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Review



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The metabolic control of DNA replication: mechanism and function

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Metabolism and DNA replication are the two most fundamental biological functions in life. The catabolic branch of metabolism breaks down nutrients to produce energy and precursors used by the anabolic branch of metabolism to synthesize macromolecules. DNA replication consumes energy and precursors for faithfully copying genomes, propagating the genetic material from generation to generation. We have exquisite understanding of the mechanisms that underpin and regulate these two biological functions. However, the molecular mechanism coordinating replication to metabolism and its biological function remains mostly unknown. Understanding how and why living organisms respond to fluctuating nutritional stimuli through cell-cycle dynamic changes and reproducibly and distinctly temporalize DNA synthesis in a wide-range of growth conditions is important, with wider implications across all domains of life. After summarizing the seminal studies that founded the concept of the metabolic control of replication, we review data linking metabolism to replication from bacteria to humans. Molecular insights underpinning these links are then presented to propose that the metabolic control of replication uses signalling systems gearing metabolome homeostasis to orchestrate replication temporalization. The remarkable replication phenotypes found in mutants of this control highlight its importance in replication regulation and potentially genetic stability and tumorigenesis.

1. Background

Complex mechanochemical molecular machines, known as replisomes, carry out genome replication for ensuring faithful genome duplication by accurately synthesizing two identical copies of the parental DNA. Replisomes assemble at specific replication initiation sites known as origins, from where they initiate and carry out DNA synthesis. To ensure that chromosomes replicate once per cell cycle, overlapping regulatory mechanisms tightly control the initiation phase of replication and prevent unscheduled re-initiation events [1–3]. Failures in this control increase the risk of replication errors and double-stranded DNA breaks, compromising genetic stability and cell viability, and eventually causing diseases like cancer [4–7].

Since Schaechter's seminal studies in *Salmonella typhimurium* in 1958, it is now well established that replication is under metabolic control (this process is termed metabolic control of replication, MCR). In bacteria, this process impacts both the initiation and elongation phases of replication, resulting in a reproducible temporal compartmentalization of DNA synthesis in the cell cycle in a wide range of nutritional conditions and growth rates [8–12]. This system depends on the energy and precursors derived from nutrients rather than on the actual nature of the carbon and nitrogen sources, as *Escherichia coli* cells growing at the same rate in different media exhibit similar temporal replication patterns [9,11]. In lower eukaryotes, the compartmentalization of

replication (S phase) also varies with growth and nutritional conditions [13,14]. Moreover, the cell cycle from yeast to human cells exhibits an autonomous metabolic/redox oscillation, and the entry and progression of the S phase occur at the most reductive phase of this cycle [15–17]. The metabolic oscillation also triggers global changes in the metabolome, including central carbon pathways [18–27]. Interestingly, metabolic oscillation may also be an intrinsic behaviour of bacteria as the cell cycle of *Caulobacter crescentus* is underpinned by robust metabolite fluctuations [28].

Soon after the Schaechter's seminal studies, MCR was assumed to be geared by a passive mechanism involving limiting factors. Initially, the pools of nucleotide precursors that increase with growth rate was proposed to directly determine the activity of replication enzymes and the rate of DNA synthesis. Although appealing, this hypothesis is challenged by data showing that artificial changes (balanced or imbalanced) in nucleotide pools increase replication errors, double-strand DNA breaks and ultimately genetic instability [4]. Subsequently, the ATP-bound form of the initiator DnaA that peaks near the time of initiation was proposed to be the limiting factor of MCR in bacteria [29]. However, this hypothesis is questioned by data showing that the timing of initiation poorly correlates to the amount of ATP-DnaA (or DnaA) per origin [30–35]. Hence, although ATP-DnaA is the motor of initiation, other factors are needed for proper temporalization of initiation with respect to nutritional conditions. Since the 2000s, MCR is thought to involve sophisticated signalling pathways that chemically sense the cellular metabolic state for temporalizing DNA synthesis in a broad range of nutritional conditions [36–40]. In this model, MCR continuously adjusts the size of precursor pools to the rate of DNA synthesis, preventing aberrant mutagenic feeding of replication enzymes.

The catabolic branch of metabolism, also called central carbon metabolism (CCM), encompasses approximately 40 ancestral, highly conserved enzymes organized in pathways (figure 1) [41,42]. It extracts from nutrients the precursors and energy needed for macromolecular synthesis and biomass production. By sensing the supply and demand in biosynthetic reactions, it also orchestrates metabolome homeostasis [43–45]. Because of its strategic position, CCM was proposed to play an important role in MCR. This hypothesis is supported by an ever-increasing body of data in both prokaryotic and eukaryotic cells linking CCM to replication (see below and figure 1 for a summary) and showing that CCM determinants often operate as signalling and sensor molecules ensuring moonlighting functions in non-metabolic activities [46]. Note that in addition to MCR, two systems are proposed to couple replication to growth. They involve molecules whose concentration reflects the metabolic state of the cell: the nucleotide analogues guanosine tetra- and penta-phosphate [47] (see however [34,48]) and reactive oxygen species [27,49–54].

2. CCM-replication links

In bacteria, replication initiation involves assembly of multiple copies of the initiator protein DnaA at the origin (*oriC*) to form a nucleoprotein complex that triggers origin unwinding, replicative helicase loading, replisome assembly and replication firing [55,56]. Two CCM metabolites were suggested to modulate this replication step in *E. coli*: acetyl-

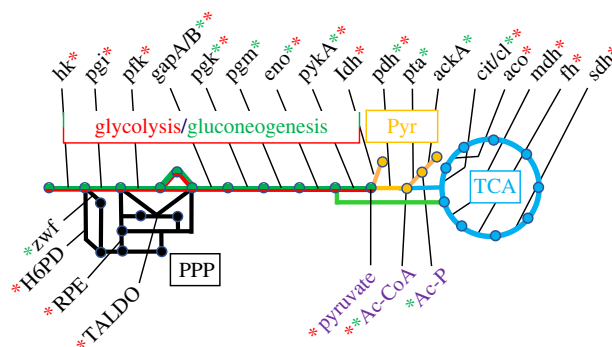


Figure 1. Schematic of central carbon metabolism and its main links to replication. Coloured lines and labels stand for glycolysis (red), gluconeogenesis (green), tricarboxylic acid cycle (TCA) (blue), pentose phosphate pathway (PPP) (dark), pyruvate metabolism (Pyr) (orange) and metabolites (violet). Key metabolic determinants (genes/enzymes and metabolites) connected to DNA replication in bacteria (green star) or in eukaryotes (red star) are listed using the following abbreviations: hk, hexokinase; pgi, glucose 6-phosphate isomerase; pfk, phosphofructokinase; gapA, glyceraldehyde 3-phosphate dehydrogenase, NAD-dependent; gapB, glyceraldehyde 3-phosphate dehydrogenase, NADP-dependent; pgk, phosphoglycerate kinase; pgm, phosphoglycerate mutase; eno, enolase; pykA, pyruvate kinase; ldh, lactate dehydrogenase; pta, phosphotransacetylase; ackA, acetate kinase; cit, citrate synthase; cl, ATP-citrate lyase; aco, aconitase; mdh, malate dehydrogenase; fh, fumarase; sdh, succinate dehydrogenase; zwf, glucose 6-phosphate dehydrogenase; H6pD, hexo-6-phosphate pyrophosphokinase; RPE, ribulose 5-phosphate epimerase; TALDO, transaldolase. Abbreviations of metabolites are as follows: Ac-CoA, acetyl-CoA; Ac-P, acetyl-phosphate.

CoA and acetyl-phosphate. These metabolites acetylate DnaA through an acetyltransferase (YfiQ) or non-enzymatically, respectively, collectively inhibiting ATP and ADP binding to DnaA and impeding formation of the active DnaA-origin nucleoprotein complex [57]. DnaA acetylation is removed by the deacetylase CobB. Although DnaA acetylation/deacetylation is documented *in vivo* and *in vitro*, its exact contribution to initiation regulation in steady-state growing cells remains uncertain, as DnaA acetylation is so far only known to peak at the stationary phase compared to the exponential growth phase. Interestingly, another study links the acetylation potential to DnaA activity. In that work, a general decrease in protein acetylation induced by CCM mutations depleting the pools of acetyl-CoA and acetyl-phosphate or by deleting the major acetyltransferase *yfiQ* was found to suppress initiation defects of a DnaA mutant (*dna46*) [58]. Despite this, the authors were unable to conclude on the role of DnaA acetylation in initiation regulation in exponentially growing cells, as suppression still occurs in conditions of general increase in acetylation (in *cobB* knockout cells). Instead, they proposed various scenarios where initiation is modulated by other metabolic determinants impacting formation of the DnaA-origin nucleoprotein complex. One such scenario may involve cyclic AMP which interacts *in vitro* with DnaA and promotes the production of the active DnaA-ATP form and its binding to the replication origin [59].

In addition to metabolites, key CCM enzymes are also involved in replication. In *Bacillus subtilis*, several catabolic enzymes are linked to three replication proteins essential for the initiation and elongation phases of replication: the DnaC helicase, the DnaG primase and the lagging strand

DNA polymerase DnaE. These proteins are loaded early at the origin during initiation, and may form a ternary complex in the replisome to carry out DNA melting, RNA priming and lagging strand synthesis during DNA polymerization [60,61]. The first group of CCM enzymes important for replication are subunits of the pyruvate dehydrogenase PDH and related enzymes. They interact with DnaC, DnaG and the origin region and inhibit initiation [62–65]. Recently, our laboratories reported that the pyruvate kinase PykA modulates *in vitro* the activities of DnaC, DnaG and DnaE, and that the catalytic (CAT) domain of PykA interacts with DnaE when the polymerase is bound to primed DNA templates [66,67]. *In vivo*, PykA impacts replication in two ways: the CAT domain stimulates elongation while the C-terminal PEPut (phosphoenolpyruvate utilizer) domain inhibits initiation. Interestingly, these phenotypes are independent from PykA catalytic activity and occur in conditions where the enzyme is dispensable for growth (e.g. in a gluconeogenic medium) and does not significantly contribute to the metabolome. Therefore, the replication defects in PykA mutants are a direct consequence of amino acid residue changes rather than an indirect consequence of changes in cellular metabolism. It was thus proposed that PykA typifies a new family of replication control factors that gears MCR [66].

Genetic studies have been carried out to investigate the CCM-replication links more comprehensively. In *B. subtilis*, these studies uncovered a toolbox of nutrient-dependent activated CCM-replication links important for replication temporalization. This toolbox comprises on one side, CCM reactions ensuring the 3C part of glycolysis and reactions of the downstream pyruvate metabolism, and on the other side, the replication enzymes DnaC, DnaG and DnaE. Mutations in this CCM area suppress replication defects in *dnaC*, *dnaG* and *dnaE* (but not in *dnaI*, *dnaD*, *polC*, *dnaX* and *dnaN*) conditional mutants through a process involving conformational changes in replication enzymes [68]. These mutations also alter initiation and elongation in a medium-dependent manner and disturb the metabolic control of replication [34,69]. Similar links were described in *E. coli* [70–72] and possibly *C. crescentus* [73].

In eukaryotes, CCM determinants (proteins and metabolites) found in the nucleus [74–76] often exert moonlighting replication functions. For instance, the timing of origin firing depends on histone acetylation promoted by an increase in nuclear acetyl-CoA. This increase is geared by the redox metabolic cycle in yeast, and by nuclear forms of the ATP-citrate lyase and PDH complexes in mammalian cells [77–80]. In addition, the nuclear glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) in human cells are integral parts of the transcription cofactor OCA-S [81,82]. In response to the local NAD⁺/NADH redox status, these metabolic enzymes control the association of OCA-S with the transcription factor Oct-1 for regulating histone H2B expression and S phase progression. Moreover, core histone gene expression and S phase entry are impeded by exogenous pyruvate [83] and chromatin and chromatin factors are important for origin selection, fork rate optimization and lagging strand priming [84–86]. Collectively, these data suggest that CCM metabolites and enzymes playing fundamental role in histone biology and gene expression [75,87] also ensure important MCR functions. However, eukaryote MCR may also operate through histone-independent pathways. Indeed, the phosphoglycerate kinase PGK interacts

with the protein kinase CDC7 to stimulate replication initiation by enhancing the CDC7-mediated activation of the replicative MCM helicase in human cells [88]. Also, mammalian LDH, PGK and GAPDH modulate the activity of the replicative polymerases Pol α , Pol ϵ and Pol δ *in vitro* [89–91]. We may also note here that impaired expression of CCM genes delays the entry of human fibroblasts into the S phase or decreases the number of cells in this phase [92–94]. Collectively, these data show that CCM impacts both the initiation and elongation phases of replication from bacteria to humans.

3. Basic principles of MCR

Here, we propose that MCR is orchestrated by an active process organized as a toolbox of overlapping mechanisms differently activated in response to nutritional stimuli. In this toolbox, signalling metabolites and cognate CCM proteins convey metabolic information to enzymes of the initiation and elongation replication machineries for coupling replication temporalization to metabolism (figure 2). The transmission of metabolic information is thought to involve sophisticated (indirect) mechanisms (e.g. MCM helicase activation by PGK via CDC7 [88]; initiation firing by acetyl-CoA via histone acetylation [77–80]), although simpler (direct) mechanisms could also operate (e.g. cAMP binding to DnaA [59]). Interestingly, MCR appears to have common roots with metabolism regulation, as the CCM determinants committed to replication often continuously signal and sense metabolic fluctuations for achieving metabolome homeostasis. Hence, our MCR model implies that allosteric regulation of CCM enzymes by signalling metabolites coordinates simultaneously metabolome homeostasis and replication temporalization. The numerous CCM-replication links described above potentially highlight the diversity of these coordinating systems and similar mechanisms may be at play for integrating many cellular behaviours to metabolism [46]. Molecular insights in the *B. subtilis* PykA-driven MCR are presented below.

4. PykA-driven MCR in *B. subtilis*

4.1. PykA allosteric regulation

Pyruvate kinases are ancestral, highly conserved homotetramers that carry out the last reaction of glycolysis and anaplerotic activities in gluconeogenic conditions. Almost all pyruvate kinases are homotropically activated by the substrate phosphoenolpyruvate and allosterically regulated by heterotrophic effectors [95]. The mechanism of allosteric regulation involves conformational changes within each subunit and between neighbouring subunits for stabilizing the protein in the metabolically active R-state conformation [95,96]. As mentioned above, the *B. subtilis* PykA protein comprises a CAT (catalytic) domain containing the substrate and effector binding sites, and a PEPut domain. This latter domain is highly similar to the PEP utilizer domain of pyruvate orthophosphate dikinase and other metabolic enzymes [97]. In these enzymes, the PEP utilizer moves around a flexible arm [98,99] and is phosphorylated at a conserved TSH motif at the expense of phosphoenolpyruvate and ATP to drive sugar imports and catalytic or regulatory activities [98,100–106]. The PEPut domain of PykA is not required for

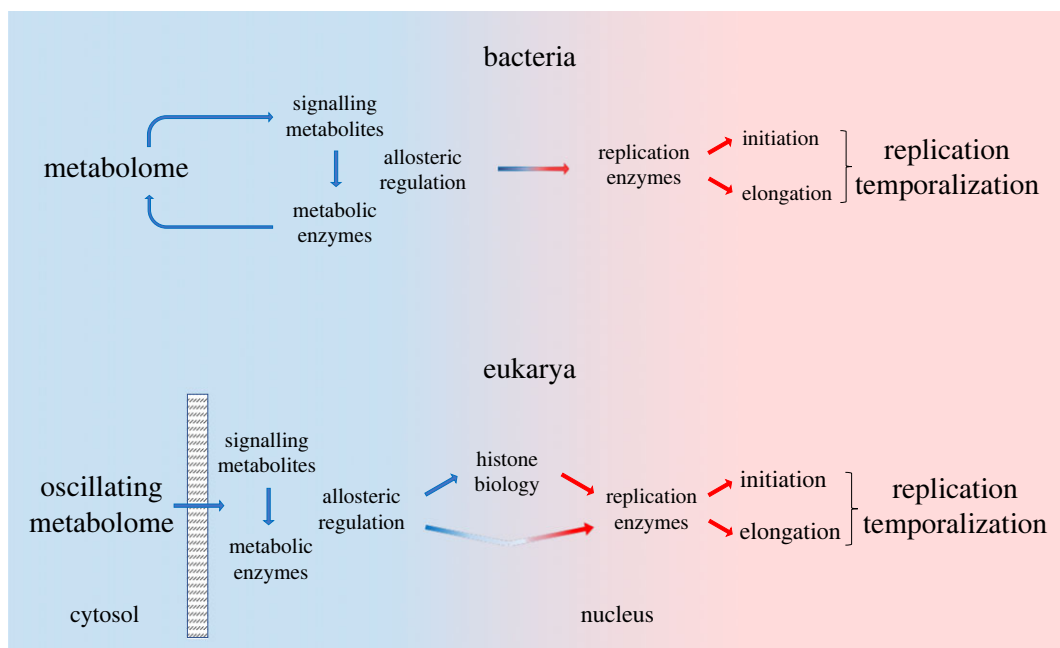


Figure 2. Basic principles of the metabolic control of replication. (a) Model for bacteria. CCM signalling metabolites allosterically regulate CCM enzymes for adjusting the metabolome to the energy afforded by available nutrients. The information contained in signalling metabolites and metabolic enzyme conformers is then conveyed to replication enzymes for regulating initiation and elongation and temporalizing replication in the cell cycle in a broad range of nutritional conditions. The transmission of information may involve physical interactions between CCM determinants and replication enzymes. (b) Model for eukaryotes. The cellular metabolism oscillates spontaneously and regulates cell cycle progression. Nuclear signalling metabolites and CCM enzymes sense and convey this information for modulating accordingly replication enzyme activity and replication temporalization through direct (physical interactions between CCM determinants and replication proteins) or indirect (through histone biology) pathways. The dashed box represents the nuclear membrane.

the catalytic activity of the enzyme [67,107]. However, it interacts with CAT via a hydrogen bond between E208 (CAT) and L536 (PEPut), as shown in a structural study of the highly homologous (72.5% identity) *Geobacillus stearothermophilus* PykA [108]. In *B. subtilis*, this interaction seems to stimulate PykA activity and to be negatively regulated by phosphorylation of the T residue of the TSH motif which maps immediately downstream of the interacting L536 amino acid (residues 537–539) [66,67]. An AlphaFold2 analysis predicts two *B. subtilis* PykA monomer structures with a highly similar spatial CAT domain and alternative PEPut positions (figure 3, top left panel) [109,110], indicating that PEPut is carried by a flexible arm, as in other PEP utilizer containing metabolic enzymes [98,99]. The tetrameric structures built from these monomeric predictions also mostly differ in the PEPut position (figure 3, middle and right panels): this peptide faces the A' domain of CAT of another subunit in the left configuration while it is directed outward of CAT in the right configuration. A close-up view (figure 3, inset) of the PEPut-A' region of the left structure suggests a H-bond interaction between L536 (PEPut) and E208' (A'), as expected from crystallography studies [108]. Hence, the AlphaFold2 study predicted two conformers of *B. subtilis* PykA tetramers in which PEPut interacts (left configuration) or not (right configuration) with CAT. These configurations are proposed to be related to the classical R- and T-state of PykA conformers, respectively [96]. The proximity of T537 with the E208'-L536 H-bond (figure 3, inset) suggests that T537 phosphorylation inhibits the CAT-PEPut interaction, R-state conformer formation and PykA activity by bringing positive charges at the CAT-PEPut interface. We infer from these results that the CAT-PEPut interaction assists the heterotrophic effectors (AMP and ribose 5-phosphate [111]) in stabilizing the active

R-state conformer and that this stimulation is negatively regulated by T537 phosphorylation [66,67,96]. Hence, PykA is a metabolic sensor that resides in individual cells in different conformations at levels depending on ligand (PEP, AMP and ribose 5-phosphate) concentrations and kinase activities that control PEPut phosphorylation and the CAT-PEPut interaction to provide the active R-state conformer at a concentration sufficient for biosynthetic needs and metabolome homeostasis (figure 4, left panel). We foresee that the pattern of PykA conformers is used by cells for dynamically characterizing nutritional conditions.

4.2. PykA-driven MCR

In addition to being a key regulator of PykA catalytic activity and metabolome homeostasis, PEPut operates as a negative regulator of replication initiation in gluconeogenic growth conditions [66]. Interestingly, this function strongly depends on determinants involved in PykA conformers regulation, namely the CAT-PEPut interaction and T537 phosphorylation. When these determinants favour formation of the R-state conformer (i.e. when PEPut efficiently interacts with CAT), a wild-type initiation activity is found while cells over-initiate in conditions favouring the accumulation of the T-state conformer (i.e. when the CAT-PEPut interaction is impeded) (figure 4, right panel). In addition to these distinct effects of PykA conformers on initiation, it was shown that (i) PykA physically interacts with the polymerase DnaE via its CAT domain when the replication enzyme is bound to primed DNA [67], (ii) PEPut modulates the strength of this interaction and its effect on the DnaE polymerase activity [66,67], and (iii) DnaE is early recruited at replication origins during initiation [61]. We thus suggest from these data that

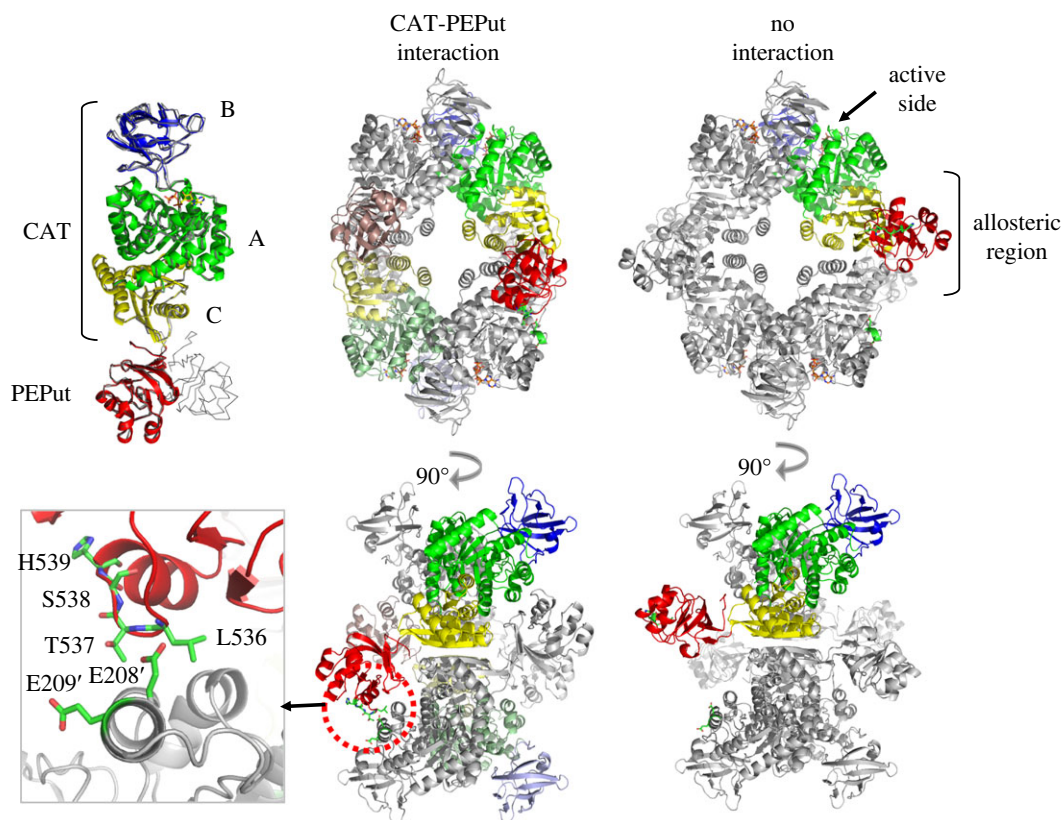


Figure 3. AlphaFold2 analysis of *B. subtilis* PyKA conformers. Top left panel: AlphaFold2 predicted structure (ranked 0) of the PyKA monomer coloured to highlight the A, B and C domains of the catalytic region (CAT) and PEPut. A monomer with a lower ranking (19) is superposed as a cartoon onto the coloured structure. Both structures are highly similar, differing mostly by an alternative PEPut position. Right panel: tetrameric structures built from the ranked 0 (leftward configuration) and ranked 19 (rightward configuration) monomer predictions (two views of each tetramer are provided). They mostly differ in the PEPut position. Inlet: Close-up view of the PEPut-A' region of the left tetrameric structure suggesting a H-bond interaction between L536 (PEPut) and E208' (A).

the MCR initiation depends on the effect of PyKA conformers on DnaE activity during initiation. Although different mechanisms can be envisioned at this stage of our knowledge, we tentatively propose a model in which only the R-state conformer interacts with DnaE at initiation sites for reducing the DnaE polymerase activity and preventing early initiation (figure 4, right panel). In an alternative model (not shown), both conformers are recruited at initiation sites by DnaE. However, while the T-state conformer dramatically increases DnaE activity and causes early initiation, the R-state conformer allows a proper polymerase activity and initiation timing. Together, these results suggest that the signalling system gearing the balance between the R- and T-state PyKA conformers controls simultaneously and dynamically PyKA activity, metabolome homeostasis and MCR initiation. However, although cells allosterically regulate PyKA catalytic activity in a broad range of nutritional conditions, the PEPut-driven initiation function of PyKA is active in gluconeogenic conditions but inactive in glycolytic media [66]. This suggests that the initiation function of PyKA conformers depends on the polarity of the carbon flux travelling CCM and that cells temporalize initiation through different mechanisms in glycolytic and gluconeogenic conditions.

As mentioned above, CAT stimulates DNA elongation in gluconeogenic growth conditions and this moonlighting activity depends on substrates (ADP and phosphoenolpyruvate) binding but not on PyKA catalytic activity [66]. Moreover, CAT physically interacts with DnaE when the polymerase is bound to primed DNA and DnaE is frequently recruited to and released from replication forks to initiate

DNA synthesis from the RNA primers produced during lagging strand synthesis [60,112–114]. Together, these results suggest that PyKA is repeatedly recruited by DnaE in the replisome, allowing a highly dynamic transmission of the metabolic information contained in PyKA conformers to replication forks. By modulating DnaE activity and fork speed, this system is proposed to gear the MCR elongation.

5. Conclusion and perspectives

In both prokaryotic and eukaryotic cells, CCM does more than simply producing precursors and energy for anabolic reactions. It also drives fundamental biological processes. To do so, many CCM metabolites and enzymes operate as signalling and sensor molecules that not only carry out metabolome homeostasis but also ensure moonlighting functions in non-metabolic activities [46]. DNA synthesis is demanding in energy and precursors. It must also be completed once initiated to prevent DNA damage that risk cell survival and health. It is therefore not surprising that DNA replication is under a metabolic control. The ever-increasing number of reports since the 1960s describing the impact of nutritional conditions on DNA replication temporalization and tight links between CCM and replication from bacteria to humans highlights the importance of such a control in all living organisms. However, despite significant efforts, the molecular mechanism and biological function of MCR remain largely unknown. This is likely due to the fact that this control involves a toolbox of complex and overlapping processes

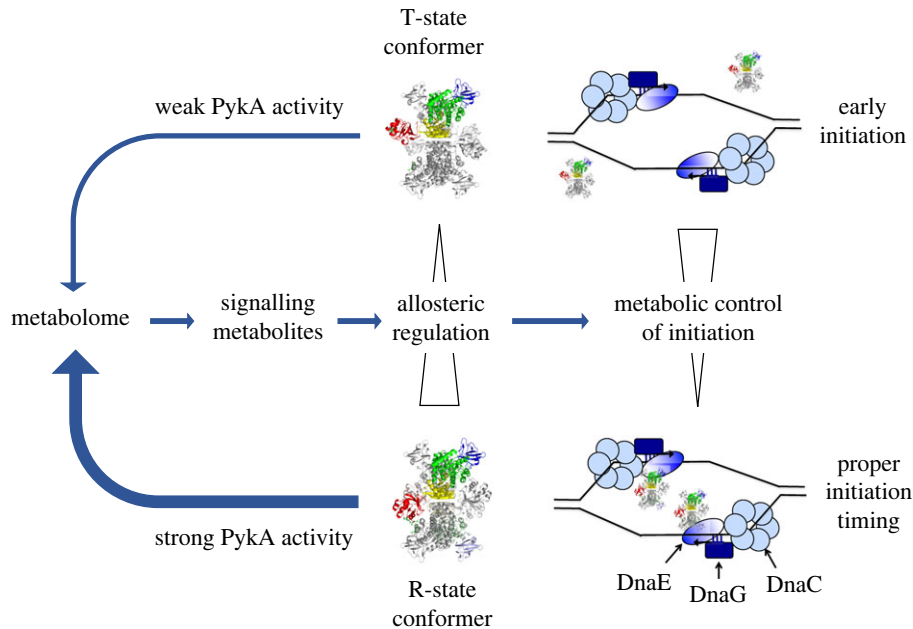


Figure 4. Roles of PykA conformers in metabolome homeostasis and replication compartmentalization. Left panel: CCM signalling metabolites allosterically regulate the ratio between the active (R-state) versus inactive (T-state) PykA conformers for achieving metabolome homeostasis. Right panel: the R-state PykA conformer is recruited by DnaE bound to primed DNA at initiation sites for ensuring a wild-type initiation timing. The T-state-conformer does not interact with DnaE at initiation sites allowing early initiation firing.

differently activated in response to nutritional stimuli. Because of their genetic tractability, relative cellular simplicity and robust cell cycle response to nutrient fluctuations, bacteria are attractive models for unraveling the mechanism and function of MCR. The recent discovery that side chain mutations in the *B. subtilis* PykA dramatically impact replication temporalization independently of their effect on PykA catalytic activity and cellular metabolome validates this notion and paves the way for further studies in this bacterium as well as in distantly related organisms. Insights from this and other organisms suggest that (i) CCM elements that signal and sense the cellular metabolic state for ensuring metabolome homeostasis are key players in MCR, and that (ii) the metabolic information contained in signalling molecules is conveyed to the replication machinery through protein-protein interactions physically connecting metabolic and replication enzymes and/or by inducing conformational changes in histone proteins via post-translational modifications. We foresee that the CCM-replication links discussed above highlight new PykA-like multifunctional regulators from bacteria to humans. Finally, it is interesting to note here that the MCR model proposed here suggests that DNA replication depends on a few key signalling metabolites in addition to the well-characterized classical replication control functions [1–3].

Surprisingly, *B. subtilis* mutants of MCR suffer from profound initiation and elongation defects, causing dramatic changes in replication compartmentalization and cell cycle [66]. As these phenotypes occur in cells fully proficient in ‘classical’ replication control functions [1–3], these results show that MCR plays an important role in DNA synthesis and that the control geared by ‘classical’ replication functions is not sufficient to properly time DNA synthesis in a broad range of nutritional conditions. Regulation of DNA replication is of prime importance for faithful propagation of the genetic material to daughter cells. Failures in this control cause chromosomal lesions (double-strand DNA breaks, nucleotide misincorporations) with a high mutagenic potential that increases the risk of genetic diseases like

cancer [4–7]. Towards the beginning of the twentieth century, it was shown that cancer cells exhibit chromosomal abnormalities, elevated glucose uptake and accelerated glycolysis flux to lactate even in the presence of sufficient oxygen (Warburg effect). We know now that genome instability and altered metabolism are two hallmarks of cancer [115]. Genome instability has been investigated continuously since its discovery leading to the proposal that it is due to DNA replication stresses caused by deregulation of initiation and fork speed. A large core of data shows that these replication abnormalities are induced by tumorigenesis drivers like oncogenes, tumour suppressors and/or mutator genes [116–119]. On the other side, altered metabolism has been rejuvenated as a research area only recently and since then, it was shown that the Warburg effect is a crucial component of malignancy that satisfies bioenergetic and biosynthetic demands, mitigates oxidative stress and contributes to cell transformation and metastasis [120,121]. Nowadays, cancer is thought to originate from mutations caused by replication stresses, and other hallmarks of cancer, including the altered metabolism, are thought to be a consequence of these mutations rather than the cause [116,122,123]. However, recent studies have shown that altered metabolism impacts genetic stability. Indeed, mutations in CCM enzymes and changes in the level of CCM metabolites were shown to directly cause mutagenic DNA damage, impede DNA repair and trigger nucleotide imbalance [124–126]. Moreover, CCM mutations disrupting MCR may cause replication phenotypes known to increase genetic instability, and alteration of the synchrony between the reductive stage of the metabolic cycle and the S phase in yeast increases the rate of spontaneous mutagenesis [66,127]. Collectively, these data suggest that CCM changes underpinning the Warburg effect may be an additional root cause of genetic instability and cancer initiation. It is of interest to note here that the past two decades have shown that many metabolites produced by the gut microbiome of mammals enter the bloodstream, impacting the blood metabolome and the

chemical environment of tissues and host cells [128–130]. In humans, this transport is a major determinant of blood metabolite variability and human health [131–135]. Moreover, the cross-talk between bacterial metabolites, histone biology and cancer highlights the links between altered microbiomes (dysbiosis) and intracellular pools of chemicals involved in DNA damage, DNA repair and potentially MCR [136–138]. Finally, it is becoming increasingly clear that metabolism plays an active role through moonlighting functions in cellular processes as diverse as transcription, posttranslational modifications, cell division, apoptosis, pathogenicity, innate immunity and development [22,36,74–76,139–147]. Therefore, CCM may provide a toolbox of links connecting determinants gearing metabolome homeostasis to a wide range of cellular processes in addition to DNA replication. Future efforts should focus on revealing the extent and mechanisms by which these determinants may actively drive and tune main cellular functions.

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All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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