance of R388 in *E. coli* and its activities requires the *stbDRs* (Guynet et al., 2011).

To understand the segregation mechanisms of R388, I used a *parS*- ParB-GFP system to study the subcellular localization of the plasmid in living cells (Li and Austin, 2002; Nielsen et al., 2006); **Figure 11A**). Wild-type R388 plasmid copies are distributed orderly throughout the cell (Guynet et al., 2011). Unlike the wild-type plasmid, R388 $\Delta$ *stbA* plasmid foci, on average two per cell, are excluded from the nucleoid and preferentially located at the center and poles of the cells. This localization defect seems to be the main cause of the observed instability, as plasmid copies are not distributed in the cellular spaces corresponding to daughter cells. The distribution of R388 $\Delta$ *stbB* plasmid foci appears similar to that of the wild-type plasmid, except in polar regions. Indeed, unlike non-conjugative mini-F and mini-P1 plasmids that localize in a restricted cell space corresponding to the nucleoid (Gordon et al., 1997; Li and Austin, 2002; Niki and Hiraga, 1997), a significant fraction (7%) of R388 foci is located near the polar membrane (**Figure 11A**). This dissimilarity could be related to the ability of R388 to conjugate, which involves its transfer to the conjugative pore (T4SS), which we have shown to be localized at the cell poles ((Segura et al., 2014) and unpublished). The fraction of R388 $\Delta$ *stbB* foci present at the poles is significantly lower than in the case of the wild-type plasmid.

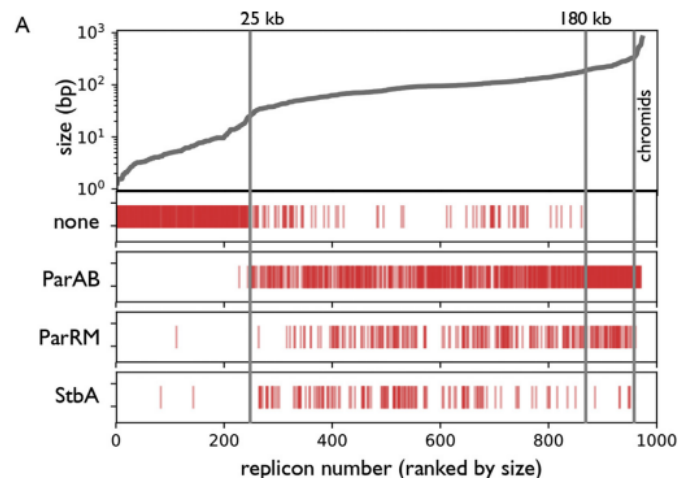


**Figure 11:** Distribution of GFP foci within the different fractions of cell length. The distance of foci to the closest cell pole was measured and sampled into five equal sections of cell length from the pole to mid-cell. R388 (n = 320); R388 $\Delta$ stbA (n = 205); R388 $\Delta$ stbB (n = 210); R388 $\Delta$ stbABC (n = 309). n, number of analyzed cells. (from (Gynet et al., 2011)).

### *Dynamics of bacterial secondary replicons*

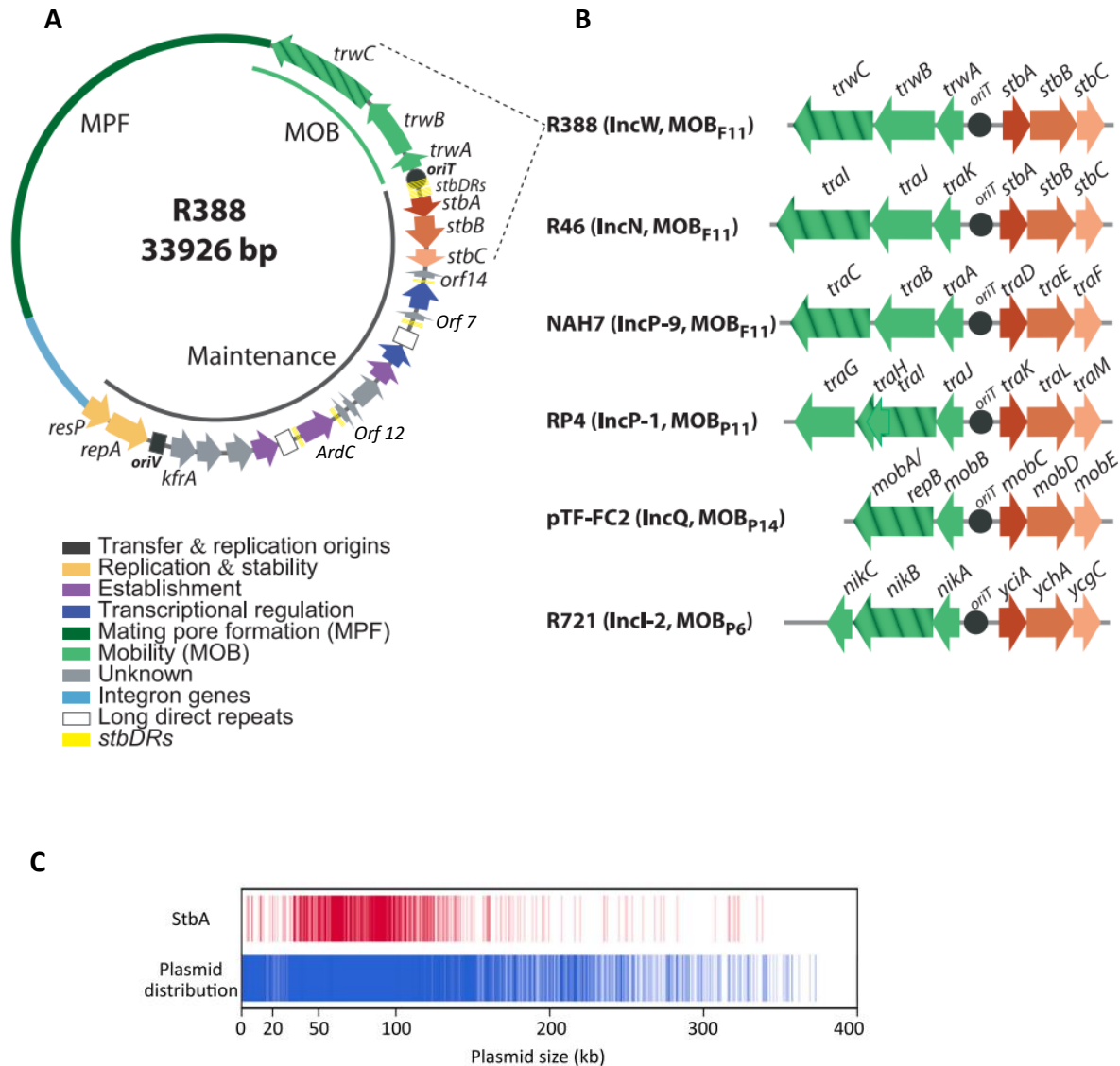
With the groups of O. Espeli (CIRB, Paris), E. Rocha (Institut Pasteur, Paris), I. Junier (TIMC, Grenoble), we combined bioinformatics, mathematical modelling and fluorescence microscopy to study the impact of the size of secondary replicon on their dynamics (Planchenault et al., 2020). We have shown that the different types of replicons - small multi-copy number plasmids, large plasmids and secondary chromosomes (chromids)- display dynamics that can be predicted by simulations of a model nucleoid. They code for subcellular positioning systems (ParAB, ParRM or StbA) according to their capacity to cross the chromosome at the nucleoid periphery. We identified two important step-size in

enterobacterial secondary replicons: 25 kb, above which replicon harbour a positioning system and 200 kb above which ParAB systems are specifically required (**Figure 12**).



**Figure 12:** Distribution of subcellular positioning systems in enterobacterial secondary replicons. The 970 replicons were ranked according to their size (x-axis; the top panel shows a cumulative plot of replicon sizes indicative of size density). The bottom four panels show event plots of the presence of the indicated system (vertical line) or absence of system detected (none). From (Planchenault et al., 2020).

We searched the StbA proteins by similarity using the six *stbA* alleles previously identified (**Figure 13AB**, (Guynet et al., 2011)), and an HMM profile was then constructed using the conserved N-terminal domains of the StbA homologs detected. Homologs of StbA were found in about 14% of the plasmids from a database containing 971 secondary replicons from Enterobacteria selected for their representativity based on the diversity of their replication and transfer machineries and the genus they belong to. We found that StbA was mostly present in medium-sized plasmids (Planchenault et al., 2020).

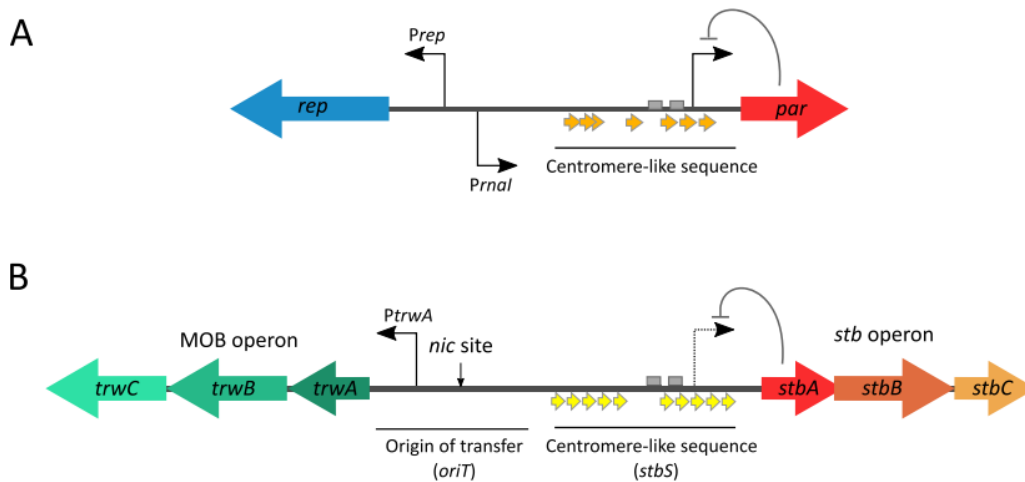


**Figure 13:** The *stbABC* operon. **A:** genetic organization of plasmid R388. ORFs and other sequence features are depicted in different colors according to the legend on the left, after assignment to functional modules based on database similarity and/or available experimental data [12]. The R388 genome is compact and organized in two major modules, one devoted to general maintenance functions and one devoted to conjugation (MOB, Mobility genes; MPF, Mating Pore Formation). *stbDRs*, located around the promoter region of the *stbABC* operon and consisting of two sets of five direct repeats of a 9 bp DNA sequence spaced by two nucleotides, are indicated. This 9 bp DNA sequence is also present in number of two or three in three other promoters of the maintenance region. **B:** Synteny conservation between the MOB-Stb region of R388 and those of other plasmids. Hatched arrows indicate the relaxase gene. Plasmid GenBank accession numbers: R388: BR000038, R46: NC\_003292, NAH7: NC\_007926, RP4: L27758, pTF-FC2: M57717.1, R721: NC\_002525. From (Guynet et al., 2011). **C:** Distribution of StbA segregation systems. The event plots represent the presence of StbA (blue vertical lines) in the 5820 enterobacterial replicons (red vertical lines) and which were ranked according to their size (x-axis). From (Siguier et al., 2023).

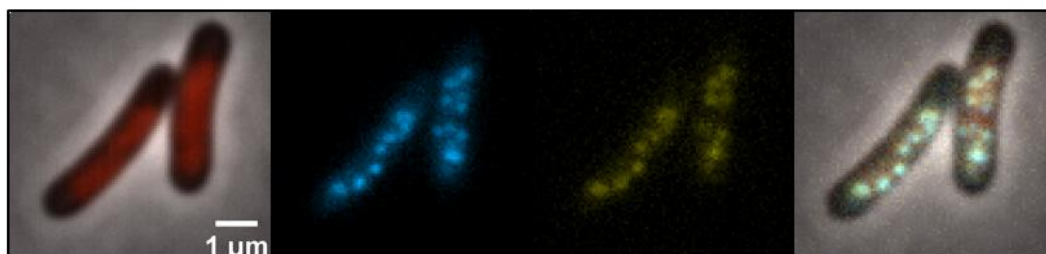
We further searched for StbA homologs in all Enterobacterial plasmids present in RefSeq (Siguier et al., 2023). In agreement with our previous results, StbA was found in 17% of plasmids (957 of 5820), the large majority (92%) ranging from 20-kb to 150-kb (**Figure 13C**). Thus, we confirmed that StbA is preferentially found in medium sized plasmids in enterobacteria. We next built a phylogenetic tree based on amino acid sequences of homologs of StbA, from previously identified alleles (Guynet et al., 2011). StbA family members fall into four major groups. Groups 1, 2 and 4 seem to be restricted to  $\gamma$ - and  $\beta$ -proteobacteria and group 3 also includes cyanobacteria. Overall, these data indicate that StbA proteins are widespread, at least in Proteobacterial species. They also suggest that their function is well adapted to medium size plasmids (i.e., ranging from 20-kb to 150-kb, (Siguier et al., 2023)).

#### ***A new type of segregation system: all in one?***

Plasmid R388 involves a single plasmid-encoded DNA-binding protein, which acts as a centromere-binding protein (CBP) that is not associated with a plasmid-encoded NTPase (Guynet et al., 2011; Guynet and de la Cruz, 2011). The staphylococcal plasmid pSK1 shares the same particularity, although the pSK1 and R388 CBPs, Par and StbA, respectively, have no homology to each other or to other known partitioning proteins (Siguier et al., 2023; Simpson et al., 2003). Plasmids R388 and pSK1 are low-copy-number plasmids. The copy number of pSK1 has not been determined experimentally, but pSK1 minireplicons are, similarly to R388, maintained at about 5 copies per chromosome (Grkovic et al., 2003). This range of copy number, which is theoretically not high enough for faithful vertical transmission to rely on a stochastic distribution (Nordström and Austin, 1989), requires an active segregation process. Both StbA and Par proteins act in *trans* with a specific centromere-like DNA sequence, which in the case of R388 consists of two sets of five direct repeats of a 9-bp sequence (*stbDRs*) located in the promoter region of the *stb* operon and which we call *stbS* (**Figure 14**).



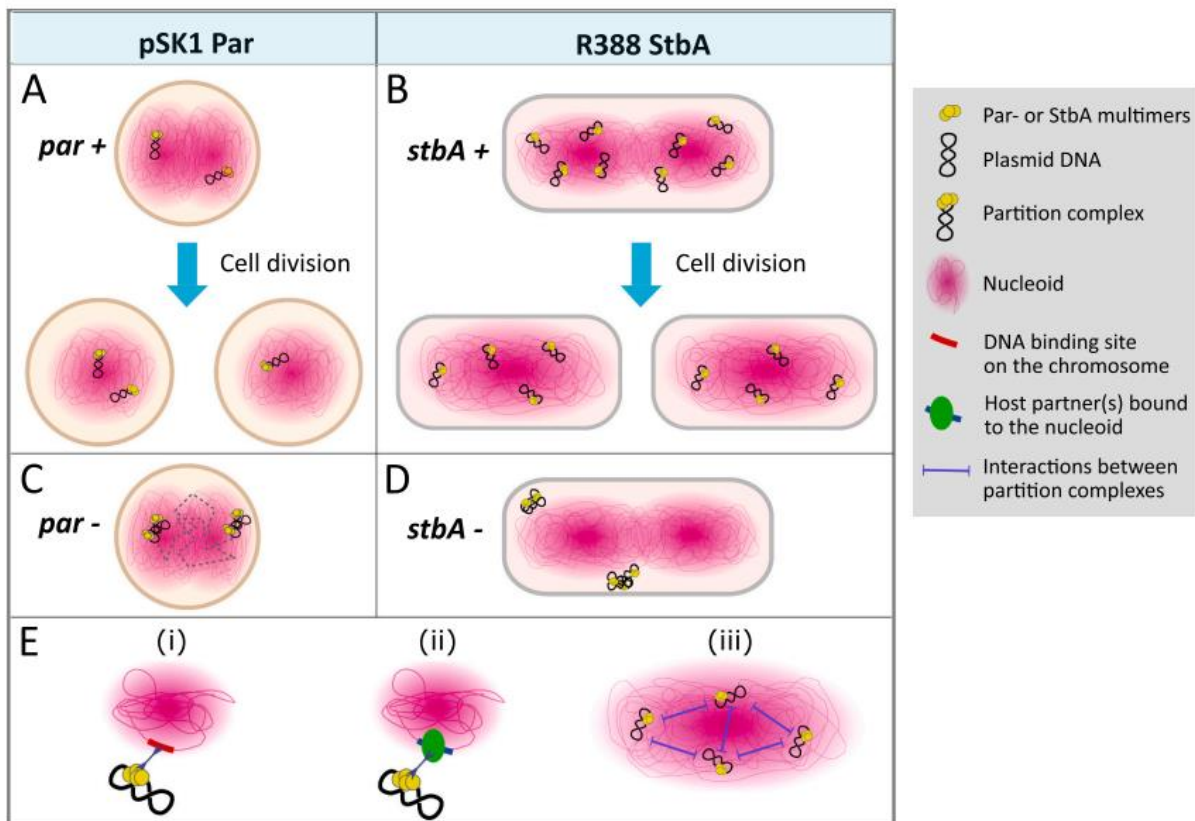
**Figure 14:** Genetic organization of *par* (A) and *stb* (B) regions of plasmids pSK1 and R388, respectively. Promoters are indicated by black bent arrows, dotted black the putative promoter P<sub>stb</sub> (predicted with SAPPHIRE, (Coppens and Lavigne, 2020)). Gray boxes represent – 10 and – 35 sequences of P<sub>par</sub> and the putative P<sub>stb</sub>. As indicated, Par and StbA repress their own promoter. Direct repeats of the centromere-like sites are represented by orange and yellow (*stbDRs*) solid arrows, respectively. The origin of conjugative transfer of plasmid R388 is shown as a vertical arrow. The scale is not respected. (From (Siguier et al., 2023)).



**Figure 15:** Plasmid R388 and StbA co-localize in live *E. coli* cells.

Live cell imaging of *E. coli* strain LN2666 containing plasmid R388 harboring *parS<sub>F</sub>* and mvenus-StbA and expressing mturquoise-Δ30ParB. Scalebar = 1 μm. From left to right, HU-mcherry (red) merged with phase contrast, mturquoise-ParB-Δ30ParB-tagged plasmids (blue), mvenus-StbA (yellow), merge.

In both cases, deletion of the CBP results in a decrease in the number of fluorescent-tagged plasmids and leads to their aberrant localization or altered dynamics, contributing to plasmid instability. Without Par, most cells lack any foci, and in cells that do have foci, these are highly mobile and do not segregate properly, resulting in a failure to ensure faithful inheritance to daughter cells upon division (**Figure 16C**). Similarly, the absence of StbA, which does not affect plasmid copy number, is associated with a significant reduction in the number of foci (ranging from 1 to 3), which tend to cluster in nucleoid-free regions, primarily at one cell pole (**Figures 11&16D**). Observation of the intracellular positioning of R388 DNA molecules and StbA in live *E. coli* cells showed that they co-localize as discrete foci evenly distributed, both in nucleoid and cytosol areas with no evidence for preferential localization (**Figure 15**; unpublished; (Gynet et al., 2011)).



**Figure 16:** Models for plasmid segregation mediated by single-protein systems. Symbols are indicated in the legend of the figure (right panel). **A-D.** Schematics showing the sub-cellular positioning of pSK1 minireplicons and R388 plasmids in *S. aureus* and *E. coli* cells, respectively. pSK1 minireplicons are confined in restricted areas in the presence of Par and separate into two or more foci in dividing cells (**A**), whereas in the absence of Par they are highly mobile (shown as a gray dotted line) and do not separate (**C**). Plasmids R388 are evenly distributed in the nucleoid area in the presence of StbA (**B**), whereas they are clustered and excluded from the nucleoid in the absence of StbA (**D**). (**E**) Proposed models for positioning and segregation

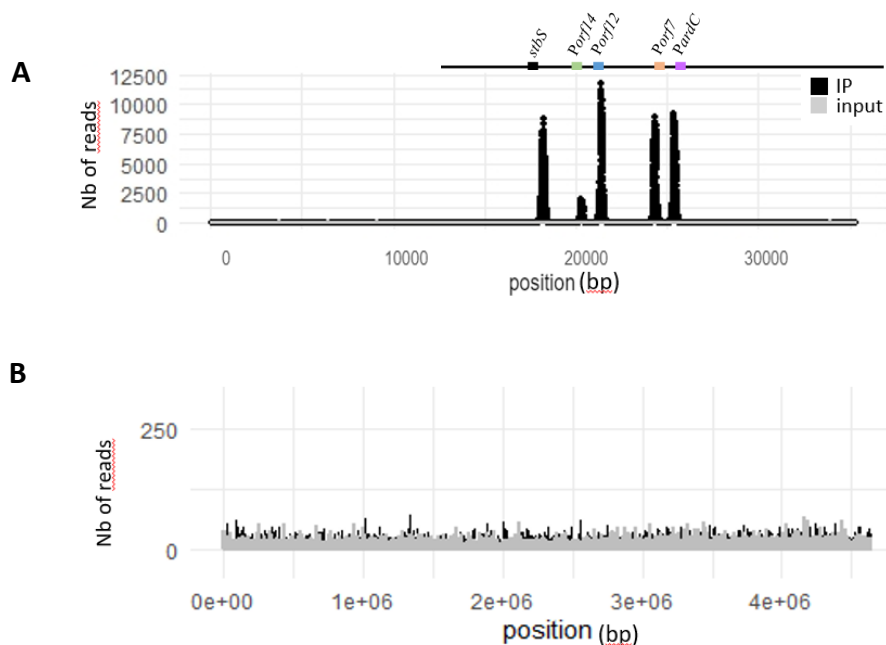


mediated with a single-protein system. (i) and (ii) represent a hitchhiking mechanism, in which plasmid molecules are attached on the bacterial nucleoid either through direct interactions between the segregation protein and the chromosomal DNA (i), or through interactions with one or more host proteins that bind to the nucleoid (ii). The plasmids thus take advantage of the segregation of the chromosomes to distribute themselves between the two daughter cells during bacterial division. In (iii), the partition complexes would interact with each other rather than with the nucleoid to partition plasmids into the nucleoid space and ensure daughter cells to receive at least one copy of the plasmid. (From (Sigulier et al., 2023)).

Plasmid localization studies in live cells have used a TetR-GFP/tetO system in *S. aureus* for pSK1 minireplicons and a ParB<sub>P1</sub>-GFP/*parS* system in *E. coli* for R388 plasmids (Chan et al., 2022; Guynet et al., 2011). The presence of Par correlates with limited mobility of pSK1 minireplicon foci, usually two per cell, which are confined and segregate during cell division. For plasmid R388, cells typically exhibit 4 to 6 evenly distributed foci, corresponding to its copy number. Deletion of the CBP in both systems results in fewer foci, aberrant localization, and increased plasmid instability. Without Par, most cells lack foci, and any remaining foci are highly mobile and fail to separate properly (**Figure 16C**). Similarly, the absence of StbA does not affect plasmid copy number but results in fewer foci, which cluster in nucleoid-free spaces, mainly at one cell pole (**Figures 11&16D**). These findings highlight the role of Par and StbA as sub-cellular positioning systems that localize plasmids to the nucleoid, potentially facilitating efficient segregation by exploiting entropic forces.

To date, the mechanism by which these proteins achieve plasmid segregation is not understood. Because StbA is neither an NTPase motor nor an NTP/NDP exchange factor, R388 segregation may involve either a motor provided by the host cell, or may not need a motor at all. Rather, it may take advantage of the host chromosome segregation as vehicles for segregation, this is the 'hitchhiking' or 'pilot-fish' model (**Figure 16E**). The stable propagation of plasmids through their physical association with the host chromosome parallels the partitioning systems seen in eukaryotic extra-chromosomal elements, such as the 2-micron yeast plasmid and certain mammalian viral episomes (reviewed in (Sau et al., 2019)). In pSK1 and R388 plasmids, Par and StbA centromere-binding proteins likely bind to their respective centromeres, forming a segregation complex that associates with the nucleoid either through direct chromosomal interactions or *via* unknown host-associated factors. So far, no potential binding sites in host genomes and no potential host-factors have been identified for any of

the systems. In addition, this model may pose challenges given the broad host range of plasmids like R388. In the case of R388, our Chip-seq analyses did not reveal any StbA binding site on the *E. coli* chromosome (**Figure 17B**, unpublished data). This is in agreement with the fact that, even if more than 350 matches to the 9-bp *stbDR* repeats sequences are found on the *E. coli* chromosome, the combination of two *stbDR*s in direct repetition separated by 2 bp, required to detect binding *in vitro* (Quèbre et al., 2022), is not found. We have also failed so far to identify StbA host partners in *E. coli* by searching for proteins using bacterial two-hybrid assays (BACTH), and our plasmid stability assays using Keio mutants suggest that none of the *E. coli* NAPs (Nucleoid Associated Proteins) are required for R388 segregation.

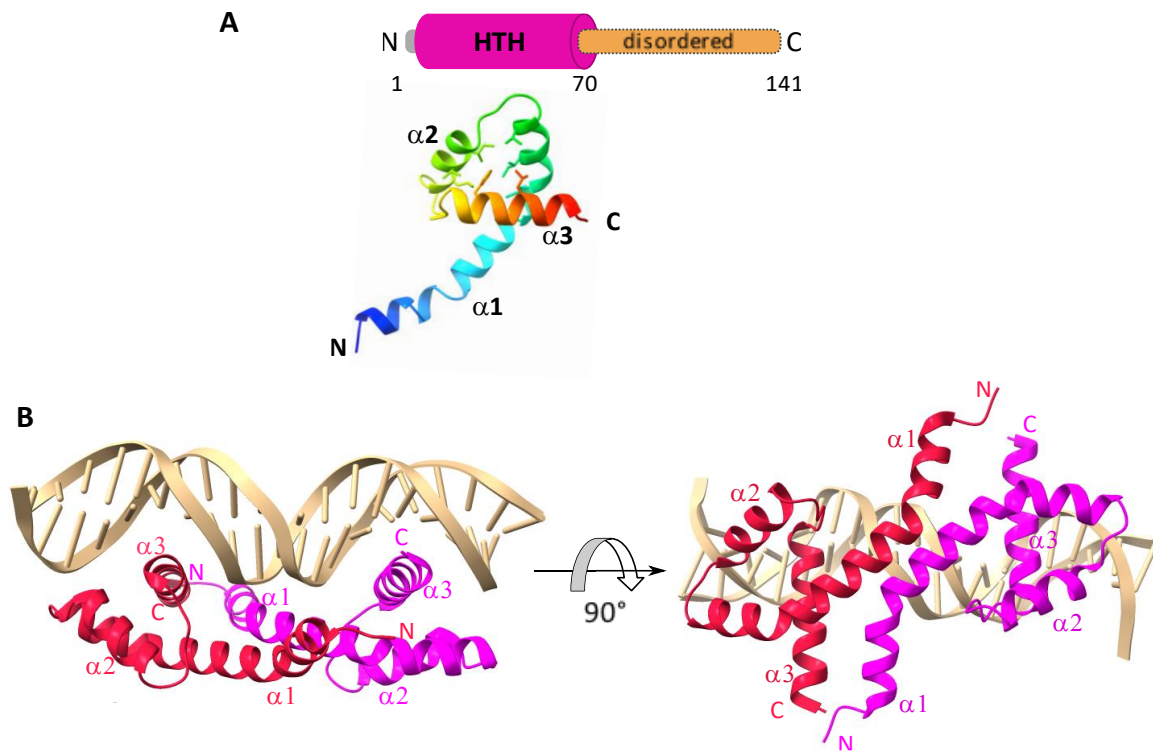


**Figure 17:** Interaction profile of StbA on R388 and on the *E. coli* chromosome. ChIP sequencing of StbA at a sequencing depth of  $\approx 11.5$  million reads. Average library fragment size is 194bp. Immunoprecipitated (IP) DNA was sequenced and the number of reads for each base is plotted. Total DNA sequenced (input) represents the background noise of the analysis. To increase accuracy, sense and antisense reads were positioned at the center of each fragment (based on library size). **A.** ChIP sequencing of StbA on plasmid R388. A schematic diagram of the genomic organization of interest is shown above the graph. **B.** ChIP sequencing of StbA on the *E. coli* chromosome (MG1655) (unpublished).

As an alternative to these models, plasmids could position themselves through repulsion forces between partition complexes, occupying the largest possible volume within the nucleoid, ensuring their proper distribution during cell division (**Figure 16E**).

### ***What does StbA tell us about its functions?***

To unravel the molecular basis of R388 segregation, we characterized its DNA binding domain (Quèbre et al., 2022). In order to assess the diversity of the StbA proteins and gain structural information, we performed BLASTP searches among all complete prokaryotic genome sequences available using R388 StbA as a query. It turned out that the N-terminal half of StbA, which is a small protein of 110 residues, displays high degree of conservation, while the C-terminal half of the protein is very poorly conserved. Limited proteolysis further confirmed that StbA is organized in two domains. We determined the crystal structure of the pretty conserved N-terminal domain of StbA (StbA<sub>1-75</sub>) to 1.9 Å resolution (**Figure 18A**). It folds into an HTH DNA-binding domain, structurally related to that of the PadR subfamily II of transcriptional regulators. This domain, which is a typical HTH with three  $\alpha$  helices, of which  $\alpha$ 3 is supposed to be the recognition helix responsible for the specific binding to the major groove of DNA, contains the DNA binding activity required for specific binding to the *stbDR* sequences and for plasmid segregation. *In vitro* experiments (EMSA) further strongly suggest that FL StbA, as well as StbA<sub>1-75</sub>, binds to the *stbDRs* with high cooperativity resulting in the binding of two StbA HTH domains to every two *stbDRs*. Since no structure of StbA in the presence of DNA is yet available, we attempted to generate a model of StbA<sub>1-75</sub> bound to DNA (**Figure 18B**). In the model, StbA assembles as a head-to-tail dimer and, as expected, the recognition  $\alpha$ 3-helix of each monomer inserts into the major groove of the DNA. Noteworthy, both monomers contact the DNA on the same side, which is consistent with the organization of *stbS* in two arrays of five 9-bp *stbDRs* spaced by 2-bp, thus corresponding to a complete helix turn.



**Figure 18:** StbA DNA binding domain.

Crystal structure of StbA N-terminal domain. **A.** Schematic of the secondary structures elements of StbA showing that it is organized in two domains, and ribbon diagram of the monomeric tri-helical HTH structure of StbA<sub>1-75</sub> showing the HTH fold formed by the three  $\alpha$ -helices in the molecule (PDB ID: 7PC1). **B.** Model of StbA DNA-binding domain bound to DNA. StbA<sub>1-75</sub> (PDB ID: 7PC1) and the replication terminator protein of *Bacillus subtilis* bound to DNA (PDB ID: 1F4K) structures were superimposed.  $\alpha$ -helices of the HTH domain are indicated. The display of structures and analyses were performed with UCSF Chimera. Adapted from (Quèbre et al., 2022) and (Siguier et al., 2023).

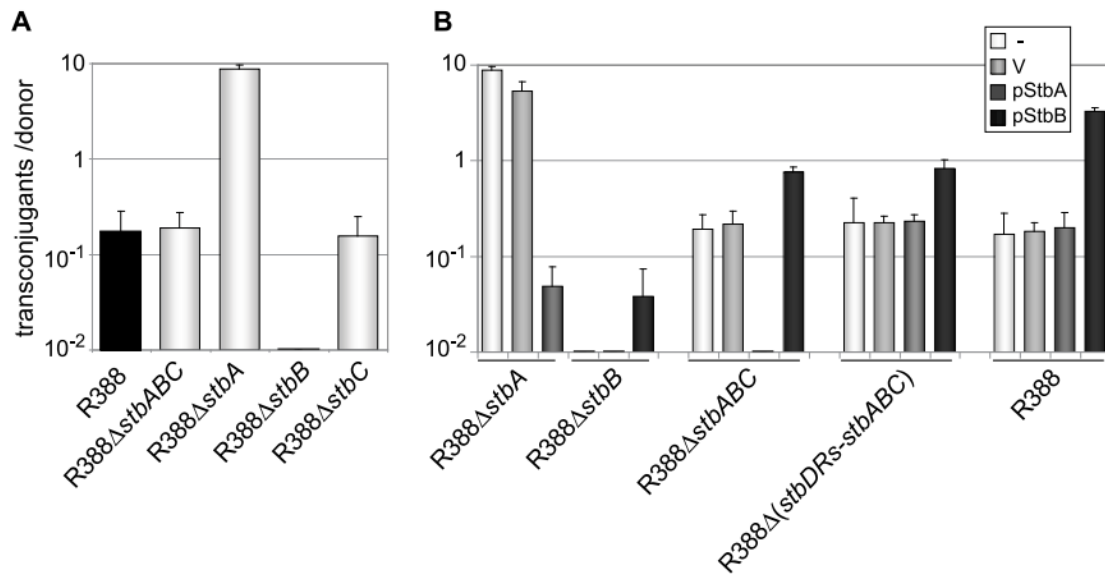
The non-conserved C-terminal half harbors a predicted disordered region (residues 69 to 108) and its function is unknown (**Figure 18A**; (Quèbre et al., 2022)). A truncated version of StbA, deleted of its C-terminal domain (StbA<sub>1-75</sub>), displays only partial activities *in vivo*, indicating that the non-conserved C-terminal domain is required for efficient segregation as well as subcellular plasmid positioning. One might expect that, as with the characterized PadR family proteins to which StbA is structurally related, it would mediate dimerization through interactions with the HTH domain of the other monomer, but there is no evidence for this yet and the contribution of StbA C-terminal domain in the oligomerization of the protein is unknown. Its disordered nature probably correlates with the failure to crystallize FL StbA

despite numerous attempts, as well as the very low confidence in all predictions with AlphaFold 2, which does not allow to propose a 3D structure model of the FL protein. Although the C-terminal domain is not required for the formation of specific StbA-*stbDRs* complexes *in vitro*, it is clearly necessary for StbA activities *in vivo*. Indeed, StbA<sub>1-75</sub> exhibits only partial activities in segregation and subcellular positioning, as well as reduced activity in repressing the *stbDR*-carrying promoters of plasmid R388. The C-terminus of StbA might thus stabilize interactions between StbA and the *stbDR* sites, and/or promote interactions with other partners (Quèbre et al., 2022).

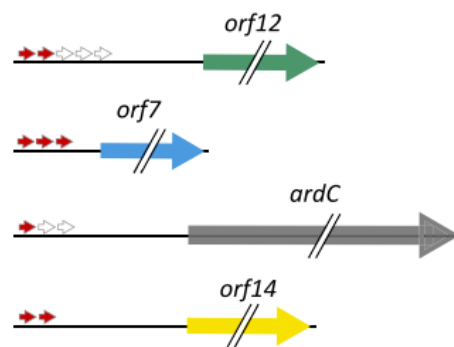
### ***Stb and the control of conjugation***

StbA and StbB control conjugation with opposite but interdependent effects, which correlates with variations in intracellular positioning of plasmid DNA (**Figures 11&19**; (Guynet et al., 2011; Quèbre et al., 2022)). Indeed, while deletion of the entire *stb* operon does not affect conjugation, deletion of *stbA* leads to a (50-fold) increase in conjugation frequency correlated with the exclusion of plasmid foci from nucleoid areas. Conversely, deletion of *stbB* leads to a complete inhibition of conjugation frequency, correlated with the absence of plasmid foci near the cell poles, where DNA transport channel assembly occurs ((Segura et al., 2014) and unpublished).

In addition to its role in plasmid segregation, StbA acts as a transcriptional repressor of the expression of some R388 genes present in the leading region of R388 (*stbA*, *ardC*, *orf7*, *orf12* and *orf14*, **Figures 13A&20**) (Fernandez-Lopez et al., 2014; Quèbre et al., 2022). We have shown that StbA binds to the promoters of these genes (**Figure 17A**). All StbA-regulated promoters include two or more *stbDRs*. No function has yet been assigned to *orf7*, *orf12* or *orf14*. *ardC* encodes the antirestriction protein ArdC, which is required for conjugation from *E. coli* to *Pseudomonas putida* (Belogurov et al., 2000; González-Montes et al., 2020). We can assume that these genes, by virtue of their position in the leading region of R388, encode functions linked to conjugative transfers. This would make the repressor activity of StbA a means of controlling conjugation.



**Figure 19:** Conjugation frequencies of plasmid R388 derivatives. Plasmids contained in the different donor strains are indicated on the X axis. The figures shown represent the mean and standard deviation of at least four independent assays. **A:** Donor strains contain the R388 derivatives shown; **B:** Complementation analysis. Donor strains contain the same R388 derivatives plus a helper complementing plasmid, as shown (V = empty vector) (from (Guynet et al., 2011)).



**Figure 20:** Genes of which promoters are regulated by StbA. stbDRs are represented by gray arrows and consensus ones are colored in red.

All these data suggested that StbA and StbB control plasmid R388 subcellular positioning and conjugation by a mechanism that we did not understand, and that StbA acts as an atypical segregation system that does not involve a plasmid-encoded NTPase partner (Guynet and de la Cruz, 2011). This study was the first evidence for a mechanistic interplay between plasmid vertical and horizontal transmission, and the starting point of my current

projects as it is one of the important and challenging questions remaining in the field concerning the spatiotemporal dynamics of secondary replicons.

#### 2.2.4 "Handling" of the *Caenorhabditis elegans* model gut microbiota

***With a view to studying horizontal gene transfer in a complex bacterial community, we have initiated a study to better characterize the synthetic microbiota of Caenorhabditis elegans (CeMBio, (Dirksen et al., 2020)). This is part of the results of R. Dusfour-Castan's current thesis. Two Master 2 students made a significant contribution to this project (R. Miranda-Capet and C. Hall).***

*C. elegans* is a bacterivore capable of multiplying and reproducing in a variety of bacterial regimes. Recent studies indicate that *C. elegans* carries a complex microbiota, composed of a wide diversity of bacterial species, with the dominant group comprising several  $\gamma$ -proteobacteria (Enterobacteriaceae, Pseudomonaceae and Xanthomonadaceae) (Dirksen et al., 2016; Zhang et al., 2017). *C. elegans* is also a model of choice for our study for technical reasons: ease of cultivation, short life cycle, hermaphroditism allowing homozygous cultures to be maintained, and transparency in light microscopy allowing direct visualization of bacterial cells *in vivo*. Importantly, it is a bacterivore and its microbiota is made up of the bacteria present in the food plate where it develops from sterile eggs. We use the CeMBio bacteria as a simplified natural microbiota of *C. elegans* (Dirksen et al., 2020). It comprises a dozen environmental bacterial strains selected from meta-analyses of the nematode's natural microbiome.

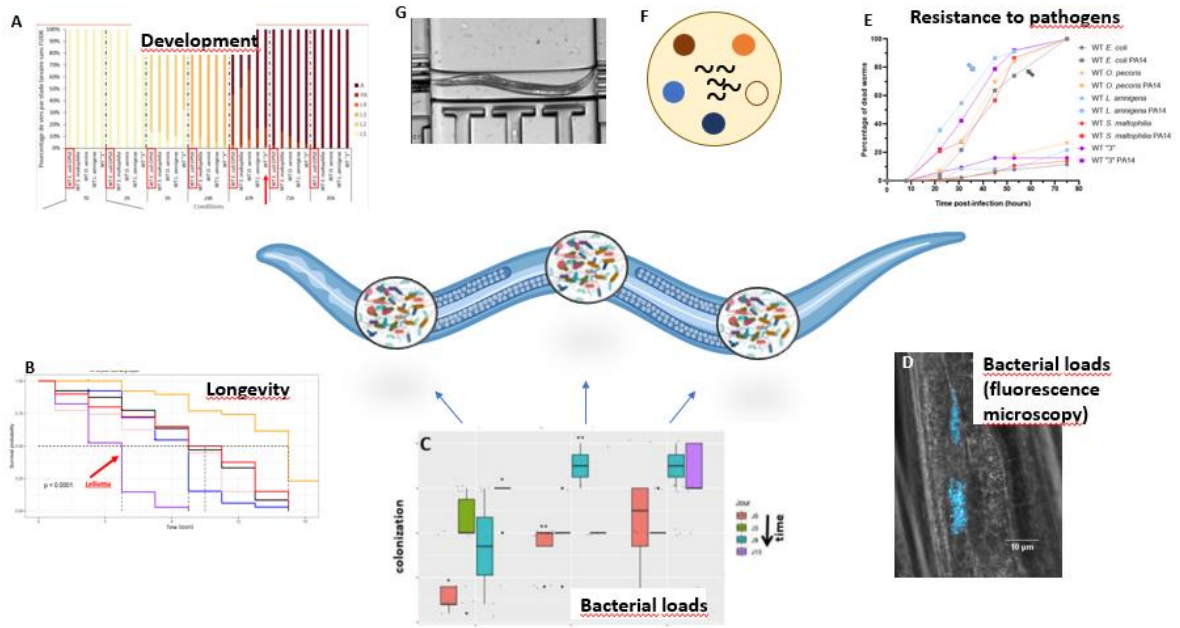
We have analyzed the impact of microbiota heterogeneity on its host by using simplified microbiotas derived from the CeMBio community, comprising different compositions and varying degrees of complexity (microbiotas with 1, 3, or 6 strains). We have developed all the protocols needed to quantify the colonization rate of strains in the gut by microbiota extraction and fluorescence microscopy, and assessed the impact of microbiota composition and complexity on certain physiological parameters of *C. elegans* (longevity, development, and offspring, **Figure 21**). Regardless of the microbiota's composition and complexity, we observe that the colonization rate of CeMBio strains in the intestine remains

stable over time. This stability is observed both at the global scale of the intestine and within its different sections.

Recent studies (Birlutiu et al., 2023) have shown that *Lelliottia amnigena*, present in the CeMBio community, can sometimes be a human opportunistic pathogen. We decided to pay particular attention to this strain as it could have clinical relevance and might also be a candidate for gene exchange, potentially becoming a multi-drug-resistant opportunistic pathogen. Our results demonstrate that *L. amnigena* affect *C. elegans*' lifespan, fertility and development of *C. elegans* compared to other CeMBio bacteria and that it depends on the composition and complexity of the microbiota. In addition, thanks to a grant from the CBI, we are developing a project involving a collaboration with two groups of the CBI, A. Pérez-Escudero (CRCA-CBI) for studying the behavior of worms when faced with a food choice using a high-throughput system that allows tracking several thousand worms simultaneously (**Figure 21F**), and A. Mattout (MCD-CBI) for her expertise on *C. elegans* (**Figure 21E**). We further show that worms preconditioned with *L. amnigena* are more sensitive to the pathogen *P. aeruginosa* and die earlier and that, regardless of the microbiota's complexity and composition, worms tend to preferentially choose *L. amnigena* when faced with a food choice (manuscript in preparation).

Finally, in collaboration with M. Delarue's team (LAAS, Toulouse), we have contributed to the development of custom-made microfluidic chips for culturing worms in individual chambers, including an immobilization system for acquisitions ((Ben Meriem et al., 2023), **Figure 21F**). Although there are many issues to be resolved, such as efficient removal of eggs from the chamber and improved immobilization of the worms, this technology could enable us to monitor the microbiota in real time under the most controlled conditions possible.





**Figure 21:** Various types of assays to characterize *C. elegans*' microbiota. We routinely monitor and record several parameters over time, including **A.** development, **B.** longevity, **C.** bacterial loads and their localization in the gut, using microscopy (**D**), **E.** survival rates upon infection with a pathogen. **F.** Behavior of worms when faced with a food choice. **G.** Setting up of microfluidic devices for worm imaging.

## 4. RESEARCH PROJECT

*This section can be read independently of the previous ones.*

Antimicrobial resistance (AMR) is recognized as a major and ever-increasing burden, not only because it is a leading cause of human mortality worldwide but also because it threatens the health of animals and plants, with effects on food safety and the environment. AMR can occur by spontaneous mutation but is mainly acquired through horizontal gene transfer from the bacterial gene pool. This is achieved by the action of mobile genetic elements (MGEs), which carry the most resistance and virulence determinants (Partridge et al., 2018). Some MGEs, including transposons and integrons, move within and between DNA molecules of a bacterial genome, while others, such as conjugative plasmids, are able to spread the formers across different species, genera, orders and even phyla. By acting as natural cargo ships of MGEs, conjugative plasmids are largely responsible for disseminating AMR. Numerous examples demonstrate their considerable impact on the spread of genes encoding AMRs, especially in well-studied human pathogens (Carattoli, 2013; von Wintersdorff et al., 2016). It is, therefore, urgent to improve our knowledge of their intimate propagation mechanisms to identify new potential strategic lines to limit the spread of AMR.

In this context, I use approaches ranging from molecular and cellular biology to the fate of MGEs in complex ecosystems, with the following main objectives:

- **Examine the interplay between vertical and horizontal transfer**

I aim to reach a mechanistic and molecular understanding of the process by which conjugative plasmids face the trade-off between two modes of transmission: vertical from mother to daughter cells (segregation), and horizontal from donor to recipient cells (conjugation). This is one of the important and challenging questions remaining in the field concerning the spatiotemporal dynamics of secondary replicons. **I co-supervise the thesis of C. Hall (started 12/2023, 50% with F. Cornet) on this project.**

- **Enlarge our knowledge on horizontal gene transfer to a complex ecosystem**

Plasmid propagation can occur in any environment, such as wastewater, animal and human gut or soil. Yet, most studies have mainly focused on *in vitro* experiments, and little is known about the spread of the resistance gene *in vivo*. My goal is to investigate the dynamic feature of conjugative plasmids and transposons both at the population and cellular levels within the simple eukaryotic intestine model *Caenorhabditis elegans*. Indeed, tight analyses at the cellular scale and above all in natural environments would provide important breakthrough in the field. **I co-supervise the thesis of R. Dusfour-Castan (started 10/2021, 50% with B. Ton-Hoang) on this project.**

Another project has recently emerged from a collaboration with M. Campos in the team, aiming to **Investigate the genetic bases of the control of cell proliferation in *Caenorhabditis elegans*' gut.**

Bacteria are highly efficient at proliferating, a capability central to their pathogenicity, yet our understanding of how this proliferation is regulated within complex communities remains limited. Using *C. elegans* and its synthetic gut microbiota (CeMBio) as a model system, we aim to explore the interactions, proliferation, and host influence of bacterial populations, specifically through the perturbation of the microbiota with various *Escherichia coli* strains. By characterizing colonization patterns and employing Massive Allelic Exchange to create genetic hybrids, we will identify the genetic *loci* that enable *E. coli* to adopt a commensal role, or become pathogenic, thereby advancing our understanding of bacterial community dynamics and pathogenic mechanisms.

## 4.1 Interplay between plasmid segregation and conjugation

### 4.1.1 State of the art

On the one hand, conjugative plasmids are faithfully transmitted to daughter cells by specific systems required to distribute their replicated copies to the two daughter cells during cell division (vertical transfer). These so-called partition systems separate and position

plasmids at specific subcellular positions, usually at the nucleoid (for a review: (Bouet and Funnell, 2019)).

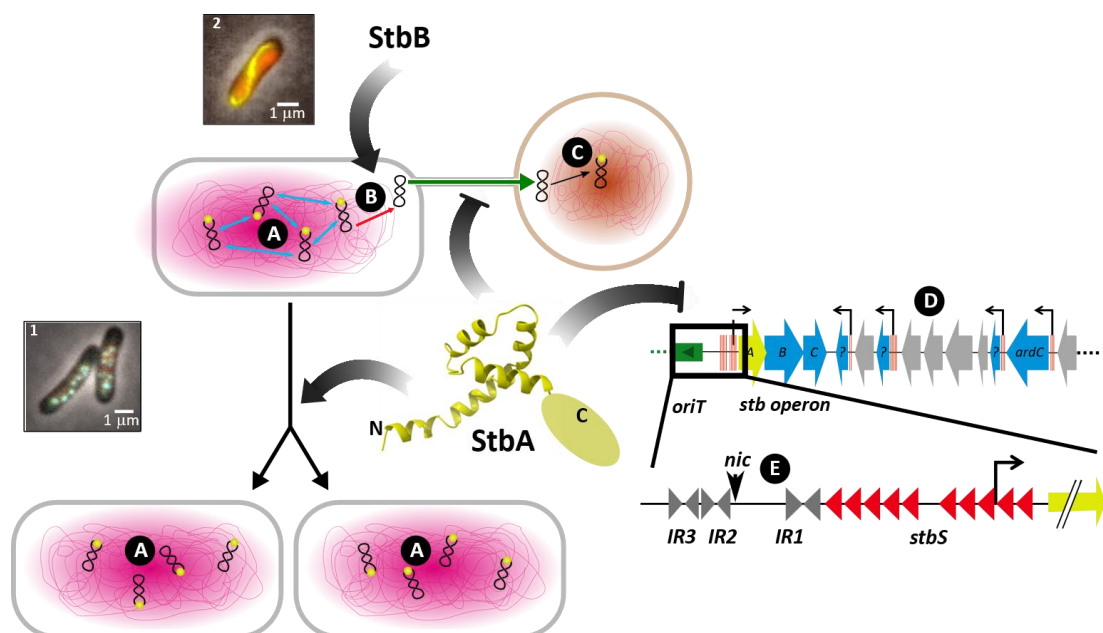
On the other hand, conjugative plasmids are propagated within bacterial populations by conjugation. Conjugation involves the unidirectional transfer of plasmid DNA from a donor bacterium to a recipient bacterium through a conjugative pore (T4SS, type IV secretion system) which is established at the membrane between the two cells in physical contact (horizontal transfer) (Cabezón et al., 2015; Llosa et al., 2002; Viroille et al., 2020).

Clearly, these two modes of transfer involve and require mutually exclusive subcellular localization of the plasmids, and how they switch from one to the other is a sorely missing piece of information. This is true not only in the donor bacterium but also in the recipient bacterium that has just acquired a conjugative plasmid and becomes a new stable donor, thus contributing to the exponential spread of AMR. Yet, this checkpoint in plasmid trafficking has received very little attention.

I am especially interested in PTU-W plasmids, including the three typical members R388, pSa and R7K, which are of great interest: (i) they are among the broadest host range plasmids in Proteobacteria. Their successful transfer and stable transmission have been reported in many bacterial species, including several of the most critical multidrug-resistant pathogens that have been identified as the greatest threat to human health by the World Health Organization, including *Acinetobacter*, *Pseudomonas*, and various *Enterobacteriaceae* (such as *Klebsiella*, *Shigella* and *Salmonella*) (Fernández-López et al., 2006; Venter, 2019); (ii) they are a good examples of recent acquisition of AMR genes as a consequence of the extensive use of antibiotics (Revilla et al., 2008), (iii) they show a compact genetic backbone organized in clusters of only three essential gene sets devoted to conjugation, replication, and segregation (Fernández-López et al., 2006), which facilitate their study; and finally (iv) we previously showed that plasmid R388, which is a major model for the study of the conjugation process, encodes a peculiar system, Stb, consisting of two proteins (StbA and StbB), which act to balance two modes of transmission of the plasmid, vertical by segregation and horizontal by conjugation (Guynet et al., 2011); **figure 22** and **section 2.2.3**). The Stb system is found in more than 15% of the sequenced plasmids from *Enterobacteriaceae* belonging to various groups (PTU-N1, P1, P9, Q1 and I2, (Guynet et al., 2011) and unpublished data). It is the only system reported that regulates the trade-off between the segregation and conjugation processes. In

addition, StbA is the key protein of a new type of segregation system based on a single plasmid-encoded protein (Guynet et al., 2011; Quèbre et al., 2022; Siguier et al., 2023). StbA is a multifunctional protein that has a central role in the physiology of plasmid R388. It is not only the sole plasmid-encoded protein involved in plasmid segregation, but also it has a major role in the regulation of the expression of several R388 genes and in the control of conjugation (Figure 22).

Our previous work on the Stb system led to two strong hypotheses (see also section 2.2.3) that we will address: i) StbA inhibits conjugation either by confining plasmids at nucleoid areas, by acting on the *oriT* region, by regulating the expression of genes involved in conjugation or a combination of these three actions; ii) StbB stimulates conjugation by releasing plasmid copies from their StbA-mediated confinement and potentially drives them to the T4SS. Importantly, we will also (iii) study the impact of this system on stability and transfer of plasmids in a model gut microbiota.



**Figure 22: The Stb system and the interplay between vertical and horizontal transfers**

Black circles indicate the gaps in our knowledge, which will be addressed in the GeTBac project. The pictures show live *E. coli* cells of functional fluorescent fusions of StbA and StbB

(unpublished): **1.** StbA-mvenus and ParB-GFP tagged R388 colocalization and **2.** StbB-mvenus. **Left:** bacterial cells are represented by rounded rectangles. The nucleoid is represented, plasmids in black and the StbA protein by yellow circles. A spherical bacterium is shown as a recipient cell in orange. **Center:** a schematic of the StbA structure composed of an N-ter domain, of which the structure has been solved (Quèbre et al., 2022) and an unstructured C-ter domain. **Top right:** the leading region of R388, including *oriT* and the *stb* operon. Genes are shown by arrows and those of which expression is regulated by StbA are in blue, *stbDRs* are represented by vertical red bars. **Bottom right:** a zoom of the *oriT* region showing *stbS*, composed of two sets of five direct repeats *stbDRs* (red triangles), three inverted repeats (IR), and the *nic* site.

### ***StbA as a segregation system***

StbA is one of the two known segregation systems that require a single protein. Most well-studied low copy-number plasmids, which are maintained at less than ~10 copies per chromosome, rely on specific segregation/partition systems for faithful inheritance. These systems assemble a dedicated nucleoprotein complex, namely the segrosome, around a centromere sequence and then separate and position them at specific subcellular positions, allowing each daughter cell to receive at least one copy after cell division. Partition systems described so far include a *cis*-acting centromere site and contain two genes organized in tandem in an autoregulated *par* operon. In all cases, one gene encodes an essential NTPase, which provides the physical forces to separate plasmid copies and position them at specific subcellular locations. The other gene encodes a DNA-binding protein that binds to the centromere site and connects the plasmid DNA and the NTPase (for a recent review: (Bouet and Funnell, 2019)).

By contrast to these typical partition systems, StbA does not require a plasmid-encoded NTPase partner to ensure the segregation of *E. coli* conjugative plasmid R388 (Guynet et al., 2011; Guynet and de la Cruz, 2011). The unrelated Par system of the staphylococcal plasmid pSK1 is the only other system described so far that can also do without an NTPase (Chan et al., 2022; Simpson et al., 2003). These systems raise the question of how a single DNA-binding protein can ensure both the assembly of the segrosomes and the addressing of the plasmid to both daughter cells.

Most cells carrying R388 exhibit between 4 and 6 foci of fluorescent-tagged plasmid that are evenly assorted within the nucleoid area (**Figure 22A**). This roughly corresponds to the copy number of R388, suggesting that most foci contain a single copy of the plasmid. The absence of StbA, which does not affect plasmid copy number, correlates with a significant decrease in the number of foci (1 to 3), which are observed as clusters in nucleoid-free spaces, mostly at one cell pole, and with plasmid instability (Guynet et al., 2011). This non-uniform plasmid distribution in the cell in the  $\Delta stbA$  may be driven by entropic forces that tend to physically separate plasmids from the chromosome. This results in plasmid exclusion from nucleoid space, similar to what is observed for high-copy-number plasmids, DNA circles devoid of partition system. Additionally, plasmids become unmixed from large DNA molecules when mixed in cavities provided by artificial nanofluidic model systems (Hsu and Chang, 2019; Liu et al., 2022; Planchenault et al., 2020; Wang, 2017). In this view, the StbA protein can be considered as a sub-cellular positioning system that act to localize plasmid molecules to the nucleoid, which could allow plasmids to hijack entropy. This is reminiscent of the so-called 'hitchhiking' model proposed for eukaryotic extra-chromosomal elements, such as the 2-micron yeast plasmid, in which plasmids utilize the chromosomes as vehicles for segregation (for review: (Sau et al., 2019)). A potential strategy for efficient segregation would thus be the association of plasmids to the bacterial nucleoid by physical interactions, which would be ensured by StbA. Although the StbA binding activity to the centromere sequence *stbS* has been characterized recently (Quèbre et al., 2022), the mechanism by which would do so is not understood (**Figure 22A&C**).

### ***StbA as an inhibitor of conjugation***

StbA inhibits conjugation, so that its inactivation makes R388 a superspreader plasmid with up to 50-fold higher transfer frequency than the wild type (wt). It is not known how StbA controls conjugation, but several links between the protein and conjugation are possible leads. First, the role of StbA in plasmid copy positioning could prevent conjugative transfer. Indeed, StbA-mediated segregation of R388 and inhibition of conjugation correlates with the confinement of plasmids to nucleoid zones. In turn, StbA inactivation coincides with the exclusion of plasmids from the nucleoid and the superspreader phenotype. Second, the DNA-

binding activity of StbA could interfere with conjugative transfer. R388 StbA is encoded in the *stb* operon, which is divergently transcribed from the *trwABC* mobility operon (*MOB*) involved in conjugative DNA processing (Fernández-López et al., 2006; Guynet et al., 2011). Upstream to the *MOB* operon is the origin of conjugative transfer (*oriT*), where the relaxosome assembles. This involves the relaxase TrwC that initiates DNA cleavage at the *nic* site prior to transfer, the membrane coupling protein TrwB at the T4SS, and the accessory protein TrwA. The intergenic region of about 450 bp between the two operons thus contains both *oriT* and *stbS*, the essential centromere site, which is composed of two sets of five direct repeats of a 9-bp DNA sequence (*stbDR*). The segrosome formation may thus interfere with the relaxosome assembly and/or the conjugative DNA processing. Third, StbA could regulate the expression of genes involved in the control of conjugation. In addition to its role in plasmid segregation, StbA acts as a transcriptional repressor of the *stbABC* operon promoter and of promoters of four other R388 genes present in the leading region (i.e. the first region transferred during conjugation), of which *ardC*, that encodes an anti-restriction protein required for conjugation from *E. coli* to *P. putida* (Fernandez-Lopez et al., 2014; González-Montes et al., 2020), and three genes of unknown function (**Figure 22D**). In addition to *stbA* and *stbB*, the operon contains another gene, *stbC*, which is not involved in plasmid segregation in *E. coli* (Guynet et al., 2011) and whose functions remain unknown.

### ***StbB is not involved in segregation but stimulates conjugation***

The StbB protein, which is predicted to be an ATPase, is not involved in segregation, but stimulates conjugation. Importantly, it is strictly required for transfers in the presence of StbA (**Figure 22B**). Again, this control of conjugation by StbB correlates with the subcellular localization of plasmids. StbB stimulation of conjugation could be due to the presence of plasmid copies in nucleoid-free zones, which are near the membrane T4SS, while StbB inactivation coincides with the confinement of plasmids in the nucleoid and the absence of transfer.



### 4.1.2 Understand how StbA activities lead to conjugation inhibition

To gain a molecular and cellular understanding of how a single DNA-binding protein can mediate segregation complex assembly and plasmid addressing to daughter cells during division, and the transcriptional regulation of multiple genes, we will investigate how these activities modulate conjugative transfers.

#### *Mechanism of StbA-mediated plasmid segregation (Figure 21 A&C)*

##### **Rationale and preliminary data**

In our previous work, we found that the Fertility plasmid utilizes the nucleoid structure to position itself dynamically with the host cell cycle (Le Gall et al., 2016). Interestingly, we have observed a similar nuclear association for the R388 plasmids. Here, we hypothesize that the dynamic localizations of StbA-GFP and ParB-GFP tagged R388 plasmids may also depend on the nucleoid structure and the host cell cycle. In that line, the complex formed by StbA bound to its centromere *stbS* would associate with the nucleoid. This could be done through direct interactions with chromosomal DNA sequences (**Figure 16E(i)**), or through indirect interactions involving one or more unknown chromosome-associated host factors (**Figure 16E(ii)**). Alternatively, plasmid copies may position relative to each other to optimize their distribution. Such a process could rely on repulsion forces between partition complexes by an unknown mechanism (**Figure 16E(iii)**). Plasmid copies would thus occupy the largest possible volume, while remaining bound to the nucleoid by non-specific interactions, ensuring adequate positioning upon cell division. Our Chip-sequencing analyses did not reveal any StbA binding site on the *E. coli* chromosome (unpublished), and we have failed so far to identify StbA host partners in *E. coli* by searching for proteins using bacterial two-hybrid assays (BACTH) (unpublished).

##### **Positioning and dynamics of R388 and StbA**

In order to gain insights into the mechanism by which StbA mediates plasmid segregation, we will examine the dynamics of R388 and StbA by **(i)** analyzing the behavior of plasmids in relation to each other, to the nucleoid (DAPI stain or HU-GFP), fluorescently-labeled chromosomal loci, and the membrane, in the presence or absence of StbA or *StbS*, **(ii)**

determining whether StbA not only positions the plasmids at the nucleoid but also prevents their aggregation, and **(iii)** studying the effect of altering the nucleoid dynamics and the cell cycle on plasmid positioning and on the activities of StbA. To do so, we will perform time-lapse epifluorescence and multicolor 3D-SIM imaging in various conditions (in cells with altered nucleoid dynamics and cell cycle, in round-shape cells). By analyzing the trajectories of single plasmids with high temporal resolution (~seconds), we will characterize their type of movement occurring before, during and after segregation events. To further identify the proportions of StbA molecules in clusters, and to determine how the localization and dynamics of StbA are affected in the *stbS* sites (wt versus R388 $\Delta$ *stbS*), we will perform spt-PALM experiments using photo-activable probes to track the localization and dynamics of single StbA molecules over time scales ranging from seconds to minutes (Guilhas et al., 2020; Sanchez et al., 2015).

#### **Search for host factors involved in StbA-mediated plasmid segregation**

We will use the spatially resolved proteomic mapping assay based on APEX2-dependent proximity biotinylation (Santin et al., 2018). StbA will be genetically fused to the *apex2* coding sequence and used as bait in *E. coli* in the presence or absence of plasmid R388. Proximity-biotinylated potential StbA interactants will then be enriched on streptavidin and identified by mass spectrometry. Potential candidates will be verified for their interaction with StbA by BACTH, and tested for their role in R388 stability by genetic and cell biology approaches developed in other sections.

#### ***Interactions between StbA, stbS and the oriT region (Figure 21E)***

##### **Rationale and preliminary data:**

R388 StbA is a small protein of 110 residues harboring a fairly conserved N-terminal half composed of a HTH DNA-binding domain, and a non-conserved C-terminal half with a predicted disordered region (Quèbre et al., 2022). Although the C-terminal domain is not required for the specific and cooperative binding to the *stbDRs in vitro*, it is clearly necessary for StbA activities *in vivo* (for segregation, subcellular positioning, as well as transcriptional regulation, (Quèbre et al., 2022)), potentially by interacting with other proteins. A body of evidence suggests that StbA forms oligomers. However, it is not known whether the N-ter

and/or the C-ter domains are involved. StbA operator regions consist of arrays of two to five *stbDRs*, with the exception of *stbS*, which contains ten *stbDRs* arranged in two sets in the *stbA* promoter. The presence of only the other *stbDRs* carried in the StbA-regulated promoters (i.e. R388 $\Delta$ *stbS-stbABC* in the presence of StbA *in trans*), as well as the centromere composed of only one set of *stbDRs*, is not sufficient to ensure R388 stability (unpublished data and (Guynet et al., 2011), indicating that this arrangement in two sets of *stbDRs* sets is important for segregation. Besides, the synteny of the *stb* and MOB operons is very conserved (unpublished *in silico* data), which strongly suggests that the intergenic region may be a key DNA sequence in regulating the two modes of transfer. Consistent with this hypothesis, our chip sequencing data and BACTH assays suggest an interaction between StbA and *oriT*, particularly the IR1 site (**Figure 22E**), and interactions between StbA, StbB, TrwA, TrwB and TrwC, respectively (unpublished data).

### **StbA binding to the centromere *stbS***

We aim to understand how *stbS* is recognized as a centromere site compare to the other *stbDRs*-carrying promoters in plasmid R388. To do so, we will dissect the binding of StbA to its centromere *stbS* classical *in vitro* techniques (EMSA, DNase I footprinting) with StbA and StbA<sub>1-75</sub>. We will analyse the importance of the organization of StbA operators in sets of 9-bp *stbDRs* spaced by 2-bp thus corresponding to a complete helix turn, as well as the role of the IR1 sequence, which forms a cruciform structure and whose deletion would lead to a decrease in the conjugation rate (*com. pers.* G. Moncalian and (Llosa et al., 1991)). We will also investigate the possibility of forming a sandwich complex that would bring together the two sets of five *stbDRs* (separated by 43 bp), as suggested by the organization of *stbS*. Although we did not detect complexes containing two separate DNA molecules carrying five *stbDRs in vitro*, this does not rule out the possibility of forming the complex inside the same DNA molecule *in vivo*. We will address this question by constructing derivatives of R388 lacking one set of *stbDRs* but retaining the one containing the P*stbA* – 35 and -10 sequences (in order not to disturb the expression of the *stb* genes), as well as deletions in the sequence between the two sets of *stbDRs*. We will verify the transcriptional activity of P*stbA* (Quèbre et al., 2022) and determine the frequencies of loss and transfer as well as the sub-cellular localization of the mutants *in vivo* (stability and conjugation assays).

### **Impact of the binding of StbA on the activities of the relaxase**

To examine the importance of having *oriT* and *stbS* in close proximity, we will determine conjugation frequencies and stability of R388 derivatives in which *stbS* and *oriT* are physically decoupled. In addition, the dynamics of the segrosomes and the relaxosome in live cells be monitored using fluorescently labelled proteins (StbA, TrwA, TrwB, and TrwC) and R388 labelled with a ParB-FP/*parS* system. We will also analyze the effect of StbA on TrwC activities *in vivo* (i.e. in mobilization assays of plasmids carrying the *trwABC-stb* intergenic region in the presence of R388 $\Delta$ *oriT*) *in vitro*, in the presence of StbA (in cleavage and strand transfer at *nic* assays using single stranded DNA substrates, and in relaxation assays using plasmid DNAs carrying the *trwABC-stb* intergenic region (Lucas et al., 2010)).

### **Structure-function study of StbA**

We will undertake a structure-function study by searching for StbA variants, carrying small deletions or specific mutations, that are impaired for oligomerization in the N-ter DNA-binding domain, based on our models derived from structural and modelling data (unpublished), and in the C-ter domain. For the C-ter disordered domain, we will also use cysteine-scanning and thiol modification to probe StbA interactions with itself, with StbB and other identified proteins (Vecchiarelli and Funnell, 2013). We will also attempt to crystallize full-length StbA in the presence of chosen DNA substrates.

### ***Impact of StbA transcriptional regulation on conjugation (Figure 21D)***

### **Rationales and preliminary data**

StbA acts as a transcriptional repressor of four promoters controlling the expression of seven genes located in the leading region that might play an important role in conjugation. Indeed, upon entry of the plasmid into recipient cells devoid of StbA, an enhanced expression of these genes might be observed due to the unrepressed activity of the StbA-regulated promoter. This phenomenon, referred to as zygotic induction, is a well-known mechanism to express leading genes rapidly and transiently in transconjugant cells. While some conjugative

plasmids use single-stranded promoters for zygotic induction (Couturier et al., 2023), R388 plasmid might have developed another strategy to express leading genes through a StbA-dependent repression, as shown for the Reg576 repressor of the *Bacillus subtilis* p576 plasmid (Val-Calvo et al., 2018). The role of StbA-regulated genes in conjugation is unclear and among these, three are of unknown function (*orf7*, *orf12* and *orf14*). In *E. coli*, the deletion of these genes does not affect conjugation (unpublished). However, as for *ardC*, genes regulated by StbA might be important for conjugation depending on the species involved.

### **Timing of StbA-regulated gene expression**

We will evaluate the production level of sfGFP translational fusion of the StbA-regulated genes by performing time-course experiments where microscopy snapshot images of donor, recipient and transconjugant populations will be acquired at several time points after mixing donor and recipient cells. Then, to obtain the timing of sfGFP in individual transconjugants, we will perform time-lapse imaging of conjugation in a microfluidic chamber in combination with reporters to monitor the step of ssDNA transfer and its ss-to-dsDNA conversion (Couturier et al., 2023). This analysis will reveal the specific production timings with respect to plasmid processing steps.

### **Functions of StbA-regulated genes**

To identify a putative role of *orf7*, *orf12* and *orf14*, we will perform conjugation assays using different donor and recipient species (CeMbio and natural hosts of plasmid R388 such as *Stenotrophomonas maltophilia*, *P. aeruginosa* and *V. cholerae*, etc), and in conditions where these genes are absent or overexpressed in the donor or the recipient cells (by providing *in trans* a plasmid carrying the gene under the control of an inducible promoter). Genes proving a role in conjugation will then be studied using the same tools as the Stb proteins.

#### **4.1.3 Role of StbB in the stimulation of conjugation (Fig 20B)**

Based on our hypothesis that StbB function is to release plasmid copies from their StbA-mediated confinement and potentially drives them to the T4SS, we will explore the diversity

and search specific features of StbB proteins *in silico*, characterize StbB activities *in vitro* and *in vivo*, and investigate putative interactions between StbB and StbA, DNA, and the MOB proteins. We aim to provide an understanding of the role of StbB during the conjugation process and in the trade-off with StbA activities.

### **Characterization of StbB activities**

#### **Rationales and preliminary data**

StbB lacks signature lysine residue of the P-loop involved in ATP binding that defines the deviant Walker A box the ParA family (**Figure 23**, (Guynet *et al.*, 2011)). A distant lysine could enable ATP-dependent nucleotide sandwich dimer formation as observed for McdA (Schumacher *et al.*, 2019). The other characteristic Walker motifs (A' and B) are not detectable in StbB. Purified StbB protein exhibits a weak ATPase activity *in vitro*, we have not yet detected any DNA binding activity (non-specific or specific to the *stbDRs*), as observed for ParA and McdA proteins, and our BACTH assays suggest that StbB interacts with StbA, and the three MOB proteins (unpublished).

<b>StbB</b>	<b>S</b> G N V G <b>K</b> S
<b>ParA (P1)</b>	<b>K</b> G G V G <b>K</b> T
<b>SopA (F)</b>	<b>K</b> G G V Y <b>K</b> T
<b>McdA</b>	S G G T G <b>K</b> T
<b>Soj</b>	<b>K</b> G G V G <b>K</b> T
<b>MipZ</b>	<b>K</b> G G A G <b>K</b> S

**Figure 23:** Walker A box of various ATPases.

#### **in silico study of StbB proteins**

As for our previous survey on StbA ((Planchenault *et al.*, 2020; Siguier *et al.*, 2023) and unpublished), we will search for StbB homologs. This will allow us to identify and groups and conserved features/residues of StbB proteins and compare them to other Walker-type proteins involved in the positioning of cellular cargoes (i.e. ParA, Soj, McdA, MipZ...), as well as to examine whether StbB and StbA proteins co-evolved in order to identify potential residues involved in interactions between the proteins.

#### **StbB interactions with StbA and the relaxosome**

To examine StbB interaction network, we will follow the dynamics of FP-tagged StbB, StbA, MOB proteins and R388 or R388 $\Delta$ stbA. To characterize StbB action with respect to the DNA processing for conjugation (formation of the relaxosome/*oriT* cleavage), we will compare the subcellular localization of wt R388 and derivatives defective for DNA cleavage at *oriT* (carrying TrwC[Y18F] and/or R388 $\Delta$ *oriT*). We will also use a system in which R388 $\Delta$ stbB is co-transformed with a second compatible plasmid expressing *stbB* under the control of an inducible promoter (*Para*), to follow in real-time the interplay between segregation and conjugation upon induction with arabinose (time-lapse microscopy coupled with microfluidics).

### ***StbB bundle structures (picture 1 in Figure 22)***

Initial fluorescence images of StbB strikingly revealed the formation of high-dimensional bundle structures spanning the cell body. We believe that these structures are involved in the recognition and capture of plasmids for the conjugation process at the membrane. To investigate the dimensions and localization of these StbB bundles with respect to the bacterial membrane and the nucleoid, we will use 3D-SIM imaging (Lesterlin et al., 2014). These experiments should help decorticate when and where these structures assemble/disassemble and how they interact with the plasmids and the cell membrane/nucleoid. In addition to static characterization, we will also use 3D deconvolved time-lapse epifluorescence microscopy to monitor the dynamics stages covering the nucleation, extension, and disassembly of these structures and relate their coordination with the localization of StbA and plasmids.

Importantly, we will include wt and R388 $\Delta$ stbA to evaluate if StbB dynamics is dependent of StbA, and we will perform these experiments under various growth conditions to affect host cell physiology and in bacterial strains of different shapes, to examine whether the function of StbB is related to host cell processes or dependent on the bacterial morphology itself.

Finally, to decorticate where and how StbB functions during the different stages of conjugation, we will quantify the distribution of StbB molecules associated to the membrane/treads/nucleoid using spt-PALM experiments with photo-activable probes to

follow the localization and dynamics of single StbB molecules, in the presence or absence of StbA (wt R388 versus R388 $\Delta$ stbA), and in a vegetative state and during conjugation.

### ***StbB production profile***

We will first address the role of StbA in **(i)** the production and localisation of a StbB-sfGFP translational fusion and in **(ii)** the profile and timing production of StbB in the context of conjugation. StbA regulates negatively the *stbABC* promoter. In absence of StbA, the production of StbB might be increased altering its localization pattern. Using snapshot and time-lapse images, we will determine the production level and the dynamics of a StbB-sfGFP translational fusion in wt R388 and in R388 $\Delta$ stbA mutant.

#### **4.1.4 Impact of Stb on plasmid prevalence in bacterial populations**

We aim to explore the impact of the Stb system in nature, through in silico and wet experiments. We will analyse the organization and diversity of Stb systems, as well as identify mutations in the Stb system that would evolve into superspreaders. In addition, we will study the control of plasmid spreading by Stb in a complex bacterial community, which will also pave the way to study environmental factors that may influence conjugation between host-associated bacteria.

### ***Identification of mutations leading to superspreader plasmids***

The  $\Delta$ stbA mutation, which makes plasmid R388 a superspreader in *E. coli*, indicates that the Stb system is a locus susceptible to genetic changes that can dramatically increase the conjugation capacity of plasmids and thereby worsen the spread of AMR worldwide. One way to improve the propagation of plasmids is to broaden their host range. In that line, our preliminary research based on sequence comparison and conjugation capabilities of the PTU-W group of plasmids, indicates that the *stb-oriT-trwA* region may also be involved in the host



range of these plasmids. ((Revilla et al., 2008) and unpublished). Two types of sequences have already been identified: TrwA binding sites and IHF protein binding sites.

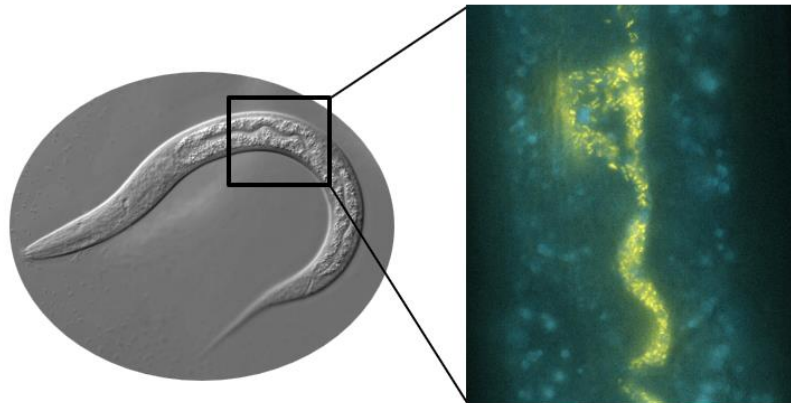
We will construct mutants from our *in silico* analyses and set up genetic screens to identify mutants of the *stbS-oriT-trwA* region and the *stbA* gene that are deficient in conjugation inhibition and/or enlarge the host spectrum in different PTU-W plasmids (R388, pIE321, pSA, R7K). The mutant plasmids will be tested in conjugation assays between different hosts and used in other selected assays.

### ***Ability of Stb to spread plasmids in a model gut microbiota***

Plasmid propagation can occur in any environment, such as wastewater, animal and human gut or soil. Yet, most studies have mainly focused on *in vitro* experiments, and little is known about the spread of the resistance gene HGT *in vivo*. In that line, we will study of the impact of the Stb system on plasmid spreading in the model gut microbiota of *Caenorhabditis elegans*. The *C. elegans* model is unique because it allows tracking of plasmid transfers both at the population scale, due to the ease of obtaining large numbers of worms with a controlled microbiota, as well as at the cellular scale.

The natural microbiota of *C. elegans* has only recently been described, opening up the possibility of using this simple yet powerful model system in studies of the host microbiota (Zhang et al., 2017). The composition of the microbiota, which is composed of a high diversity of bacterial species, the dominant group comprising several  $\gamma$ -proteobacteria (*Enterobacteriaceae*, *Pseudomonaceae* and *Xanthomonadaceae*), was shown to tune *C. elegans* health (Haçariz et al., 2021) and pathogenesis (Radeke and Herman, 2021). *C. elegans* is a model of choice for our study: **(i)** it is bacterivorous and its eggs are sterile, so the composition of its microbiota is controlled exclusively by the bacteria on the plates; **(ii)** it is easy to cultivate (short life cycle, selfing hermaphroditism allowing maintenance of homozygous cultures) with the possibility to generate large numbers of nearly genetically identical individuals with identical life histories, and **(iii)** importantly, it is transparent under light microscopy allowing direct visualization of bacterial cells *in vivo*. We will use the CeMBio bacteria as a simplified natural *C. elegans* microbiota (Dirksen et al., 2020). This includes a dozen of environmental bacterial strains that have been selected from meta-analyses of the

natural microbiome of the nematode. We have already transferred R388 plasmid by conjugation to 8 of the 12 CeMBio strains, and introduced a cassette containing the mcherry FP and ParB-mturquoise fusion in the chromosome of 4 of the CeMBio strains, which exhibit a blue focus upon the entry of a plasmid carrying the *parS* sequence on plates. Finally, we are able to detect fluorescent plasmid foci inside the transparent worm gut under light microscopy (unpublished, **Figure 24**).



**Figure 24:** *Ochrobactrum pecoris* carrying R388::*parS-parBmvenus* inside *C. elegans*'s gut.

Although the sequences are available at NCBI, some are not assembled and plasmid sequences are missing. We will thus first investigate plasmid composition of all the strains (extraction and Pac Bio sequencing).

We will then follow the fate of plasmid R388 in the CeMBio strains both *in vitro* on agar plates or in liquid cultures and *in vivo*, in the gut of *C. elegans* intestine. We will analyse conjugation events in space and time by visualization under the microscope, and by quantification of bacteria after mechanical disruption of worms (protocol adapted from (Ortiz et al., 2021)), either by plating and/or RT-PCR. This will be done by varying the complexity of the microbiota (i.e. the number of different bacterial strains), and using either the CeMBio bacteria or by transient gut invaders as the donor bacterium (i.e. *E. coli* or worm's pathogens such as *P. aeruginosa*, *Vibrio cholerae* and *Serratia marcescens*). Finally, we will compare wt R388 and chosen plasmids identified in our study (i.e. R388 $\Delta$ *stbA* and other superspreaders). This approach will also generate data on environmental factors that may influence HGT

(temperature, presence of antibiotics, heterogeneity of the microbiota ...).

### ***Role of Stb in relation to other known segregation systems***

Many enterobacterial plasmids carry a combination of segregation systems (i.e. Stb with Par type I or/and II/III, unpublished data). This is the case of the RP4/RK2 plasmid, which carries a Stb system (*traKLM*) and a type I Par system (*inc-korB*). Our very preliminary data indicate that the insertion of RP4 Par system in R388 leads to a decrease in conjugation rates, while the insertion of the one of plasmid F (for which no Stb or analogous system has been identified) has no effect (unpublished). We have already constructed several PTU-W plasmids, as well as RP4 with a FROS system to localize them using fluorescence microscopy.

We will continue our thorough phylogenetic analysis of the Stb system to determine its distribution among conjugative and mobilizable plasmids, and in other conjugative elements (ICE), and analyze of the other components of the region (*oriT*, *MOB* genes, and *stbC*) and of the plasmids (T4SS encoding region, and other features such as the partition and replication modules). This should provide important information on the characteristics of the StbC proteins, and allow identification of clinically important plasmids that could be included in our study.

We also plan to search for functionally equivalent systems of Stb but phylogenetically unrelated, first by identifying conjugative plasmids devoid of any known segregation system and then according to different criteria (presence of a putative DNA-binding protein, synteny conservation with the conjugative machinery encoding region, presence of a putative NTPase). In addition, we will pursue genetics (deletions and exchanges of segregation and replication modules) to determine if the Stb system intriguing properties are conserved in plasmids closely related to R388 (PTU-W), and even more interestingly, in a plasmid carrying a classical Par system in addition to Stb.

## 4.2 Horizontal gene transfer in complex bacterial communities

Bacteria can rapidly evolve to adapt to their environment, exemplified by the emergence of multi-drug-resistant bacteria, a global health issue resulting from the extensive use of antibiotics since the 1940s. This evolvability is primarily due to their ability to share and combine MGEs, which carry most clinically significant antibiotic resistance genes (ARGs). MGEs include plasmids, transposons, and integrons, capable of moving intracellularly (between bacterial chromosomes and plasmids) or intercellularly (within or between species). Conjugative plasmids are extrachromosomal MGEs that replicate autonomously and transfer through direct contact between a donor and a recipient bacterium via a conjugation pore. They are considered as the most active disseminators of resistance genes, central to the horizontal transfer of other MGEs via recombination and transposition. Transposable elements, including insertion sequences (IS) and transposons, transfer to different loci within a bacterium (between chromosomes, plasmids, or both) without requiring homologous sequences between the transposon and insertion site. They encode a transposase enzyme that catalyzes the necessary DNA strand cuts and transfers for transposition, spreading antibiotic resistance genes and modulating their expression.

While the molecular basis of EGMs propagation mechanisms has been widely described, there is still a significant lack of knowledge about these processes and the factors influencing them *in situ*, within complex natural bacterial communities such as microbiota. The information available comes mainly from numerous metagenomic analyses, in different microbiota or ecosystems, which do not give access to the spatio-temporal dynamics of these processes. In this context, our goal is to study the dynamics of the exchange of these EGMs within a complex ecosystem, the model intestinal microbiota of the nematode *C.elegans*.

In addition, conjugative plasmids have gained significant interest in recent years for the development of non-antibiotic strain-specific antimicrobial treatments. Such strategies would allow targeting killing of pathogenic bacteria without harming host microbiota and to limit antimicrobial resistance. These systems induce efficient killing of targeted bacteria by using the RP4 conjugative plasmid to deliver DNA with antibacterial activities, which can be a toxin (López-Igual et al., 2019) or CRISPR/cas systems (Reuter et al., 2021), to specific bacterial

strains. However, these conjugation-based systems, which have been tested mainly *in vitro*, face limitations due to low transfer rates, as well as the uncontrolled fate of the donor bacterium. In this context, the properties of the Stb system hold promise for the development of potentially highly efficient ('superspreader') and controlled (unstable) genetic tools for the delivery of antibacterial activities. In addition, M. Robledo and col. recently introduced a synthetic conjugation enhancer based on the use of bacterial adhesion system that optimize plasmid delivery to the target bacteria with high selectivity (Robledo et al., 2022).

#### **4.2.1 Study of the spatio-temporal dynamics of conjugation and transposition in the CeMbio bacterial community *in vitro* and *in vivo***

We will analyze conjugation in the bacterial population. This will be done *in vitro* on agar plates and in liquid cultures, as well as *in vivo* in the gut of *C. elegans*. The set up for detection of conjugative transfers under the microscope is based on the ParB fluorescent protein (ParB-FP, produced in the future recipient cell), which binds specifically to the *parS* DNA sequence carried by the plasmid of interest. Transconjugant cells, i.e. those that have acquired the plasmid by conjugation, then display fluorescent foci. This system has proved effective *in vitro*, but diffuse basal fluorescence of ParB-FP in the recipient cells prevents clear detection of transconjugant bacteria within *C. elegans* gut microbiota. We are currently optimizing the system by introducing a repression loop: *parB-FP* will be under the control of a promoter regulated by a repressor (R1) encoded by the recipient cell, and the incoming plasmid will encode a second repressor (R2) that regulates the promoter of R1. ParB-FP will thus be produced only in the presence of the plasmid in transconjugants. We will prioritize IncW family plasmids because they have a broad host range, encompassing most proteobacteria classified as high priority by the WHO.

We will analyse conjugation events in space and time by visualization under the microscope, and by quantification of bacteria after mechanical disruption of worms (protocol adapted from (Ortiz et al., 2021)), either by plating, RT-PCR, and/or sequencing. This will be done by varying the complexity of the microbiota (i.e. the number of different bacterial strains), and using either the CeMbio bacteria or by transient gut invaders as the donor

bacterium (i.e. *E. coli* MG1655, which is a poor colonizer of *C. elegans* (unpublished), or worm's pathogens such as *P. aeruginosa*, *Vibrio cholerae* and *Serratia marcescens*). We will specifically determine: the frequency of conjugative transfer in the nematode intestine for different strains; where conjugation preferentially occurs in the nematode's digestive tract; the dynamics of the transition from recipient to donor state in bacteria; and whether transient invaders can genetically modify the microbiota through conjugation.

We will also take advantage of the systems we have set up for the study of conjugation *in vivo* and our knowledge of transposons to characterize genetic exchanges by transposition in the CeMbio bacterial community *in vitro* and *in vivo*. This axis will benefit from the presence of the ISFinfer database in the team (Siguier et al., 2006). We will focus on 3 families of transposons that use different transposition mechanisms (IS630/Tc1/Mariner families: "cut and paste" (Tellier et al., 2015), IS3: "copy and paste" (Chandler et al., 2015) and IS200/605: "peel paste and copy" (He et al., 2015; Karvelis et al., 2021). These families are chosen for their representativeness, knowledge of their transposition and ease of visualization of certain transpositional activities.

The IS630/Tc1/Mariner elements are among the most widespread in the prokaryotic and eukaryotic world. Their transposition "cut and paste" allows transposition to be detected after removal of the conjugative plasmid carrying them. Transposition of IS3 and IS200 elements, despite fundamental differences in their mechanism, involves an intermediate excision step by circularization whose efficiency reflects transpositional activity and which is easily detectable by phenotypic tests using markers (ARG, gene encoding a fluorescence protein...). Indeed, circularization leads to the assembly of a strong hybrid promoter (IS3; (Duval-Valentin et al., 2001)) or to the precise reconstitution of the donor sequence (IS200-605; (Ton-Hoang et al., 2010)). The second transposition step, involving the insertion of these elements into a target, is rarer, but can be detected by sensitive papillation-type assays (e.g. via the ignition of a promoterless marker gene after insertion upstream of a promoter) (Guynet et al., 2020a).

We will construct several synthetic transposons derived from these elements, which will be placed on conjugative plasmids and which can be counter-selected if necessary. Several such systems have been described in the literature, often developed as genetic engineering tools (e.g.: (Conte et al., 2019; Nazareno et al., 2021)). We will then monitor their

fate, first *in vitro* in each strain, then in the CeMbio community *in vitro* and *in vivo*, using papillation, mating out (Guynet et al., 2020a) or fluorescence microscopy (in bacteria extracted from the microbiota or directly in nematodes).

This part should generate data on environmental factors that may influence HGT (comparison of liquid/solid media and parts of the gut, temperature, presence of antibiotics or other compounds, heterogeneity of the microbiota ...).

#### **4.2.2 Efficiency and fate of conjugative devices engineered to control microbiota**

Our set-up of *C. elegans*' intestine microbiota will allow us to analyze the effects *in vivo* of several tools that are engineered in order to genetically modify microbiota, such as synthetic cell to cell adhesion systems, which provide a way to greatly improve conjugation rates as well as specificity by the expression of specific nanobodies and their cognate antigen to induce cell-cell docking (Robledo et al., 2022), and conjugation-based antimicrobials (López-Igual et al., 2019; Reuter et al., 2021). Depending on the results obtained in the sections described previously, we could design variants derived from R388 superspreaders (R388 $\Delta$ stbA-based). We will first perform conjugation assays *in vitro* with selected CeMBio strains and pathogens, and then infection assays on *C. elegans* to study the effects of treatment with these antimicrobials on the recovery of the worms and on the composition of their microbiota, as well as their fate after infection with natural *C. elegans* pathogens (*Pseudomonas aeruginosa* and *Vibrio Cholerae*).

#### **4.3 Investigate the genetic bases of the control of cell proliferation in *Caenorhabditis elegans*' gut**

This part, which will be done with Manuel Campos, is still in the design phase, but preliminary experiments should soon be underway.

Extra-intestinal pathogenic *E. coli* (ExPEC) serves as an effective infection model in *C. elegans*, where pathogen accumulation in the intestine leads to gut distension and worm

death, mirroring pathogenicity observed in humans and mice. This pathogenicity is predominantly influenced by general bacterial physiological traits, such as capsule protection, gut adhesion via pili and adhesins, biofilm formation, and nutrient scavenging abilities like iron uptake. These features are predictive of *E. coli* pathogenicity in pure cultures. We have demonstrated that various combinations of CeMBio strains can form a microbiota, providing different degrees of protection to *C. elegans* against *P. aeruginosa* (manuscript in preparation, **section 2.2.4**). We observed that the worm's lifespan negatively correlates with the bacterial load of the dominant strain in the microbiota, supporting the hypothesis that pathogenicity in *C. elegans* is primarily driven by the uncontrolled growth of a bacterial strain, rather than specific virulence mechanisms.

Bacteria are remarkably adept at proliferation, and this ability is fundamental to their pathogenicity. Although we have a detailed understanding of many molecular aspects of bacterial cell growth and division, our knowledge of the control of their proliferation within complex systems, such as communities, remains limited. To fill this gap, we will use *C. elegans*, with its simple synthetic gut microbiota (CeMBio), as a tractable system to explore how bacterial populations interact, proliferate, and influence the host, and *E. coli*, a well-studied model organism, as an ideal candidate to perturb the gut microbiota of *C. elegans*.

*E. coli*, and specifically, extra-intestinal pathogenic *E. coli*, serves as an effective infection model in *C. elegans*, where pathogen accumulation in the intestine leads to gut distension and worm death, mirroring pathogenicity observed in humans and mice (Diard et al., 2007). This pathogenicity is predominantly influenced by general bacterial physiological traits, such as capsule protection, gut adhesion via pili and adhesins, biofilm formation, and nutrient scavenging abilities like iron uptake. These features are predictive of *E. coli* pathogenicity in pure cultures. However, the pathogenicity of *E. coli* strains boils down to their ability to settle and develop uncontrollably in the worm's intestine. In addition, we have found that the worm's lifespan is inversely related to the bacterial load of the dominant strain within the microbiota, and that different combinations of CeMBio strains can be established as a microbiota and that these different bacterial communities can protect the worm at different degrees against the pathogen *P. aeruginosa* (unpublished data). These results reinforce this project's hypothesis that in *C. elegans*, pathogenicity is mainly due to the uncontrolled proliferation of a bacterial strain rather than any specific virulence factors.



By using *E. coli* strains to introduce perturbations in the microbiota, we will investigate how different bacterial strains colonize the worm's gut, integrate into the microbiota, potentially become pathogenic and displace the preestablished microbiota. Our main objectives are (i) to assess the microbiota's impact on *E. coli* colonization and proliferation control, (ii) characterize the dynamics of the CeMBio bacterial community in *C. elegans*' gut when challenged with a pathogenic or commensal *E. coli* strain, (iii) Identify genetic switches turning *E. coli* into a commensal or pathogen in the microbiota and (iv) Investigate the influence of genetic context potentializing these genetic modules, with a focus placed on mobile genetic elements as they have the potential to alter the fitness of other genetic loci, even core genome loci (Coluzzi et al., 2023).

## 5. REFERENCES

- Altae-Tran, H., Kannan, S., Demircioglu, F.E., Oshiro, R., Nety, S.P., McKay, L.J., Dlakić, M., Inskeep, W.P., Makarova, K.S., Macrae, R.K., Koonin, E.V., Zhang, F., 2021. The widespread IS200/IS605 transposon family encodes diverse programmable RNA-guided endonucleases. *Science* 374, 57–65. <https://doi.org/10.1126/science.abj6856>
- Antar, H., Soh, Y.-M., Zamuner, S., Bock, F.P., Anchimiuk, A., Rios, P.D.L., Gruber, S., 2021. Relief of ParB autoinhibition by parS DNA catalysis and recycling of ParB by CTP hydrolysis promote bacterial centromere assembly. *Sci Adv* 7, eabj2854. <https://doi.org/10.1126/sciadv.abj2854>
- Barabas, O., Ronning, D.R., Guynet, C., Hickman, A.B., Ton-Hoang, B., Chandler, M., Dyda, F., 2008. Mechanism of IS200/IS605 family DNA transposases: activation and transposon-directed target site selection. *Cell* 132, 208–220. <https://doi.org/10.1016/j.cell.2007.12.029>
- Belogurov, A.A., Delver, E.P., Agafonova, O.V., Belogurova, N.G., Lee, L.Y., Kado, C.I., 2000. Antirestriction protein Ard (Type C) encoded by IncW plasmid pSa has a high similarity to the “protein transport” domain of TraC1 primase of promiscuous plasmid RP4. *J Mol Biol* 296, 969–977. <https://doi.org/10.1006/jmbi.1999.3493>
- Ben Meriem, Z., Mateo, T., Faccini, J., Denais, C., Dusfour-Castan, R., Guynet, C., Merle, T., Suzanne, M., Di-Luoffo, M., Guillermet-Guibert, J., Alric, B., Landiech, S., Malaquin, L., Mesnilgrente, F., Laborde, A., Mazenq, L., Courson, R., Delarue, M., 2023. A microfluidic mechano-chemostat for tissues and organisms reveals that confined growth is accompanied with increased macromolecular crowding. *Lab Chip* 23, 4445–4455. <https://doi.org/10.1039/d3lc00313b>
- Birlutiu, V., Birlutiu, R.-M., Dobritoiu, E.S., 2023. Lelliottia amnigena and Pseudomonas putida Coinfection Associated with a Critical SARS-CoV-2 Infection: A Case Report. *Microorganisms* 11, 2143. <https://doi.org/10.3390/microorganisms11092143>
- Bouet, J.-Y., Funnell, B.E., 2019. Plasmid Localization and Partition in Enterobacteriaceae. *EcoSal Plus* 8. <https://doi.org/10.1128/ecosalplus.ESP-0003-2019>
- Bouvier, M., Demarre, G., Mazel, D., 2005. Integron cassette insertion: a recombination process involving a folded single strand substrate. *EMBO J* 24, 4356–4367. <https://doi.org/10.1038/sj.emboj.7600898>
- Cabezón, E., Ripoll-Rozada, J., Peña, A., de la Cruz, F., Arechaga, I., 2015. Towards an integrated model of bacterial conjugation. *FEMS Microbiol Rev* 39, 81–95. <https://doi.org/10.1111/1574-6976.12085>
- Carattoli, A., 2013. Plasmids and the spread of resistance. *Int J Med Microbiol* 303, 298–304. <https://doi.org/10.1016/j.ijmm.2013.02.001>
- Chan, H.Y., Jensen, S.O., LeBard, R.J., Figgett, W.A., Lai, E., Simpson, A.E., Brzoska, A.J., Davies, D.S., Connolly, A.M., Cordwell, S.J., Travis, B.A., Salinas, R., Skurray, R.A., Firth, N., Schumacher, M.A., 2022. Molecular Analysis of pSK1 par: A Novel Plasmid Partitioning System Encoded by Staphylococcal Multiresistance Plasmids. *J Mol Biol* 434, 167770. <https://doi.org/10.1016/j.jmb.2022.167770>
- Chandler, M., Fayet, O., Rousseau, P., Ton Hoang, B., Duval-Valentin, G., 2015. Copy-out-Paste-in Transposition of IS911: A Major Transposition Pathway. *Microbiol Spectr* 3. <https://doi.org/10.1128/microbiolspec.MDNA3-0031-2014>
- Coluzzi, C., Guillemet, M., Mazzamurro, F., Touchon, M., Godfroid, M., Achaz, G., Glaser, P., Rocha, E.P.C., 2023. Chance Favors the Prepared Genomes: Horizontal Transfer Shapes

- the Emergence of Antibiotic Resistance Mutations in Core Genes. *Mol Biol Evol* 40, msad217. <https://doi.org/10.1093/molbev/msad217>
- Conte, E., Mende, L., Grainge, I., Colloms, S.D., 2019. A Mini-ISY100 Transposon Delivery System Effective in  $\gamma$  Proteobacteria. *Front Microbiol* 10, 280. <https://doi.org/10.3389/fmicb.2019.00280>
- Coppens, L., Lavigne, R., 2020. SAPPHIRE: a neural network based classifier for  $\sigma$ 70 promoter prediction in *Pseudomonas*. *BMC Bioinformatics* 21, 415. <https://doi.org/10.1186/s12859-020-03730-z>
- Corneloup, A., Caumont-Sarcos, A., Kamgoue, A., Marty, B., Le, P.T.N., Siguier, P., Guynet, C., Ton-Hoang, B., 2021. TnpAREP and REP sequences dissemination in bacterial genomes: REP recognition determinants. *Nucleic Acids Res* 49, 6982–6995. <https://doi.org/10.1093/nar/gkab524>
- Couturier, A., Virolle, C., Goldlust, K., Berne-Dedieu, A., Reuter, A., Nolivos, S., Yamaichi, Y., Bigot, S., Lesterlin, C., 2023. Real-time visualisation of the intracellular dynamics of conjugative plasmid transfer. *Nat Commun* 14, 294. <https://doi.org/10.1038/s41467-023-35978-3>
- de la Cruz, F., Frost, L.S., Meyer, R.J., Zechner, E.L., 2010. Conjugative DNA metabolism in Gram-negative bacteria. *FEMS Microbiol Rev* 34, 18–40. <https://doi.org/10.1111/j.1574-6976.2009.00195.x>
- Diard, M., Baeriswyl, S., Clermont, O., Gouriou, S., Picard, B., Taddei, F., Denamur, E., Matic, I., 2007. *Caenorhabditis elegans* as a simple model to study phenotypic and genetic virulence determinants of extraintestinal pathogenic *Escherichia coli*. *Microbes and Infection* 9, 214–223. <https://doi.org/10.1016/j.micinf.2006.11.009>
- Dirksen, P., Assié, A., Zimmermann, J., Zhang, F., Tietje, A.-M., Marsh, S.A., Félix, M.-A., Shapira, M., Kaleta, C., Schulenburg, H., Samuel, B.S., 2020. CeMbio - The *Caenorhabditis elegans* Microbiome Resource. *G3 (Bethesda)* 10, 3025–3039. <https://doi.org/10.1534/g3.120.401309>
- Dirksen, P., Marsh, S.A., Braker, I., Heitland, N., Wagner, S., Nakad, R., Mader, S., Petersen, C., Kowallik, V., Rosenstiel, P., Félix, M.-A., Schulenburg, H., 2016. The native microbiome of the nematode *Caenorhabditis elegans*: gateway to a new host-microbiome model. *BMC Biol* 14, 38. <https://doi.org/10.1186/s12915-016-0258-1>
- Durrant, M.G., Perry, N.T., Pai, J.J., Jangid, A.R., Athukoralage, J.S., Hiraizumi, M., McSpedon, J.P., Pawluk, A., Nishimasu, H., Konermann, S., Hsu, P.D., 2024. Bridge RNAs direct programmable recombination of target and donor DNA. *Nature* 630, 984–993. <https://doi.org/10.1038/s41586-024-07552-4>
- Duval-Valentin, G., Normand, C., Khemici, V., Marty, B., Chandler, M., 2001. Transient promoter formation: a new feedback mechanism for regulation of IS911 transposition. *EMBO J* 20, 5802–5811. <https://doi.org/10.1093/emboj/20.20.5802>
- Fernandez-Lopez, R., Del Campo, I., Revilla, C., Cuevas, A., de la Cruz, F., 2014. Negative feedback and transcriptional overshooting in a regulatory network for horizontal gene transfer. *PLoS Genet* 10, e1004171. <https://doi.org/10.1371/journal.pgen.1004171>
- Fernández-López, R., Garcillán-Barcia, M.P., Revilla, C., Lázaro, M., Vielva, L., de la Cruz, F., 2006. Dynamics of the IncW genetic backbone imply general trends in conjugative plasmid evolution. *FEMS Microbiol Rev* 30, 942–966. <https://doi.org/10.1111/j.1574-6976.2006.00042.x>
- González-Montes, L., Campo, I. del, Garcillán-Barcia, M.P., Cruz, F. de la, Moncalián, G., 2020. ArdC, a ssDNA-binding protein with a metalloprotease domain, overpasses the

- recipient hsdRMS restriction system broadening conjugation host range. *PLOS Genetics* 16, e1008750. <https://doi.org/10.1371/journal.pgen.1008750>
- Gordon, G.S., Sitnikov, D., Webb, C.D., Teleman, A., Straight, A., Losick, R., Murray, A.W., Wright, A., 1997. Chromosome and low copy plasmid segregation in *E. coli*: visual evidence for distinct mechanisms. *Cell* 90, 1113–1121. [https://doi.org/10.1016/s0092-8674\(00\)80377-3](https://doi.org/10.1016/s0092-8674(00)80377-3)
- Grkovic, S., Brown, M.H., Hardie, K.M., Firth, N., Skurray, R.A., 2003. Stable low-copy-number *Staphylococcus aureus* shuttle vectors. *Microbiology (Reading)* 149, 785–794. <https://doi.org/10.1099/mic.0.25951-0>
- Guilhas, B., Walter, J.-C., Rech, J., David, G., Walliser, N.O., Palmeri, J., Mathieu-Demaziere, C., Parmeggiani, A., Bouet, J.-Y., Le Gall, A., Nollmann, M., 2020. ATP-Driven Separation of Liquid Phase Condensates in Bacteria. *Mol Cell* 79, 293-303.e4. <https://doi.org/10.1016/j.molcel.2020.06.034>
- Guynet, C., Achard, A., Hoang, B.T., Barabas, O., Hickman, A.B., Dyda, F., Chandler, M., 2009. Resetting the site: redirecting integration of an insertion sequence in a predictable way. *Mol Cell* 34, 612–619. <https://doi.org/10.1016/j.molcel.2009.05.017>
- Guynet, C., Cuevas, A., Moncalián, G., de la Cruz, F., 2011. The stb operon balances the requirements for vegetative stability and conjugative transfer of plasmid R388. *PLoS Genet* 7, e1002073. <https://doi.org/10.1371/journal.pgen.1002073>
- Guynet, C., de la Cruz, F., 2011. Plasmid segregation without partition. *Mob Genet Elements* 1, 236–241. <https://doi.org/10.4161/mge.1.3.18229>
- Guynet, C., Hickman, A.B., Barabas, O., Dyda, F., Chandler, M., Ton-Hoang, B., 2008. In vitro reconstitution of a single-stranded transposition mechanism of IS608. *Mol Cell* 29, 302–312. <https://doi.org/10.1016/j.molcel.2007.12.008>
- Guynet, C., Le, P.T.N., Chandler, M., Ton-Hoang, B., 2020a. Detection and Characterization of Transposons in Bacteria. *Methods Mol Biol* 2075, 81–90. [https://doi.org/10.1007/978-1-4939-9877-7\\_5](https://doi.org/10.1007/978-1-4939-9877-7_5)
- Guynet, C., Siguier, P., Chandler, M., Ton-Hoang, B., 2020b. Nonhomologous Recombination: Bacterial Transposons, in: Reference Module in Life Sciences. Elsevier, p. B9780128194607002000. <https://doi.org/10.1016/B978-0-12-819460-7.00122-5>
- Haçariz, O., Viau, C., Karimian, F., Xia, J., 2021. The symbiotic relationship between *Caenorhabditis elegans* and members of its microbiome contributes to worm fitness and lifespan extension. *BMC Genomics* 22, 364. <https://doi.org/10.1186/s12864-021-07695-y>
- He, S., Corneloup, A., Guynet, C., Lavatine, L., Caumont-Sarcos, A., Siguier, P., Marty, B., Dyda, F., Chandler, M., Ton Hoang, B., 2015. The IS200/IS605 Family and “Peel and Paste” Single-strand Transposition Mechanism. *Microbiol Spectr* 3. <https://doi.org/10.1128/microbiolspec.MDNA3-0039-2014>
- He, S., Guynet, C., Siguier, P., Hickman, A.B., Dyda, F., Chandler, M., Ton-Hoang, B., 2013. IS200/IS605 family single-strand transposition: mechanism of IS608 strand transfer. *Nucleic Acids Res* 41, 3302–3313. <https://doi.org/10.1093/nar/gkt014>
- Hickman, A.B., James, J.A., Barabas, O., Pasternak, C., Ton-Hoang, B., Chandler, M., Sommer, S., Dyda, F., 2010. DNA recognition and the precleavage state during single-stranded DNA transposition in *D. radiodurans*. *EMBO J* 29, 3840–3852. <https://doi.org/10.1038/emboj.2010.241>
- Hiraizumi, M., Perry, N.T., Durrant, M.G., Soma, T., Nagahata, N., Okazaki, S., Athukoralage, J.S., Isayama, Y., Pai, J.J., Pawluk, A., Konermann, S., Yamashita, K., Hsu, P.D.,

- Nishimasu, H., 2024. Structural mechanism of bridge RNA-guided recombination. *Nature* 630, 994–1002. <https://doi.org/10.1038/s41586-024-07570-2>
- Hsu, T.-M., Chang, Y.-R., 2019. High-Copy-Number Plasmid Segregation-Single-Molecule Dynamics in Single Cells. *Biophys J* 116, 772–780. <https://doi.org/10.1016/j.bpj.2019.01.019>
- Karvelis, T., Druteika, G., Bigelyte, G., Budre, K., Zedaveinyte, R., Silanskas, A., Kazlauskas, D., Venclovas, Č., Siksnys, V., 2021. Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease. *Nature* 599, 692–696. <https://doi.org/10.1038/s41586-021-04058-1>
- Kersulyte, D., Akopyants, N.S., Clifton, S.W., Roe, B.A., Berg, D.E., 1998. Novel sequence organization and insertion specificity of IS605 and IS606: chimaeric transposable elements of *Helicobacter pylori*. *Gene* 223, 175–186. [https://doi.org/10.1016/s0378-1119\(98\)00164-4](https://doi.org/10.1016/s0378-1119(98)00164-4)
- Kersulyte, D., Mukhopadhyay, A.K., Shirai, M., Nakazawa, T., Berg, D.E., 2000. Functional organization and insertion specificity of IS607, a chimeric element of *Helicobacter pylori*. *J Bacteriol* 182, 5300–5308. <https://doi.org/10.1128/JB.182.19.5300-5308.2000>
- Kersulyte, D., Velapatiño, B., Dailide, G., Mukhopadhyay, A.K., Ito, Y., Cahuayme, L., Parkinson, A.J., Gilman, R.H., Berg, D.E., 2002. Transposable Element ISHp608 of *Helicobacter pylori*: Nonrandom Geographic Distribution, Functional Organization, and Insertion Specificity. *J Bacteriol* 184, 992–1002. <https://doi.org/10.1128/jb.184.4.992-1002.2002>
- Koonin, E.V., Ilyina, T.V., 1993. Computer-assisted dissection of rolling circle DNA replication. *Biosystems* 30, 241–268. [https://doi.org/10.1016/0303-2647\(93\)90074-m](https://doi.org/10.1016/0303-2647(93)90074-m)
- Koonin, E.V., Makarova, K.S., Zhang, F., 2017. Diversity, classification and evolution of CRISPR-Cas systems. *Current Opinion in Microbiology, Environmental microbiology \* CRISPRcas9* 37, 67–78. <https://doi.org/10.1016/j.mib.2017.05.008>
- Le Gall, A., Cattoni, D.I., Guilhas, B., Mathieu-Demazière, C., Oudjedi, L., Fiche, J.-B., Rech, J., Abrahamsson, S., Murray, H., Bouet, J.-Y., Nollmann, M., 2016. Bacterial partition complexes segregate within the volume of the nucleoid. *Nat Commun* 7, 12107. <https://doi.org/10.1038/ncomms12107>
- Lesterlin, C., Ball, G., Schermelleh, L., Sherratt, D.J., 2014. RecA bundles mediate homology pairing between distant sisters during DNA break repair. *Nature* 506, 249–253. <https://doi.org/10.1038/nature12868>
- Li, Y., Austin, S., 2002. The P1 plasmid in action: time-lapse photomicroscopy reveals some unexpected aspects of plasmid partition. *Plasmid* 48, 174–178. [https://doi.org/10.1016/s0147-619x\(02\)00104-x](https://doi.org/10.1016/s0147-619x(02)00104-x)
- Liu, Z., Capaldi, X., Zeng, L., Zhang, Y., Reyes-Lamothe, R., Reisner, W., 2022. Confinement anisotropy drives polar organization of two DNA molecules interacting in a nanoscale cavity. *Nat Commun* 13, 4358. <https://doi.org/10.1038/s41467-022-31398-x>
- Llosa, M., Bolland, S., de la Cruz, F., 1991. Structural and functional analysis of the origin of conjugal transfer of the broad-host-range IncW plasmid R388 and comparison with the related IncN plasmid R46. *Mol Gen Genet* 226, 473–483. <https://doi.org/10.1007/BF00260661>
- Llosa, M., Gomis-Rüth, F.X., Coll, M., de la Cruz Fd, F., 2002. Bacterial conjugation: a two-step mechanism for DNA transport. *Mol Microbiol* 45, 1–8. <https://doi.org/10.1046/j.1365-2958.2002.03014.x>

- López-Igual, R., Bernal-Bayard, J., Rodríguez-Patón, A., Ghigo, J.-M., Mazel, D., 2019. Engineered toxin-intein antimicrobials can selectively target and kill antibiotic-resistant bacteria in mixed populations. *Nat Biotechnol* 37, 755–760. <https://doi.org/10.1038/s41587-019-0105-3>
- Lucas, M., González-Pérez, B., Cabezas, M., Moncalian, G., Rivas, G., de la Cruz, F., 2010. Relaxase DNA Binding and Cleavage Are Two Distinguishable Steps in Conjugative DNA Processing That Involve Different Sequence Elements of the *nic* Site. *J Biol Chem* 285, 8918–8926. <https://doi.org/10.1074/jbc.M109.057539>
- Marraffini, L.A., 2015. CRISPR-Cas immunity in prokaryotes. *Nature* 526, 55–61. <https://doi.org/10.1038/nature15386>
- Messing, S.A.J., Ton-Hoang, B., Hickman, A.B., McCubbin, A.J., Peaslee, G.F., Ghirlando, R., Chandler, M., Dyda, F., 2012. The processing of repetitive extragenic palindromes: the structure of a repetitive extragenic palindrome bound to its associated nuclease. *Nucleic Acids Res* 40, 9964–9979. <https://doi.org/10.1093/nar/gks741>
- Morero, N.R., Zuliani, C., Kumar, B., Bebel, A., Okamoto, S., Guynet, C., Hickman, A.B., Chandler, M., Dyda, F., Barabas, O., 2018. Targeting IS608 transposon integration to highly specific sequences by structure-based transposon engineering. *Nucleic Acids Res* 46, 4152–4163. <https://doi.org/10.1093/nar/gky235>
- Nazareno, E.S., Acharya, B., Dumenyo, C.K., 2021. A mini-Tn5-derived transposon with reportable and selectable markers enables rapid generation and screening of insertional mutants in Gram-negative bacteria. *Lett Appl Microbiol* 72, 283–291. <https://doi.org/10.1111/lam.13423>
- Nielsen, H.J., Ottesen, J.R., Youngren, B., Austin, S.J., Hansen, F.G., 2006. The *Escherichia coli* chromosome is organized with the left and right chromosome arms in separate cell halves. *Mol Microbiol* 62, 331–338. <https://doi.org/10.1111/j.1365-2958.2006.05346.x>
- Niki, H., Hiraga, S., 1997. Subcellular distribution of actively partitioning F plasmid during the cell division cycle in *E. coli*. *Cell* 90, 951–957. [https://doi.org/10.1016/s0092-8674\(00\)80359-1](https://doi.org/10.1016/s0092-8674(00)80359-1)
- Nordström, K., Austin, S.J., 1989. Mechanisms that contribute to the stable segregation of plasmids. *Annu Rev Genet* 23, 37–69. <https://doi.org/10.1146/annurev.ge.23.120189.000345>
- Ortiz, A., Vega, N.M., Ratzke, C., Gore, J., 2021. Interspecies bacterial competition regulates community assembly in the *C. elegans* intestine. *ISME J* 15, 2131–2145. <https://doi.org/10.1038/s41396-021-00910-4>
- Partridge, S.R., Kwong, S.M., Firth, N., Jensen, S.O., 2018. Mobile Genetic Elements Associated with Antimicrobial Resistance. *Clin Microbiol Rev* 31, e00088-17. <https://doi.org/10.1128/CMR.00088-17>
- Pasternak, C., Dulermo, R., Ton-Hoang, B., Debuchy, R., Siguier, P., Coste, G., Chandler, M., Sommer, S., 2013. ISDra2 transposition in *Deinococcus radiodurans* is downregulated by TnpB. *Mol Microbiol* 88, 443–455. <https://doi.org/10.1111/mmi.12194>
- Pasternak, C., Ton-Hoang, B., Coste, G., Bailone, A., Chandler, M., Sommer, S., 2010. Irradiation-Induced *Deinococcus radiodurans* Genome Fragmentation Triggers Transposition of a Single Resident Insertion Sequence. *PLoS Genet* 6, e1000799. <https://doi.org/10.1371/journal.pgen.1000799>
- Paterson, E.S., Moré, M.I., Pillay, G., Cellini, C., Woodgate, R., Walker, G.C., Iyer, V.N., Winans, S.C., 1999. Genetic analysis of the mobilization and leading regions of the IncN

- plasmids pKM101 and pCU1. *J Bacteriol* 181, 2572–2583. <https://doi.org/10.1128/JB.181.8.2572-2583.1999>
- Perutka, J., Wang, W., Goerlitz, D., Lambowitz, A.M., 2004. Use of Computer-designed Group II Introns to Disrupt *Escherichia coli* DExH/D-box Protein and DNA Helicase Genes. *Journal of Molecular Biology* 336, 421–439. <https://doi.org/10.1016/j.jmb.2003.12.009>
- Planchenault, C., Pons, M.C., Schiavon, C., Siguier, P., Rech, J., Guynet, C., Dauverd-Girault, J., Cury, J., Rocha, E.P.C., Junier, I., Cornet, F., Espéli, O., 2020. Intracellular Positioning Systems Limit the Entropic Eviction of Secondary Replicons Toward the Nucleoid Edges in Bacterial Cells. *J Mol Biol* 432, 745–761. <https://doi.org/10.1016/j.jmb.2019.11.027>
- Quèbre, V., Del Campo, I., Cuevas, A., Siguier, P., Rech, J., Le, P.T.N., Ton-Hoang, B., Cornet, F., Bouet, J.-Y., Moncalian, G., de la Cruz, F., Guynet, C., 2022. Characterization of the DNA Binding Domain of StbA, A Key Protein of A New Type of DNA Segregation System. *J Mol Biol* 434, 167752. <https://doi.org/10.1016/j.jmb.2022.167752>
- Radeke, L.J., Herman, M.A., 2021. Take a Walk to the Wild Side of *Caenorhabditis elegans*-Pathogen Interactions. *Microbiol Mol Biol Rev* 85, e00146-20. <https://doi.org/10.1128/MMBR.00146-20>
- Reuter, A., Hilpert, C., Dedieu-Berne, A., Lematre, S., Gueguen, E., Launay, G., Bigot, S., Lesterlin, C., 2021. Targeted-antibacterial-plasmids (TAPs) combining conjugation and CRISPR/Cas systems achieve strain-specific antibacterial activity. *Nucleic Acids Res* 49, 3584–3598. <https://doi.org/10.1093/nar/gkab126>
- Revilla, C., Garcillán-Barcia, M.P., Fernández-López, R., Thomson, N.R., Sanders, M., Cheung, M., Thomas, C.M., de la Cruz, F., 2008. Different pathways to acquiring resistance genes illustrated by the recent evolution of IncW plasmids. *Antimicrob Agents Chemother* 52, 1472–1480. <https://doi.org/10.1128/AAC.00982-07>
- Robledo, M., Álvarez, B., Cuevas, A., González, S., Ruano-Gallego, D., Fernández, L.Á., de la Cruz, F., 2022. Targeted bacterial conjugation mediated by synthetic cell-to-cell adhesions. *Nucleic Acids Res* 50, 12938–12950. <https://doi.org/10.1093/nar/gkac1164>
- Ronning, D.R., Guynet, C., Ton-Hoang, B., Perez, Z.N., Ghirlando, R., Chandler, M., Dyda, F., 2005. Active site sharing and subterminal hairpin recognition in a new class of DNA transposases. *Mol Cell* 20, 143–154. <https://doi.org/10.1016/j.molcel.2005.07.026>
- Sanchez, A., Cattoni, D.I., Walter, J.-C., Rech, J., Parmeggiani, A., Nollmann, M., Bouet, J.-Y., 2015. Stochastic Self-Assembly of ParB Proteins Builds the Bacterial DNA Segregation Apparatus. *Cell Syst* 1, 163–173. <https://doi.org/10.1016/j.cels.2015.07.013>
- Santin, Y.G., Doan, T., Lebrun, R., Espinosa, L., Journet, L., Cascales, E., 2018. In vivo TssA proximity labelling during type VI secretion biogenesis reveals TagA as a protein that stops and holds the sheath. *Nat Microbiol* 3, 1304–1313. <https://doi.org/10.1038/s41564-018-0234-3>
- Sasnauskas, G., Tamulaitiene, G., Druteika, G., Carabias, A., Silanskas, A., Kazlauskas, D., Venclovas, Č., Montoya, G., Karvelis, T., Siksnys, V., 2023. TnpB structure reveals minimal functional core of Cas12 nuclease family. *Nature* 616, 384–389. <https://doi.org/10.1038/s41586-023-05826-x>
- Sau, S., Ghosh, S.K., Liu, Y.-T., Ma, C.-H., Jayaram, M., 2019. Hitchhiking on chromosomes: A persistence strategy shared by diverse selfish DNA elements. *Plasmid* 102, 19–28. <https://doi.org/10.1016/j.plasmid.2019.01.004>

- Schumacher, M.A., Henderson, M., Zhang, H., 2019. Structures of maintenance of carboxysome distribution Walker-box McdA and McdB adaptor homologs. *Nucleic Acids Res* 47, 5950–5962. <https://doi.org/10.1093/nar/gkz314>
- Segura, R.L., Aguila-Arcos, S., Ugarte-Urbe, B., Vecino, A.J., de la Cruz, F., Goñi, F.M., Alkorta, I., 2014. Subcellular location of the coupling protein TrwB and the role of its transmembrane domain. *Biochim Biophys Acta* 1838, 223–230. <https://doi.org/10.1016/j.bbamem.2013.08.016>
- Siguier, P., Campos, M., Cornet, F., Bouet, J.-Y., Guynet, C., 2023. Atypical low-copy number plasmid segregation systems, all in one? *Plasmid* 127, 102694. <https://doi.org/10.1016/j.plasmid.2023.102694>
- Siguier, P., Perochon, J., Lestrade, L., Mahillon, J., Chandler, M., 2006. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 34, D32–36. <https://doi.org/10.1093/nar/gkj014>
- Simpson, A.E., Skurray, R.A., Firth, N., 2003. A single gene on the staphylococcal multiresistance plasmid pSK1 encodes a novel partitioning system. *J Bacteriol* 185, 2143–2152. <https://doi.org/10.1128/jb.185.7.2143-2152.2003>
- Soh, Y.-M., Davidson, I.F., Zamuner, S., Basquin, J., Bock, F.P., Taschner, M., Veening, J.-W., De Los Rios, P., Peters, J.-M., Gruber, S., 2019. Self-organization of parS centromeres by the ParB CTP hydrolase. *Science* 366, 1129–1133. <https://doi.org/10.1126/science.aay3965>
- Stanley, T.L., Ellermeier, C.D., Slauch, J.M., 2000. Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects *Salmonella enterica* serovar typhimurium survival in Peyer's patches. *J Bacteriol* 182, 4406–4413. <https://doi.org/10.1128/JB.182.16.4406-4413.2000>
- Taylor, J.A., Seol, Y., Budhathoki, J., Neuman, K.C., Mizuuchi, K., 2021. CTP and parS coordinate ParB partition complex dynamics and ParA-ATPase activation for ParABS-mediated DNA partitioning. *Elife* 10, e65651. <https://doi.org/10.7554/eLife.65651>
- Tellier, M., Bouuaert, C.C., Chalmers, R., 2015. Mariner and the ITm Superfamily of Transposons. *Microbiol Spectr* 3, MDNA3-0033-2014. <https://doi.org/10.1128/microbiolspec.MDNA3-0033-2014>
- Ton-Hoang, B., Guynet, C., Ronning, D.R., Cointin-Marty, B., Dyda, F., Chandler, M., 2005. Transposition of ISHp608, member of an unusual family of bacterial insertion sequences. *EMBO J* 24, 3325–3338. <https://doi.org/10.1038/sj.emboj.7600787>
- Ton-Hoang, B., Pasternak, C., Siguier, P., Guynet, C., Hickman, A.B., Dyda, F., Sommer, S., Chandler, M., 2010. Single-stranded DNA transposition is coupled to host replication. *Cell* 142, 398–408. <https://doi.org/10.1016/j.cell.2010.06.034>
- Ton-Hoang, B., Siguier, P., Quentin, Y., Onillon, S., Marty, B., Fichant, G., Chandler, M., 2012. Structuring the bacterial genome: Y1-transposases associated with REP-BIME sequences. *Nucleic Acids Res* 40, 3596–3609. <https://doi.org/10.1093/nar/gkr1198>
- Val, M.-E., Bouvier, M., Campos, J., Sherratt, D., Cornet, F., Mazel, D., Barre, F.-X., 2005. The single-stranded genome of phage CTX is the form used for integration into the genome of *Vibrio cholerae*. *Mol Cell* 19, 559–566. <https://doi.org/10.1016/j.molcel.2005.07.002>
- Val-Calvo, J., Luque-Ortega, J.R., Crespo, I., Miguel-Arribas, A., Abia, D., Sánchez-Hevia, D.L., Serrano, E., Gago-Córdoba, C., Ares, S., Alfonso, C., Rojo, F., Wu, L.J., Boer, D.R., Meijer, W.J.J., 2018. Novel regulatory mechanism of establishment genes of conjugative plasmids. *Nucleic Acids Res* 46, 11910–11926. <https://doi.org/10.1093/nar/gky996>



- Vecchiarelli, A.G., Funnell, B.E., 2013. Probing the N-terminus of ParB using cysteine-scanning mutagenesis and thiol modification. *Plasmid* 70, 86–93. <https://doi.org/10.1016/j.plasmid.2013.02.002>
- Venter, H., 2019. Reversing resistance to counter antimicrobial resistance in the World Health Organisation's critical priority of most dangerous pathogens. *Biosci Rep* 39, BSR20180474. <https://doi.org/10.1042/BSR20180474>
- Virolle, C., Goldlust, K., Djermoun, S., Bigot, S., Lesterlin, C., 2020. Plasmid Transfer by Conjugation in Gram-Negative Bacteria: From the Cellular to the Community Level. *Genes (Basel)* 11, E1239. <https://doi.org/10.3390/genes11111239>
- von Wintersdorff, C.J.H., Penders, J., van Niekerk, J.M., Mills, N.D., Majumder, S., van Alphen, L.B., Savelkoul, P.H.M., Wolffs, P.F.G., 2016. Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Front Microbiol* 7, 173. <https://doi.org/10.3389/fmicb.2016.00173>
- Wang, Y., 2017. Spatial distribution of high copy number plasmids in bacteria. *Plasmid, SI: ISPB Plasmid* 2016 91, 2–8. <https://doi.org/10.1016/j.plasmid.2017.02.005>
- Yoon, P.H., Skopintsev, P., Shi, H., Chen, L., Adler, B.A., Al-Shimary, M., Craig, R.J., Loi, K.J., DeTurk, E.C., Li, Z., Amerasekera, J., Trinidad, M., Nisonoff, H., Chen, K., Lahiri, A., Boger, R., Jacobsen, S., Banfield, J.F., Doudna, J.A., 2023. Eukaryotic RNA-guided endonucleases evolved from a unique clade of bacterial enzymes. *Nucleic Acids Res* 51, 12414–12427. <https://doi.org/10.1093/nar/gkad1053>
- Zhang, F., Berg, M., Dierking, K., Félix, M.-A., Shapira, M., Samuel, B.S., Schulenburg, H., 2017. *Caenorhabditis elegans* as a Model for Microbiome Research. *Front Microbiol* 8, 485. <https://doi.org/10.3389/fmicb.2017.00485>
- Ziegelin, G., Pansegrau, W., Lurz, R., Lanka, E., 1992. TraK protein of conjugative plasmid RP4 forms a specialized nucleoprotein complex with the transfer origin. *J Biol Chem* 267, 17279–17286.

## 6. PAST RESEARCH SUMMARY

### 6.1 Research activity summary (English)

My research is oriented towards the understanding of how MGEs operate, i.e. their intimate propagation mechanisms (modes of transfer, integration, and regulation), *in vitro* as well as *in vivo*, in the cell and more recently within bacterial communities. I have had the opportunity to study several types of elements, combining various and multi-scale approaches, such as biochemical and structural studies, genetics, genome-wide molecular genetics, bioinformatics, fluorescence microscopy and more recently a eucaryotic model microbiota. My PhD (2004-2008) was dedicated to the characterization of IS608, the model element of a widespread family of atypical bacterial transposons. We identified an entirely novel transposition pathway, involving exclusively single stranded DNA, associated with a new class of transposases (Y1). My post-doctorate in F. de la Cruz's group (2008-2011) was devoted to study another major process of bacterial genetic diversity, conjugation. I initiated work on the *stb* operon and have shown that it controls the balance between two modes of transmission of plasmid R388: vertical transmission by segregation to daughter cells, and horizontal transmission by conjugation.

In 2011, I joined M. Chandler's group as a Chargée de Recherche at the CNRS, where I participated in different aspects of IS608 transposition regulation, from the choice of target sites to detailed conformational changes in the transposase during strands exchange leading to IS excision and integration. During this period, I also contributed to a project led by B. Ton-Hoang devoted to the study of the dissemination of REP sequences in bacterial genomes, and on which I co-supervised Alix Corneloup's thesis (2012-2016). REP are short palindromic DNA sequences present in large numbers in many bacterial genomes. This work, via the development of an assay to visualize and characterize TnpAREP transposase activity *in vitro* for the first time, has enabled us to propose a model for REP dissemination/amplification.

I then joined J.-Y. Bouet's team in 2016, which merged later with the team of F. Cornet in 2019 (LMGM) to develop projects focused on deciphering spatiotemporal dynamics of secondary replicons (plasmids). In particular, I am interested in understanding how conjugation events are integrated into the maintenance functions of the plasmid (replication, segregation), which is one important remaining question in the field. We have shown that the

Stb system, present in over 15% of plasmids in enterobacteria, mechanistically links these two processes. It involves the StbA protein, of which we have characterized the DNA-binding domain, and whose activities make it both a new type of segregation system, through its role in positioning plasmid molecules in the bacterium, and an inhibitor of conjugation. This work is part of Valentin Quèbre's PhD (co-supervised 2019-2023), and I co-supervise Charlotte Hall's PhD that on this project since December 2023. I also aim to enlarge our knowledge on horizontal gene transfer to a more complex ecosystem by investigating the dynamic feature of conjugative plasmids and transposons both at the population and cellular levels within the simple eukaryotic intestine model *Caenorhabditis elegans*. Romane Dusfour-Castan's thesis work (2021-now) shows that *Lelliottia amnigena* influences the lifespan, fertility and development of *C. elegans* compared with other CeMBio bacteria, with these effects depending on the composition and complexity of the microbiota.

## 6.2 Résumé de l'activité de recherche (Français)

Mes recherches sont orientées vers la compréhension du fonctionnement des Éléments Génétiques Mobiles (EGMs), c'est-à-dire leurs mécanismes intimes de propagation (modes de transfert, intégration et régulation), *in vitro* et *in vivo*, dans la cellule et plus récemment au sein des communautés bactériennes. J'ai eu l'opportunité d'étudier plusieurs types d'éléments, en combinant diverses approches multi-échelles, telles que des études biochimiques et structurales, la génétique, la génétique moléculaire à l'échelle du génome, la bioinformatique, la microscopie à fluorescence et plus récemment un modèle de microbiote eucaryote.

Durant ma thèse (2004-2008), j'ai participé à la caractérisation d'IS608, l'élément modèle d'une famille répandue de transposons bactériens atypiques. Nous avons identifié une voie de transposition entièrement nouvelle, impliquant exclusivement l'ADN simple brin, associée à une nouvelle classe de transposases (Y1). Mon post-doctorat dans le groupe de F. de la Cruz (2008-2011) était consacré à l'étude d'un autre processus majeur de la diversité génétique bactérienne, la conjugaison. J'ai initié des travaux sur l'opéron *stb*, et nous avons montré qu'il contrôle l'équilibre entre deux modes de transmission du plasmide R388 : la transmission verticale par ségrégation aux cellules filles, et la transmission horizontale par conjugaison.

En 2011, j'ai rejoint le groupe de M. Chandler en tant que Chargée de Recherche au CNRS, où j'ai participé à différents aspects de la régulation de la transposition d'IS608, depuis le choix des sites cibles jusqu'aux changements conformationnels de la transposase pendant l'échange de brins conduisant à l'excision et à l'intégration de l'IS. Pendant cette période, j'ai également contribué à un projet dirigé par B. Ton-Hoang visant à étudier la dissémination des séquences REP dans les génomes bactériens, et sur lequel j'ai co-encadré la thèse d'Alix Corneloup (2012-2016). Les REP sont de courtes séquences d'ADN palindromiques présentes en grand nombre dans de nombreux génomes bactériens. Ce travail, *via* le développement d'un test pour visualiser et caractériser pour la première fois l'activité de la transposase TnpAREP *in vitro*, nous a permis de proposer un modèle pour la dissémination/amplification des REP.

J'ai ensuite rejoint l'équipe de J.-Y. Bouet en 2016, qui a fusionné plus tard avec l'équipe de F. Cornet en 2019 (LMGM) pour développer des projets axés sur le déchiffrement de la dynamique spatio-temporelle des réplicons secondaires (plasmides). Mes projets ont notamment pour but de comprendre comment les événements de conjugaison sont intégrés aux fonctions de maintenance du plasmide (réplication, ségrégation), ce qui constitue une question importante encore non résolue dans ce domaine. Nous avons montré que le système *Stb*, présent dans plus de 15% des plasmides chez les entérobactéries, lie mécaniquement ces deux processus. Il implique la protéine *StbA*, dont nous avons caractérisé le domaine de liaison à l'ADN, et dont les activités en font à la fois un nouveau type de système de ségrégation, par son rôle dans le positionnement des molécules de plasmide dans la bactérie, et un inhibiteur de la conjugaison. Ce travail fait partie de la thèse de Valentin Quèbre (co-encadrée 2019-2023), et je co-encadre la thèse de Charlotte Hall sur ce projet depuis décembre 2023. Mon objectif est également d'élargir nos connaissances sur le transfert horizontal de gènes à un écosystème plus complexe en étudiant la dynamique des plasmides conjugatifs et des transposons à la fois au niveau de la population et au niveau cellulaire dans le modèle intestinal eucaryote simple *Caenorhabditis elegans*. Les travaux de thèse de Romane Dusfour-Castan (2021-maintenant) montrent que *Lelliottia amnigena* influence la durée de vie, la fertilité et le développement de *C. elegans* par rapport à d'autres bactéries CeMBio, avec des effets dépendant de la composition et de la complexité du microbiote.

## 7. PROJECT SUMMARY

### 7.1 Project summary (English)

Since my thesis, my research has focused on the molecular mechanisms involved in the propagation in bacterial genomes of mobile genetic elements (MGEs), including transposons and plasmids. Our results on the study of the intriguing Stb system, showing that it is the only system linking plasmid subcellular localization to segregation and conjugation efficiencies, encouraged me to pursue my objectives to describe in depth the roles of Stb proteins, and their consequences on plasmid localization and fate. In addition, the development of cutting-edge technologies, such as microscopy or high-throughput sequencing techniques, is making it possible to tackle questions that previously remained unanswered. I have therefore chosen to include in my research a broader scale of study, i.e. complex ecosystems.

As carriers of MGEs, including transposons, integrons, and associated passenger genes, conjugative plasmids behave as gene shuttles able to propagate within bacterial populations. Plasmid propagation occurs both vertically by plasmid segregation to daughter cells during proliferation and horizontally to other bacteria during transfer by conjugation. These two modes of transfer involve and require mutually exclusive subcellular localization of plasmids, i.e., plasmid copies must be moved from their position, usually in the nucleoid area, to the membrane to conjugate. How they switch from one to the other is still largely elusive. This is true not only in the donor bacterium but also in the recipient bacterium that has just acquired a conjugative plasmid and becomes a new stable donor. Yet this control point in plasmid trafficking has received very little attention to date, despite being one of the key points in the dissemination of antimicrobial resistance genes. We have identified the Stb system, found in more than 15% of enterobacterial plasmids, which act to balance vertical and horizontal plasmid propagation.

My first objective is to continue examining the interplay between vertical and horizontal transfer of plasmids. This includes (i) the characterization of the Stb proteins *in vitro* (using structural biology, biochemistry, molecular biology as well as specific *in vitro* activity assays), (ii) the study of the dynamics of the plasmids along with that of the Stb proteins in live cells (using video epifluorescence microscopy and super-resolution microscopy). We also aim to investigate whether the control of vertical and horizontal spread of plasmids by Stb

requires factors encoded by the bacterial host (using genome-wide screening approaches) and follow the fate of the plasmids and transfer events in real-time in the gut microbiota of the nematode *Caenorhabditis elegans* (using epi-fluorescence microscopy).

My second objective is to enlarge our knowledge on horizontal gene transfer to complex ecosystems. We will investigate the dynamic features of conjugative plasmids and transposons both at the population and cellular levels within the simple eukaryotic intestine model *C. elegans*, which features should allow direct imaging of conjugative events in a gastrointestinal system model, opening the way to describing the fine controls of gene exchanges inside host-associated bacterial communities. This includes the set-up of alternative strategies to antimicrobials, which is one of the actions to be taken in response to the "One Health" strategic priority of curbing the silent AMR pandemic.

My third objective, which will be coordinated together with M. Campos in the team, is to investigate the genetic bases of the control of commensalism and pathogenicity in *C. elegans*' gut. We will evaluate the impact of the establishment of a given microbiota on the colonization and uncontrolled growth of a set of *Escherichia coli* strains representative of the species and the dynamics of the worm's microbiota leading to dysbiosis. We aim to define the genetic factors responsible for *E. coli* establishment in the microbiota as a commensal, as well as the new virulence factors associated with the worm's death specific to the context of a microbial community in the gut. We will pay particular attention to MGEs, as recent studies show that they have the potential to alter the fitness of other genetic *loci*, even core genome *loci*.

## 7.2 Résumé de l'activité de recherche (Français)

Depuis ma thèse, mes recherches se sont concentrées sur les mécanismes moléculaires impliqués dans la propagation dans les génomes bactériens d'éléments génétiques mobiles (EGMs), dont les transposons et les plasmides. Nos résultats sur l'étude du système Stb, qui montrent qu'il s'agit du seul système liant la localisation subcellulaire des plasmides aux efficacités de ségrégation et de conjugaison, m'ont encouragée à poursuivre mes objectifs pour décrire en profondeur les rôles des protéines Stb, et leurs conséquences sur la localisation et le devenir des plasmides. De plus, le développement de technologies de pointe, telles que la microscopie ou les techniques de séquençage à haut débit, permet d'aborder des questions qui sont toujours restées sans réponse. J'ai donc choisi d'inclure dans mes recherches une échelle d'étude plus large, à savoir les écosystèmes complexes.

En tant que porteurs d'EGMs, y compris les transposons, les intégrons et les gènes passagers associés, les plasmides conjugatifs se comportent comme des navettes génétiques capables de se propager au sein des populations bactériennes. La propagation des plasmides se fait à la fois verticalement, par ségrégation des plasmides dans les cellules filles au cours de la prolifération, et horizontalement, vers d'autres bactéries, par transfert par conjugaison. Ces deux modes de transfert impliquent et nécessitent une localisation subcellulaire mutuellement exclusive des plasmides, c'est-à-dire que les copies de plasmides doivent être déplacées de leur position, généralement dans la zone du nucléoïde, vers la membrane pour conjuguer. La manière dont elles passent de l'une à l'autre reste encore largement obscure. Cela est vrai non seulement pour la bactérie donneuse, mais aussi pour la bactérie receveuse qui vient d'acquérir un plasmide conjugatif et devient un nouveau donneur stable. Pourtant, ce point de contrôle dans le trafic des plasmides a reçu très peu d'attention jusqu'à présent, bien qu'il soit l'un des points clés dans la dissémination des gènes de résistance aux antimicrobiens. Nous avons identifié le système Stb, présent dans plus de 15 % des plasmides entérobactériens, qui contrôle la propagation verticale et horizontale des plasmides.

Mon premier objectif est de continuer à examiner l'interaction entre le transfert vertical et horizontal des plasmides. Cela comprend (i) la caractérisation des protéines Stb *in vitro* (en utilisant la biologie structurale, la biochimie, la biologie moléculaire ainsi que des tests d'activité *in vitro* spécifiques), (ii) l'étude de la dynamique des plasmides ainsi que celle des protéines Stb dans des cellules vivantes (en utilisant la vidéo-microscopie à



épifluorescence et la microscopie à super-résolution). Nous cherchons également à déterminer si le contrôle de la propagation verticale et horizontale des plasmides par *Stb* nécessite des facteurs codés par l'hôte bactérien (en utilisant des approches de criblage à l'échelle du génome) et à suivre le devenir des plasmides et les événements de transfert en temps réel dans le microbiote intestinal du nématode *Caenorhabditis elegans*.

Mon deuxième objectif est d'élargir nos connaissances sur le transfert horizontal de gènes dans les écosystèmes complexes. Nous étudierons les caractéristiques dynamiques des plasmides et transposons conjugatifs à la fois au niveau de la population et au niveau cellulaire dans le modèle simple de l'intestin eucaryote *C. elegans*. Ces caractéristiques devraient permettre l'imagerie directe des événements conjugatifs dans un modèle de système gastro-intestinal, ouvrant la voie à la description des contrôles fins des échanges de gènes à l'intérieur des communautés bactériennes associées à l'hôte. Cela inclut la mise en place de stratégies alternatives aux antimicrobiens, ce qui est l'une des actions à entreprendre en réponse à la priorité stratégique "One Health" qui consiste à enrayer la pandémie silencieuse de résistance aux antimicrobiens.

Mon troisième objectif, qui sera coordonné avec M. Campos dans l'équipe, est d'étudier les bases génétiques du contrôle du commensalisme et de la pathogénicité dans l'intestin de *C. elegans*. Nous évaluerons l'impact de l'établissement d'un microbiote donné sur la colonisation et la croissance incontrôlée d'un ensemble de souches d'*Escherichia coli* représentatives de l'espèce, et la dynamique du microbiote du ver conduisant à une dysbiose. Nous visons à définir les facteurs génétiques responsables de l'établissement d'*E. coli* dans le microbiote en tant que commensal, ainsi que des facteurs de virulence associés à la mort du ver, spécifiques au contexte d'une communauté microbienne dans l'intestin. Nous utiliserons la technique de "Mass Allelic Exchanges", une approche qui s'apparente aux outils classiques de la génétique quantitative utilisés pour cartographier les phénotypes et les génotypes chez les eucaryotes. Nous accorderons une attention particulière aux EGMs, car des études récentes montrent qu'ils ont le potentiel d'altérer l'aptitude d'autres *loci* génétiques, même des *loci* du génome central.

## 8. SHORT CURRICULUM VITAE

**Catherine GUYNET, Ph.D.**

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Date and place of birth: 24/10/1979 in Paris (France)  
Three children

### EDUCATION

- 2008 Ph.D. in Molecular Genetics and Microbiology, Paul Sabatier University, Toulouse, France
- 2002-2004 Master's degree in Biotechnologies (Magistère), *grade Bien*, University of Paris XI, Orsay, France; Master's degree in Microbiology, Physiology and Molecular Genetics, (DEA), *grade Bien*, Paul Sabatier University, Toulouse, France

### PRESENT POSITION

- From Oct. 2011 Permanent CNRS researcher (CRCN), Laboratoire de Microbiologie et Génétique Moléculaires-Centre de Biologie Intégrative (LMGM-CBI, UMR 5100), Toulouse, France
- 2019-now Dr J.-Y. Bouet et F. Cornet's group
- 2016-2019 Dr J.-Y. Bouet's group
- 2011-2016 Dr B. Ton-Hoang et M. Chandler's group

### CAREER BREAKS

- Sept. 2014-Sept. 2018 80% part-time work
- Jun. 2017 to Jan. 2018 8 months maternity leave (birth 01/08/2017)
- Nov. 2013 to Sept. 2014 11 months maternity leave (twins, birth 19/12/2013)

### PROFESSIONAL EXPERIENCE

DATE	FUNCTION	PLACE	SUPERVISION	FUNDING
Jan. 2011- Aug. 2011	Post-doctoral researcher	Instituto de Biomedicina y Biotecnología de Cantabria, departamento de Biología	F. de la Cruz	Postdoctoral contract with the University of

		Molecular, Universidad de Cantabria, Santander, Spain		Cantabria (7 months)
<b>2008-2011</b>	Post-doctoral researcher	Instituto de Biomedicina y Biotecnología de Cantabria, departamento de Biología Molecular, Universidad de Cantabria, Santander, Spain	F. de la Cruz	FRM fellowship (5 months) EMBO long-term fellowship (24 months)
<b>Oct. 2007- Aug. 2008</b>	Engineer	Laboratoire de Microbiologie et Génétique moléculaires, Toulouse, France	M. Chandler and B. Ton-Hoang	CNRS contract (10 months)
<b>Aug. – Sept. 2005</b>	PhD student	National Institutes of Health, Bethesda, USA	F. Dyda	Fellowship from the intramural of the national institute of diabetes, NIH (2 months)
<b>Oct. 2004- Sept. 2007</b>	PhD student	Laboratoire de Microbiologie et Génétique moléculaires, Toulouse, France	M. Chandler and B. Ton-Hoang	MENRT fellowship (3 years)

## INTERNSHIPS

- 2005** Two months training in Dr. Fred Dyda's group, National Institutes of Health, Bethesda, USA.  
**"Crystal structure determination of IS608 transposase bound to a DNA hairpin substrate"**
- 2004** Four months Master's training in Dr M. Arlat's group, supervised by E. Lauber, Laboratoire d'Interactions Plantes-Micro-organismes (LIPM)/ UMR441-2594 (INRA-CNRS), Castanet-Tolosan, France: **"Role of the TonB proteins during *Xanthomonas campestris* life cycle"**  
Four months Master's training in Dr M. Chandler's group, supervised by P. Rousseau Laboratoire de Microbiologie et Génétique Moléculaires (LMGM)/ CNRS UMR5100, Toulouse, France: **"Regulation of IS911: role of the domains of the transposase and its regulator"**
- 2003** Three months training period in Dr G. Meyer's group, Ecole Nationale Vétérinaire de Toulouse (ENVT)/UMR1225 IHAP, Toulouse, France : **"Expression of G glycoprotein of the bovine respiratory syncytial virus in a Baculovirus expression system"**
- 2002** Three months training period in Dr H. Bedouelle's group, Institut Pasteur/ URA2185 (CNRS), Paris, France: **"Construction and characterisation of a bivalent miniantibody for the dengue virus glycoprotein"**
- 2001** Three months training period in Dr B. Delmas's group, supervised by J.-F. Eleouët, Unité de Virologie et Immunologie Moléculaires (VIM)/ UR0892 (INRA), Jouy-en-Josas, France: **"Construction of a minigenome of the bovine respiratory syncytial virus"**

## FUNDING

- 2023** Principal coordinator of the GeTBac project (ANR, 645 k€)
- 2023** Co-coordination of a funding from the LMGM with E. Brendon (10 k€)
- 2022** Co-coordination of a funding from the CBI with A. Mattout and A. Pérez-Escudero (10k€)
- 2019** Co-coordination of the PlasMED project for partner #3 (ANR, 500 k€)
- 2019** Co-coordination of a funding from the CBI with A. Pérez-Escudero (8k€)
- 2015** Funding from the University of Toulouse (13 k€)

## SUPERVISION OF GRADUATE STUDENTS

- Déc. 2023-** Co- supervisor of Charlotte Hall PhD (50%) "**Role of the Stb System in the propagation dynamics of bacterial plasmids**"
- Oct. 2021-** Co- supervisor of Romane Dusfour-Castan PhD (50%) "**Bacterial gene exchanges in a model gut microbiota**" ([P21](#), [P23](#))
- 2019-2022** Co-supervisor of Valentin Quèbre PhD (50%) "**Bacterial DNA segregation: control of the assembly of the partition complex**" (graduated 03-2022; [P20](#), [P21](#))
- 2012-2016** Co-supervisor of Alix Corneloup PhD (50%) "**REP sequences dissemination in bacterial genomes: characterization of TnpAREP proteins activities**" (graduated 10-2016; [P12](#), [P19](#))

## SUPERVISION OF UNDER-GRADUATE STUDENTS

### Master :

- 2012** **Yoann Lacaze**, Master 2 Microbiologie (U. Toulouse III, 6 months)
- 2016** **Deghan Mahdi**, Master 2 Microbiologie (U. Toulouse III, 6 months)
- 2017** **Aurélie Groperrin**, Master 1 Biotechnologies (U. Toulouse III, 2 months)
- 2018** **Dereck de Mezquita**, Master 1 Structural and Functional Biology (U. Toulouse III, 2 months)
- 2019** **Maxence Passageon**, Master 2 Microbiologie (U. Toulouse III, 6 months)
- 2020** **Romain Miranda-Capet**, Master 2 Research Microbiologie (U. Toulouse III, 6 months)
- 2022** **Rim Khanfour**, Master 1 Biotechnologies (U. Toulouse III, 2 months)
- 2023** **Marie Bailly**, Master 1 Biologie Santé (U. Toulouse III, 4 months)
- 2023** **Charlotte Hall**, Master 2 Biologie Santé (U. Poitiers, 6 months)
- 2024** **Michael Goode**, Master 2 Molecular Microbiology (U. Toulouse III, 6 months)

### Others:

**Laure Gaizza**, Licence professionnelle (2015, 4 months); **Auriane Rakitch**, licence 3 (2017, 2 months), **Yannis Vareilles**, BTS Biotechnologies (Lycée La Découverte, Decazeville, 2021, 2 months and 2022, 2 months), **Ambre Bues**, BTS Bio-analyses et Contrôles (Lycée Jean Mermoz, Montpellier, 2023, 2 months)

## TEACHING ACTIVITIES

From 2011     Master 2 Microbiology courses, University Toulouse III (10h per year)  
2021-2022     Licence 2 genetics courses, University Toulouse III (32h)

## EXPERTISE

- **Thesis monitoring committees:** S. Djermoun (U. Lyon), M. Lambérioux (Institut Pasteur, Paris)
- **Thesis juries:** Yera Tena (2013, U. Cantabria, Spain), Irene Del Campo (2016, U. Cantabria, Spain), Raül Ruiz Gonzales (2016, U. Cantabria, Spain), C. Palencia Gandara (2021, U. Cantabria, Spain), Egill Richard (2022, Institut Pasteur, Paris), Dieudonné Pengdbamba Zongo (2024, Institut Pasteur Paris, France)
- **Reviewing for scientific journals** (Genes, Life, NAR, Pathogens, Plasmid...).

## BIBLIOMETRICS

23 articles in peer-reviewed journals, including 18 articles and 5 book chapters.  
<https://orcid.org/0000-0003-1970-3411>

## AWARD

2009     Prix de la fondation Jacques Monod, sous l'égide de la fondation de France

## TRAINING

- First aider at work (every 2 years since 2012)
- Biostat R (3 days, Jan. 2021)
- management (n+1 dossier de carrière, 2 days, 2023)
- NGS (5 days, Lyon, 2023)

## MISCELANEOUS

Elected member of the Comité de Laboratoire (CDL) of the LMGM since 2021

## 9. PUBLICATIONS

- P23** Ben Meriem Z., Mateo T., Faccini J., Denais C., Dusfour-Castan R., Guynet C., Merle T., Suzanne M., Di-Luoffo M., Guillermet-Guibert J., Alric B., Landiech S., Malaquin L., Mesnilgrete F., Laborde A., Mazonq L., Courson R., Delarue M. An easy-to-use microfluidic mechano-chemostat for tissues and organisms reveals that confined growth is accompanied with increased macromolecular crowding *Lab Chip*. 2023 Oct 10;23(20):4445-4455. doi: 10.1039/d3lc00313b. PMID: 37740366.
- P22** Siguier P., Campos M., Cornet F., Bouet J.Y., Guynet C.\* (2023) Atypical low-copy number plasmid segregation systems, all in one? *Plasmid*. 127:102694. doi: 10.1016/j.plasmid.2023.102694. Epub 2023 Jun 8. PMID: 37301314.
- P21** Cornet F., Blanchais C., Dusfour-Castan R., Meunier A., Quebre V., Sekkouri Alaoui H., Boudsoq F., Campos M., Crozat E., Guynet C., Pasta F., Rousseau P., Ton Hoang B., Bouet JY. (2023) DNA Segregation in Enterobacteria. *EcoSal Plus*. eesp00382020. PMID: 37220081.
- P20** Quèbre V., Del Campo I., Cuevas A., Siguier P., Rech J., Le P.T.N., Ton-Hoang B., Cornet F., Bouet J.-Y., Moncalian G.\*, de la Cruz F.\* and Guynet C.\* (2022) Characterization of the DNA Binding Domain of StbA, A Key Protein of A New Type of DNA Segregation System. *Journal of Molecular Biology*, 434(19):167752. doi: 10.1016/j.jmb.2022.167752. Epub ahead of print. PMID: 35868361. BioRxiv 2022.04.29.490116; doi:<https://doi.org/10.1101/2022.04.29.490116>.
- P19** Corneloup A., Caumont-Sarcos A., Kamgoue A., Marty B., Le P.T.N., Siguier P., Guynet C.\* and Ton-Hoang B.\* (2021) Tnp<sub>AREP</sub> and REP sequences dissemination in bacterial genomes: REP recognition determinants, *Nucleic Acids Research*, 49 (12): 6982–6995.
- P18** Guynet C., Siguier P., Chandler M. and Ton-Hoang B. (2021) **Nonhomologous Recombination: Bacterial Transposons**. In: Jez Joseph (eds.) *Encyclopedia of Biological Chemistry*, 3rd Edition. vol. 4, pp. 303–312. Oxford: Elsevier. DOI: [10.1016/B978-0-12-819460-7.00122-5](https://doi.org/10.1016/B978-0-12-819460-7.00122-5)
- P17** Planchenault C., Pons M., Schiavon C., Siguier P., Guynet C., Dauverd-Girault J., Cury J., Rocha E.P., Junier I., Cornet F., Espéli O. (2020) Intracellular positioning systems limit the entropic eviction of 1 secondary replicons toward the nucleoid edges in bacterial cells. *Journal of Molecular Biology*, 432(3):745-761. doi: 10.1016/j.jmb.2019.11.027.
- P16** Guynet C.\*, Ton-Hoang B., Bouet, J.-Y. and Hallet, B.\* (2020) First biochemical steps on bacterial transposition pathways. Horizontal gene transfer, *Methods in Molecular Biology*, 2075 pp.157–177.
- P15** Guynet C.\*, Le P.T.N., Chandler M., Ton-Hoang B.\*(2020) Detection and Characterization of Transposons in Bacteria. Horizontal Gene transfer, *Methods in Molecular Biology*, 2075 pp.81–90.
- P14** Morero N.R., Zuliani C., Kumar B., Bebel A., Okamoto S., Guynet C., Hickman A.B., Chandler M., Dyda F., Barabas O. (2018) Targeting IS608 transposon integration to highly specific sequences by structure-based transposon engineering *Nucleic Acids Res*. 46(8):4152-4163. doi: 10.1093/nar/gky235.
- P13** Lavatine L., He S., Caumont-Sarcos A., Guynet C., Marty B., Chandler M., Ton-Hoang B. (2016) Single strand transposition at the host replication fork. *Nucleic Acids Res*. 44(16):7866-83.

- P12** He S., Corneloup A., Guynet C., Lavatine L., Caumont-Sarcos A., Siguier P., Marty B., Dyda F., Chandler M., Ton-Hoang B. (2015) The IS200/IS605 family and “peel and paste” single-strand transposition mechanism. *Microbiol Spectrum* 3(4):MDNA3-0039-2014.
- P11** He S., Guynet C., Siguier P., Hickman A.B., Dyda F., Chandler M., Ton-Hoang B. (2013) IS200/IS605 family single-strand transposition: mechanism of IS608 strand transfer. *Nucleic Acids Res.* 41(5):3302-13.
- P10** Guynet C. and de la Cruz F. (2011) Plasmid segregation without partition, *Mob Genet Elements* 1(3):236-241.
- P09** Guynet C., Cuevas A., Moncalián G., de la Cruz F. (2011) The *stb* operon balances the requirements for vegetative stability and conjugative transfer of plasmid R388. *PLoS Genet* 7(5):e1002073.
- P08** Ton-Hoang B., Pasternak C., Siguier P., Guynet C., Hickman A.B., Dyda F., Sommer S., Chandler M. (2010) Single-stranded DNA transposition is coupled to host replication. *Cell* 142(3):398-408.
- P07** Guynet C., Achard A., Ton-Hoang B., Barabas O., Hickman A.B., Dyda F., Chandler M. (2009) Resetting the site: redirecting integration of an insertion sequence in a predictable way. *Mol Cell* 34(5):612-9.
- P06** Guynet C., Hickman A.B., Barabas O., Dyda F., Chandler M. and Ton-Hoang B. (2008) *In Vitro* Reconstitution of a Single-Stranded Transposition Mechanism of IS608. *Mol Cell* 29:1-11.
- P05** Barabas O., Ronning D.R.<sup>+</sup>, Guynet C.<sup>+</sup>, Hickman A.B., Ton-Hoang B., Chandler M. and Dyda F. (2008) Mechanism of IS200/IS605 Family DNA Transposases: Activation and Transposon-Directed Target Site Selection. *Cell* 132, 208-220. <sup>+</sup> contributed equally to the work.
- P04** Blanvillain S., Meyer D., Boulanger A., Lautier M., Guynet C., Denance N., Vasse J., Lauber E. and Arlat M. (2007) Plant Carbohydrate Scavenging through TonB-Dependent Receptors: A Feature Shared by Phytopathogenic and Aquatic Bacteria. *PLoS ONE.*: 2:e224.
- P03** Rousseau P., Loot C., Guynet C., Ah-Sang, Y., Ton-Hoang, B. and Chandler M. (2007) Control of IS911 target selection: How OrfA may ensure IS dispersion. *Mol. Microbiol.* 63 (6), 1701–1709.
- P02** Ronning D.R., Guynet C., Ton-Hoang, B., Perez, Z., Ghirlando, G., Chandler, M. and Dyda, F. (2005) Active site sharing and synaptic assembly in a new class of DNA transposases. *Mol Cell.*: 20(1):143-54.
- P01** Ton-Hoang, B., Guynet C., Ronning, D.R., Cointin-Marty, B., Dyda, F., and Chandler, M. (2005) Transposition of ISHp608, member of a novel family of bacterial insertion sequences. *EMBO J.*:24(18):3325-38.

## THESIS

Guynet C. (2008) Etude du mécanisme de transposition de la Séquence d'Insertion bactérienne IS608. Thesis from Paul Sabatier University (<http://theseups.ups-tlse.fr/415/>)









