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# H-NS-like nucleoid-associated proteins, mobile genetic elements and horizontal gene transfer in bacteria

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1 H-NS-like nucleoid-associated proteins, mobile genetic elements and  
2 horizontal gene transfer in bacteria

3

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12

13

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16 Horizontal gene transfer

17 H-NS

18 IncHI plasmids

19 Nucleoid associated proteins

20 StpA

21 Thermoregulation

22 Transcription silencing

23 ABSTRACT

24

25 Horizontal gene transfer plays an important role in the evolution of bacterial  
26 species, conferring new genetic traits on the recipient bacterium that extend its  
27 range of phenotypes and plasmids make important contributions to this process.  
28 However, the inappropriate expression of newly acquired genes may lead to a  
29 loss of competitive fitness, resulting in the elimination of the new gene-  
30 bacterium combination. It is thought that transcriptional silencing of  
31 horizontally acquired genes offers a route out of this dilemma and that nucleoid-  
32 associated proteins, especially those related to the H-NS protein, play a  
33 particularly important role in the silencing process. The discovery that many  
34 plasmids express orthologues of nucleoid-associated proteins adds an  
35 interesting dimension to current models of regulatory integration following  
36 lateral transfer of DNA. Other horizontally acquired genetic elements, such as  
37 genomic islands, also express nucleoid-associated proteins of their own. Here the  
38 interactions of H-NS-like nucleoid-associated proteins encoded by the core  
39 genome, genomic islands and plasmids are described.

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48	<b>Contents</b>
49	
50	1. Introduction
51	2. Nucleoid associated proteins
52	3. H-NS-like NAPs encoded by horizontally acquired genetic elements
53	4. H-NS-like proteins and thermal adaptation
54	5. Plasmid conjugation control by H-NS
55	6. Plasmid- and chromosome-encoded H-NS orthologues: points of distinction
56	7. Hha-like proteins and H-NS target specificity
57	8. Concluding remarks
58	Acknowledgements
59	References
60	

## 61 **1. Introduction**

62

63 Horizontal gene transfer (HGT) occurs in bacterial populations through  
64 transformation (the direct uptake of naked DNA by a competent bacterium),  
65 transduction (a process that is mediated by a bacteriophage) and conjugation  
66 (the transfer of a self-transmissible plasmid or the mobilisation of one plasmid  
67 by the transfer apparatus of a second) (Dobrindt et al., 2010; Domingues et al.,  
68 2012; Dorman, 2009a; Fortier and Sekulovic, 2013; McGinty and Rankin, 2012;  
69 Smillie et al., 2010; Wozniak and Waldor, 2010). In principle, all three  
70 mechanisms of HGT can involve plasmids. Those genes that have been acquired  
71 by HGT must be replicated if they are to be transmitted vertically in the bacterial  
72 population. Replication can be achieved straightforwardly if the genes are part of  
73 a self-replicating element such as a plasmid. Replication of multicopy plasmids  
74 occurs in parallel with, but largely independently of, that of the chromosome,  
75 relying on stochastic processes to ensure segregation at cell division (Reyes-  
76 Lamothe et al., 2014). Low copy number plasmids possess elaborate machinery  
77 to ensure their replication and segregation is coordinated with cell division so  
78 that daughter cells inherit plasmid copies with high fidelity (Bahl et al., 2009;  
79 Ebersbach and Gerdes, 2005; Gerdes et al., 2010; Ghosh et al., 2006; Hayes and  
80 Barilla, 2006; Pinto et al., 2012; Schumacher, 2012). Toxin/antitoxin systems act  
81 to eliminate plasmid-free daughter cells should these arise, ensuring the future  
82 presence of plasmid-carriers in the population (Schuster and Bertram 2013;  
83 Unterholzner et al., 2013).

84 If the genes acquired by HGT become part of the chromosome they will be  
85 replicated with that molecule, ensuring their vertical transmission. However,

86 these *physically* integrated genes share with those on autonomously replicating  
87 plasmids the problem of *regulatory* integration. How is the expression of these  
88 genetic newcomers to be controlled so that the new cell-gene combination is not  
89 compromised at the level of competitive fitness? In the case of genes that become  
90 part of the chromosome one must also consider the problem of their physical  
91 placement within the architecture of the nucleoid (Dorman, 2013). The presence  
92 in bacterial chromosomes of very large clusters of genes (genomic islands) that  
93 have been acquired by HGT shows that these problems can be overcome, but  
94 what are the rules that underlie the solutions? It seems likely that nucleoid  
95 associated proteins (NAPs) are at least part of the solution both to the regulatory  
96 and to the physical integration problems that occur following HGT. This is  
97 possible due to their lack of DNA sequence specificity for binding that allows  
98 NAPs to target a wider range of newly arrived sequences than a conventional  
99 protein with a strict preference for a particular DNA binding consensus  
100 sequence. It also arises in part from the ability of some NAPs to silence  
101 transcription, buying time for the process of gene regulatory integration to  
102 proceed (Dorman, 2007; Fang and Rimsky, 2008; Navarre et al., 2007).

103

## 104 **2. Nucleoid associated proteins**

105

106 NAPs play key roles in bacterial physiology by regulating the expression of large  
107 numbers of genes and by contributing to the structure of the nucleoid. Even the  
108 simplest of cellular microbial genomes encode at least one NAP (Dorman, 2011;  
109 Zhang and Baseman, 2011) and many bacteria express a variety (Browning et al.,  
110 2010; Dame, 2005; Dillon and Dorman, 2010; Dorman, 2009b; Rimsky and

111 Travers, 2011; Teramoto et al., 2010). The H-NS protein is a NAP that has been  
112 studied in great detail in the context of HGT but it is just one of over a dozen  
113 NAPs that can be found in the model bacterium *Escherichia coli* (Azam and  
114 Ishihama, 1999; Dillon and Dorman, 2010).

115

### 116 *2.1. The H-NS protein*

117 *E. coli* contains about 20,000 copies of H-NS, a small dimeric DNA binding protein  
118 that is encoded by the *hns* gene in the Ter macrodomain of the chromosome. H-  
119 NS binds non-specifically to DNA and has a preference for DNA with a high A+T  
120 content (Dorman, 2004; Fang and Rimsky, 2008). The sequences of its preferred  
121 binding sites contain the TpA step, lending to those DNA elements a degree of  
122 flexibility that may be important for the higher order structures that characterise  
123 H-NS nucleoprotein complexes (Bouffartigues et al., 2007; Fang and Rimsky,  
124 2008; Lang et al., 2007). DNA sequences that meet the requirements of preferred  
125 binding sites are typically found at transcription promoters and throughout  
126 HGT-acquired xenogeneic DNA elements (Lucchini et al., 2006; Navarre et al.,  
127 2006; Oshima et al., 2006). These sequences are binding-and-nucleation sites  
128 from which more extensive H-NS-DNA complexes can grow. This NAP has two  
129 binding modes on DNA. In one, H-NS binds to its nucleation site and then  
130 polymerises along the DNA, creating a coating that interferes with the binding of  
131 other proteins, including RNA polymerase (Liu et al., 2010). In the second mode,  
132 H-NS creates bridges between two DNA sequences (or two segments of the same  
133 DNA molecule) (Dame et al., 2006; Maurer et al., 2009). It can do this because  
134 each H-NS dimer has two DNA binding domains and the flexible connector that  
135 links each of these to its dimerization domain facilitates DNA-protein-DNA

136 bridge formation (Arold et al., 2010; Dorman et al., 1999; Fernandez-de-Alba et  
137 al., 2013) (Figs. 1 and 2). Each binding mode influences nucleoid architecture, at  
138 least locally, and each can also interfere with transcription (Rimsky et al., 2001).  
139 Transitions between these binding modes are thought to be sensitive to  
140 magnesium ion concentrations (Liu et al., 2010). Any gene arriving by HGT in a  
141 bacterium expressing an H-NS-like protein may have its expression silenced if it  
142 presents an appropriate DNA structure to which the protein can bind. The  
143 derepression of the silenced genes can be achieved by myriad mechanisms. Some  
144 of these rely on alterations to the shape of the DNA that are incompatible with  
145 bridge maintenance, others involve the binding of proteins that compete with H-  
146 NS or have binding modes that interfere with bridge stability (Stoebel et al.,  
147 2008). It seems that *ad hoc* solutions to the problem of H-NS-mediated  
148 transcription silencing can evolve easily (Kane and Dorman, 2011). Presumably  
149 those anti-silencing mechanisms that succeed in terms of vertical transmission of  
150 xenogeneic genes link their derepression to physiologically meaningful signals.  
151 This hypothesis seems to be supported by studies where specific examples of  
152 factor-mediated signalling and H-NS antagonism have been examined (Kane and  
153 Dorman, 2011; Turner and Dorman, 2007; Walther et al., 2007; 2011).

154

## 155 *2.2. H-NS paralogues and orthologues*

156 The StpA protein is 59% identical to H-NS and is encoded by a paralogous gene  
157 located in a separate part of the chromosome to *hns*, the *stpA* gene (Zhang and  
158 Belfort, 1992). The StpA protein is an excellent RNA chaperone and also has DNA  
159 binding activity (Deighan et al., 2000; Doetsch et al., 2011; Lim et al., 2012;  
160 Mayer et al., 2007; Sonnenfield et al., 2001; Uyar et al., 2009; Williams et al.,



161 1996; Zhang et al., 1995). StpA shares many of the properties of H-NS but is  
162 present in fewer copies and has a distinct expression pattern (Free and Dorman,  
163 1997; Sondén and Uhlin, 1996; Wang et al., 2011; Zhang et al., 1996). It is also  
164 sensitive to proteolytic degradation by the Lon protease but is protected by  
165 forming heterodimeric complexes with H-NS (Johansson and Uhlin, 1999;  
166 Johansson et al., 2001). In *E. coli*, the *hns* gene is transcribed during chromosome  
167 replication and becomes silenced by H-NS if replication fork movement is  
168 arrested (Free and Dorman, 1995). The bacterium appears to maintain a  
169 constant ratio of H-NS to DNA and it is possible that the bacterium has just  
170 enough H-NS to meet its needs during rapid, exponential growth (Doyle et al.,  
171 2007; Free and Dorman, 1995). The *stpA* gene is expressed transiently at just this  
172 phase of the growth cycle, leading to a model in which it serves to supplement  
173 the H-NS protein population. In *Salmonella*, StpA suppresses the expression of  
174 the RpoS stress-and-stationary-phase sigma factor of RNA polymerase through a  
175 complicated cascade involving anti-adaptors that protect RpoS from proteolytic  
176 degradation (Lucchini et al., 2009). It is interesting to note in this context that  
177 complete knockout mutations of the *hns* gene in *Salmonella* can only be tolerated  
178 if the *rpoS* gene is also inactivated (Lucchini et al., 2006; Navarre et al., 2006).  
179 This indicates that the relative abundances of H-NS, StpA and RpoS influence  
180 bacterial physiology in important ways (Battesti et al., 2012; Free et al., 2001;  
181 Ohta et al., 1999). If new targets for H-NS/StpA are introduced by HGT, the cell  
182 must redistribute its existing pool of these proteins to include the additional  
183 binding sites. In the case of large, A+T-rich self-replicating plasmids, it appears  
184 that simply increasing the expression of these NAPs is not sufficient to prevent  
185 the resulting disturbance to the H-NS-DNA ratio from affecting the competitive

186 fitness of the bacterium. In such cases the plasmid can overcome the fitness  
187 deficit by expressing a gene of its own that encodes an H-NS-like protein (Doyle  
188 et al., 2007; Takeda et al., 2011).

189

### 190 **3. H-NS-like NAPs encoded by horizontally acquired genetic elements**

191

192 The role of H-NS in silencing the transcription of horizontally acquired genetic  
193 elements such as pathogenicity islands is well established (Dorman 2007;  
194 Lucchini et al., 2006; Navarre et al., 2006; Navarre et al., 2007; Oshima et al.,  
195 2006). It is interesting to note that many islands themselves encode H-NS-like  
196 proteins and that some of these play roles in antagonizing the transcription  
197 silencing properties of H-NS (Cameron and Dorman, 2012; Walthers et al., 2007;  
198 2011). These island-encoded H-NS-like proteins fall broadly into two classes:  
199 those that are similar in size and amino acid sequence to H-NS and those that  
200 resemble truncated H-NS molecules lacking the carboxyl terminal domain of H-  
201 NS. The latter, truncated type is typified by H-NST of enteropathogenic *E. coli*  
202 (EPEC) and its many orthologues (Levine et al., 2014; Williamson and Free,  
203 2005). Examples of the full-length type include H-NSB and Hfp from  
204 uropathogenic *E. coli* (UPEC) strains CFT073 and 536, respectively (Müller et al.,  
205 2010; Williamson and Free, 2005). The Ler protein encoded by the Locus of  
206 Enterocyte Effacement (LEE) pathogenicity island has important similarities to  
207 full-length H-NS; however, these are confined to the DNA binding domain in the  
208 carboxyl terminal portion of the protein (Dorman et al., 1999).

209 The H-NST protein of EPEC was shown originally to reverse H-NS-  
210 mediated repression of the *proU* and *bgl* loci in *E. coli* K-12 through a mechanism

211 that relies on protein-protein interaction with H-NS (Williamson and Free,  
212 2005). At *proU* this seems to involve a subtle remodelling an H-NS nucleoprotein  
213 complex at one part of the *proU* regulatory region (Williamson and Free, 2005).  
214 These data showed that H-NST exerts a *trans*-dominant effect when co-  
215 expressed with H-NS. Taking into account the absence of an obvious counterpart  
216 to the H-NS DNA binding domain in H-NST, one interpretation of these data  
217 envisages a role for the truncated H-NS-like protein in 'doping' the H-NS  
218 population through the creation of H-NS-H-NST heterodimers that lack a  
219 complete range of DNA binding functions (Stoebel et al., 2009). Here, the  
220 heterodimer fails fully to repress transcription either through a reduction in  
221 DNA-binding-and-polymerization activity or through a failure to establish stable  
222 bridged structures in DNA (Fig. 2). In the absence of full-length H-NS, truncates  
223 of the native H-NS protein that lack the DNA binding domain can interact with  
224 the H-NS paralogue StpA to repress the H-NS target operon *bgl* (Free et al., 1998)  
225 The strength of this co-repressor activity depends on the level of expression of  
226 the H-NS truncate (Free et al., 2001). These co-repressor effects are quite distinct  
227 from the anti-repressor activities of H-NST. How can these apparently opposite  
228 influences on H-NS activity be reconciled?

229         The original investigation of H-NST antagonism of H-NS binding at the  
230 *proU* locus detected a possible H-NST nucleoprotein complex at the same  
231 location, hinting at possible DNA binding activity by H-NST, something that the  
232 isolated oligomerization domain of H-NS itself lacks (Williamson and Free,  
233 2005). Recently, evidence has emerged from electrophoretic mobility shift  
234 assays that H-NST has DNA binding activity that is independent of H-NS (Levine  
235 et al., 2014). This is an important point of distinction between the molecular

236 mechanism of H-NST and that of those truncated forms of H-NS itself that have  
237 been created by molecular genetic methods or by natural mutation events. It  
238 suggests that H-NST, encoded by a gene in a horizontally acquired genetic  
239 element, can intrude into the H-NS regulon via two mechanisms: one in which H-  
240 NST forms a complex with H-NS directly, modifying the transcription silencing  
241 activity of H-NS, and a second mechanism that depends on an independent DNA  
242 binding activity that is inherent within H-NST. These mechanisms are not  
243 mutually exclusive and may indicate a ternary complex in which H-NST remodels  
244 an existing DNA-H-NS nucleoprotein complex through both H-NS-H-NST protein-  
245 protein interaction and DNA binding by H-NST.

246         The gene that encodes H-NST is in a horizontally acquired island located  
247 at the *asnW* tRNA gene of EPEC (Williamson and Free, 2005). EPEC strains also  
248 contain in their chromosomes the LEE pathogenicity island, a cluster of  
249 horizontally acquired virulence genes that express a type III secretion system  
250 and its associated effector proteins. Levine et al. (2014) have studied the impact  
251 of H-NST on LEE gene expression in enterohaemorrhagic *E. coli* (EHEC), an  
252 organism that does not possess its own H-NST. There, H-NST acts positively on  
253 transcription within LEE independently of Ler, a second H-NS-like protein that is  
254 encoded by the *ler* gene in LEE (Levine et al., 2014). The Ler protein is itself an  
255 antagonist of H-NS-mediated transcription silencing of LEE genes and other  
256 virulence genes located elsewhere in the genome (Abe et al., 2008; Bingle et al.,  
257 2014; Garcia et al., 2012). Ler is also able to repress transcription and does so at  
258 plasmid-located virulence loci (Bingle et al., 2014).

259         H-NS antagonism by Ler raises the important question of how Ler  
260 achieves specificity, confining its effects mainly to virulence genes on

261 horizontally acquired pathogenicity islands and plasmids. This question becomes  
262 even more pertinent when one considers that the amino sequence similarity  
263 between H-NS and Ler is mainly confined to the carboxyl terminal domains of  
264 the proteins where the DNA binding motifs are located (Dorman et al., 1999). An  
265 indirect readout mechanism has been proposed in which H-NS-like proteins like  
266 Ler sense structural motifs in their DNA targets rather than relying on a direct  
267 readout mechanism where the protein docks with portions of DNA that contain a  
268 match to a specific consensus sequence (Cordeiro et al., 2011; Dillon et al., 2010).  
269 This allows proteins such as Ler to bind to subsets of H-NS targets that match  
270 their particular DNA structural preferences. Concerning Ler interaction with H-  
271 NS once the target site has been identified, recent single-molecule studies  
272 performed *in vitro* with purified protein indicate that Ler operates by a non-  
273 cooperative DNA binding mechanism to compete with H-NS and antagonize its  
274 ability to silence LEE gene transcription (Winardhi et al., 2014). Although H-NST  
275 and Ler operate independently, it has been suggested that H-NST can assist Ler  
276 indirectly through interfering with the ability of H-NS to establish a  
277 transcription-silencing complex on LEE DNA (Levine et al., 2014).

278         The horizontally acquired island at the *serU* tRNA locus in uropathogenic  
279 *E. coli* (UPEC) strain CFT073 also encodes an H-NST protein but this molecule  
280 (H-NST<sub>UPEC</sub>) differs from its EPEC counterpart (H-NST<sub>EPEC</sub>) in important respects  
281 (Williamson and Free, 2005). The UPEC orthologue has only a weak ability to  
282 antagonise H-NS-mediated transcription repression and this correlates with a  
283 poor ability to form heterodimers with H-NS. A single difference in the amino  
284 acid sequences of the two proteins is responsible for these distinct properties  
285 (Williamson and Free, 2005). Although UPEC lacks an effective H-NST activity of

286 its own, it possesses an additional H-NS-like protein, H-NSB, encoded by the  
287 same island that harbours the gene for H-NST<sub>UPEC</sub>. H-NSB has similar DNA  
288 binding and transcription silencing properties to H-NS when tested at the *proU*  
289 locus (Williamson and Free, 2005). The genes for H-NST<sub>UPEC</sub> and H-NSB lie  
290 among open reading frames potentially capable of expressing components of  
291 Type IV secretion systems, MobA/RepB-like proteins and a P4-like integrase, all  
292 indicative of a previous history of the *serU*-associated island as a mobile genetic  
293 element (Williamson and Free, 2005).

294 An H-NSB counterpart called Hfp has been characterised in UPEC strain  
295 536 (Dorman, 2010; Müller et al., 2010). Once again, the gene is located in a  
296 horizontally acquired island at the *serU* tRNA gene. Genes related to *hnsB/hfp*  
297 have been detected in many strains of extraintestinal pathogenic *E. coli* (EPEC)  
298 with UPEC forming a majority of the *hnsB/hfp*-positive isolates (Müller et al.,  
299 2010). Since all of these isolates also possess the H-NS and StpA proteins, they  
300 share a 3-H-NS-like-protein repertoire with those *S. flexneri* and *S. enterica*  
301 strains that have acquired plasmids encoding a third H-NS-like protein (see  
302 Section 6).

303 The Hfp protein can form heterodimers with H-NS and with StpA, raising  
304 the possibility that it can modulate the activities of these proteins in some way.  
305 An important role has been identified for Hfp and StpA in maintaining normal  
306 growth at low or high temperatures. Interestingly, the genes encoding Hfp and  
307 StpA have opposing expression patterns as a function of growth phase in batch  
308 culture: the *stpA* gene is expressed transiently in logarithmic growth whereas the  
309 *hfp* gene is expressed maximally at the onset of stationary phase and thereafter  
310 (Müller et al., 2010). The three genes, *hfp*, *hns* and *stpA* are subject to negative

311 auto-regulation and negative cross-regulation, showing that their regulatory  
312 networks are integrated. The presence of Hfp, the third H-NS-like protein in  
313 strain 536, appears to have extended the range of gene regulatory possibilities  
314 that are available to this pathogen as it experiences fluctuations in temperature.  
315 This is likely to be significant in the context of disease because the transition  
316 from the external environment to the human host and back again involves  
317 adaptation to changing temperature.

318

#### 319 **4. H-NS-like proteins and thermal adaptation**

320

321 The gene coding for the H-NS protein was discovered independently on a  
322 number of occasions by investigators studying bacterial adaptation to thermal  
323 stress (Atlung and Ingmer, 1997; Dorman et al., 1990; Göransson et al., 1990;  
324 White-Ziegler and Davis, 2009). For example, the plasmid-encoded virulence  
325 regulon of the dysentery bacillus *Shigella flexneri* is transcriptionally silenced by  
326 H-NS and this silencing is reversed following a temperature shift to 37°C (Beloin  
327 and Dorman, 2003; Falconi et al., 1998; Hromockyj et al., 1992; Porter and  
328 Dorman, 1994; Prosseda et al., 2004; Tran et al., 2011). H-NS has also been  
329 implicated in virulence gene thermoregulation in enteroinvasive *E. coli* (EIEC),  
330 EHEC and EPEC (Beltrametti et al., 1999; Dagberg and Uhlin, 1992; Falconi et al.,  
331 1998; Martínez-Santos et al., 2012; Umanski et al., 2002), ETEC (Jordi et al.,  
332 1992; Trachman and Yasmin, 2004; Trachman and Maas, 1998), UPEC (Madrid et  
333 al., 2002; White-Ziegler et al., 1998; 2000), *E. coli* K5 (Corbett et al., 2007; Rowe  
334 et al., 2000), *E. coli* K-12 (Dorman and Ní Bhriain, 1992; Gally et al., 1993; Kawula  
335 and Orndorff, 1991; Lithgow et al., 2007; Olsén et al., 1993), *Erwinia*

336 *chrysanthemii* (James and Hugouvieux-Cotte-Pattat, 1996), *Dickeya dadantii*  
337 (Costechareyre et al., 2010), *Proteus mirabilis* (Poore and Mobley, 2003),  
338 *Salmonella enterica* serovar Typhimurium (Duong et al., 2007), *Yersinia*  
339 *enterocolitica* (Uliczka et al., 2011), *Y. pseudotuberculosis* (Herovan et al., 2004).  
340 It has been proposed that the thermosensing activity of H-NS is an intrinsic  
341 property of the protein and that the sensing mechanism concerns the ways in  
342 which H-NS monomers are arranged within the dimer and in higher order  
343 oligomers (Arold et al., 2010; Ono et al., 2005; Stella et al., 2006). There is also  
344 evidence that changes to the structure of DNA in H-NS nucleoprotein complexes  
345 interfere with the ability of the protein to maintain transcription silencing  
346 (Prosseda et al., 2004). In addition, H-NS-mediated transcription silencing can be  
347 overcome through the intervention of other thermo-regulated DNA binding  
348 proteins that disrupt the H-NS repression complex (Stoebel et al., 2008).

349 H-NS also has an important relationship with the cold shock response  
350 (Dersch et al., 1994; La Teana et al., 1991) and this may be significant in the  
351 context of plasmid conjugation in the environment (Forns et al., 2005; Sherburne  
352 et al., 2000). The cold shock protein CspA contributes to the positive regulation  
353 of the *hns* gene, possibly at the level of *hns* transcription (Giangrossi et al., 2001;  
354 La Teana et al., 1991) and H-NS is itself an important regulator of cold shock  
355 genes (White-Ziegler and Davis, 2009). Some genes contributing to the cold  
356 shock response express mRNA that is subject to differential folding and cold  
357 shock proteins are known to modulate this folding process (Giuliodori et al.,  
358 2010; Phadtare and Severinov, 2010). In this context it is interesting to note that  
359 H-NS has been described as a cold shock protein (La Teana et al., 1991) and it  
360 has a direct role in translation control where it can reposition ribosomes on



361 mRNA templates that have sub-optimal ribosome binding sites (Park et al.,  
362 2010). Its paralogue, StpA is a well-established RNA chaperone that can facilitate  
363 both RNA strand annealing and strand displacement (Rajkowitsch and  
364 Schroeder, 2007). StpA is the chromosomally encoded H-NS-like protein that  
365 most resembles the plasmid-encoded Sfh and H-NS<sub>R27</sub> molecules expressed by  
366 IncHI1 plasmids (Beloin et al., 2003). This similarity may be relevant in the  
367 context of thermal regulation of plasmid conjugation.

368

### 369 **5. Plasmid conjugation control by H-NS**

370

371 Plasmid R27 and its relatives are capable of self-transmission by conjugation.  
372 However, this process is temperature sensitive and R27 transfers best at low  
373 temperatures (below 30°C) with conjugation rates declining precipitously with  
374 increasing temperature (Maher and Taylor, 1993). The plasmid transfer  
375 apparatus is expressed only at the permissive temperatures, indicating that  
376 thermoregulation may lie at the level of *tra* gene expression (Gilmour et al.,  
377 2001). Given the many links between H-NS-like proteins and thermoregulation  
378 of transcription and in light of the presence on R27 of a gene encoding a member  
379 of this family of proteins, the possibility that H-NS<sub>R27</sub> might control conjugation  
380 in response to temperature has been investigated (Forns et al., 2005). The same  
381 study also considered the possible contribution of the chromosomally encoded  
382 orthologue (H-NS). H-NS<sub>R27</sub> was found to interact in complex ways with the *oriT*  
383 region of R27 and with several of the *tra* genes; furthermore, the  
384 chromosomally-encoded H-NS also interacts physically with these regions of the  
385 plasmid (Forns et al., 2005). These observations lead to a consideration of the

386 differential contributions of plasmid-encoded and chromosome-encoded H-NS-  
387 like proteins to gene expression, not only within plasmids but in the wider  
388 genome too.

389 H-NS can also contribute to the control of plasmid transfer independently  
390 of a thermal signal. The best-studied examples concern the role of H-NS as a  
391 transcription repressor of transfer gene expression in the F plasmid (Will and  
392 Frost, 2006; Will et al., 2004) and in the F-like plasmid R1 (Wagner et al., 2013).  
393 Here the H-NS protein in question is the one that is encoded by the chromosome  
394 and its targets are on the plasmids, with plasmid-encoded and chromosome-  
395 encoded factors acting to overcome H-NS-imposed transcriptional silencing. For  
396 example, the plasmid encoded TraJ protein and the chromosome-encoded ArcA  
397 proteins have been shown to antagonize H-NS silencing of *tra* transcription in R1  
398 (Wagner et al., 2013; Will and Frost, 2006). In the case of ArcA, the  
399 environmental signal being transduced is microaerobiosis (Serna et al., 2010;  
400 Strohmaier et al., 1998).

401

## 402 **6. Plasmid- and chromosome-encoded H-NS orthologues: points of** 403 **distinction**

404

405 The IncHI1 plasmid R27 from *Salmonella* and its close relative pSfR27 from  
406 *Shigella* encode the H-NS/StpA-like proteins H-NS<sub>R27</sub> and Sfh, respectively (Doyle  
407 et al., 2006; Doyle and Dorman, 2007; Sherburne et al., 2000; Beloin et al., 2003;  
408 Forns et al., 2005; Paytubi et al., 2014). Although chromosomally and plasmid-  
409 encoded H-NS-like proteins are closely related, transcriptomic studies suggest  
410 that they have distinct effects on gene expression: the plasmid-encoded H-NS<sub>R27</sub>

411 has its effect predominantly among genes that have been acquired horizontally  
412 whereas the chromosomally encoded H-NS does not exhibit a preference (Baños  
413 et al., 2009b; 2011). Among the major target of H-NS in *Salmonella* are the  
414 horizontally acquired genes of its many pathogenicity islands and islets; it is  
415 thought that inappropriate up-regulation of these genes leads to a loss of  
416 competitive fitness (Doyle et al., 2006; Paytubi et al., 2014). These genes also  
417 feature among the targets of the Sfh protein from pSfR27 (the R27 plasmid from  
418 *Shigella flexneri*) and the H-NS<sub>R27</sub> protein from plasmid R27 of *Salmonella*  
419 *enterica* (Beloin et al., 2003; Deighan et al., 2003; Dillon et al., 2010).

420         How can these closely related plasmid- and chromosome-encoded  
421 proteins exert such distinct influences on host gene expression? This question  
422 has been addressed through the study of chimeric proteins in which the three  
423 functional domains of the different proteins have been exchanged (Fernandez-  
424 de-Alba et al., 2013). These domains are the oligomerization domain, the linker  
425 and the nucleic acid binding domain. The data suggest that chromosome-  
426 encoded and R27 plasmid-encoded H-NS proteins differ in the flexibilities of  
427 their linker domains, with the plasmid-encoded type being the least flexible.  
428 Differences in linker flexibility are thought to influence the versatility of an H-NS  
429 polymer during interactions with DNA with different degrees of intrinsic  
430 curvature: the chromosome-encoded protein forms a protein polymer with  
431 maximum flexibility and can adapt a greater range of DNA geometries, allowing  
432 this H-NS protein to bind stably to more targets than the plasmid-encoded  
433 orthologue with its diminished linker flexibility (Fernandez-de-Alba et al., 2013).  
434 These findings are consistent with data from chromatin immunoprecipitation on  
435 chip that show that the pSfR27-encoded Sfh protein, a plasmid-encoded H-NS

436 orthologue, shows altered target specificity and binds to just a subset of H-NS  
437 targets in *Salmonella* when H-NS is also present in the same bacterium (Dillon et  
438 al., 2010).

439

#### 440 **7. Hha-like proteins and H-NS target specificity**

441 The Hha protein is the founding member of a group of polypeptides that  
442 resemble the oligomerization domain of H-NS (Madrid et al., 2007). Hha has no  
443 nucleic acid binding activity of its own; instead it affects gene expression by  
444 interacting with H-NS to form an Hha-H-NS heterotetramer (Ali et al., 2013). Hha  
445 can alter the target specificity of H-NS, causing it to display an exaggerated  
446 preference for xenogeneic genes in *Salmonella* for example, resulting in the  
447 silencing of the virulence genes of the bacterium (Aznar et al., 2013; Baños et al.,  
448 2009a). Hha is accompanied by a paralogue, YdgT, which has many of the same  
449 properties as Hha, including the ability to interact with H-NS and StpA (Ali et al.,  
450 2013; Paytubi et al., 2004; Ueda et al., 2013); YdgT has also been named Cnu  
451 (Bae et al., 2008). Self-transmissible plasmids that encode H-NS-like proteins can  
452 also express an Hha-like polypeptide (Paytubi et al., 2013; Takeda et al., 2011).  
453 In their survey of 1382 plasmids from Gram-negative bacteria, Takeda et al.  
454 (2011) identified 65 that encoded an Hha-like protein, including 12 plasmids  
455 with genes for both Hha-like and H-NS-like proteins. The group of Juarez has  
456 recently characterized the Hha-like protein that is encoded by the virulence  
457 plasmid of *E. coli* O157:H7 (Paytubi et al., 2013). They also examined 69 plasmid-  
458 encoded Hha-like proteins and report the existence of a cluster of closely-related  
459 proteins of this class that are encoded by MOBF-like plasmids (Garcillan-Barcia  
460 et al., 2009) of the IncF incompatibility group that lack a gene for an H-NS-like

461 protein (Paytubi et al., 2013). This group of plasmids is associated with *E. coli*  
462 and *Shigella* and with virulence factor expression (Boyd et al., 1996; Johnson et  
463 al., 2007; Reisner et al., 2006).

464

## 465 **8. Concluding remarks**

466

467 This article has focused on NAPs that are related to the H-NS protein and the  
468 relationships of these NAPs with mobile genetic elements, including plasmids. It  
469 is important to point out that orthologues of other NAPs, such as Fis and HU,  
470 have also been detected on plasmids (Takeda et al., 2011) but in most cases little  
471 is known about their physiological roles. The H-NS family displays a great deal of  
472 structural versatility and can be subdivided into proteins that share amino acid  
473 sequence similarity overall with H-NS (e.g. StpA, H-NSB, Sfh), proteins of a  
474 similar size but with amino acid sequence similarity restricted to just one  
475 functional domain (Ler), proteins that resemble the oligomerization domain of  
476 H-NS and have some independent DNA binding activity (H-NST) and proteins  
477 with sequence similarity to the H-NS oligomerization domain but which depend  
478 on H-NS for DNA binding (Hha) (Figs. 1 and 2). HGT has resulted in the  
479 supplementation of the core-genome-encoded H-NS and StpA proteins by  
480 orthologues expressed from plasmids or genomic islands and all of these  
481 different proteins appear to be able to interact physically, either in solution or  
482 when bound to DNA. This confers on the group enormous potential for mutual  
483 modulation of activity and the distinct expression pattern of each protein creates  
484 a pool of H-NS-like molecules that is dynamic in composition. This dynamism is

485 likely to expand the physiological range of the bacterium by providing additional  
486 layers of gene expression modulation that can enhance competitive fitness.

487

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489

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495 **References**

496

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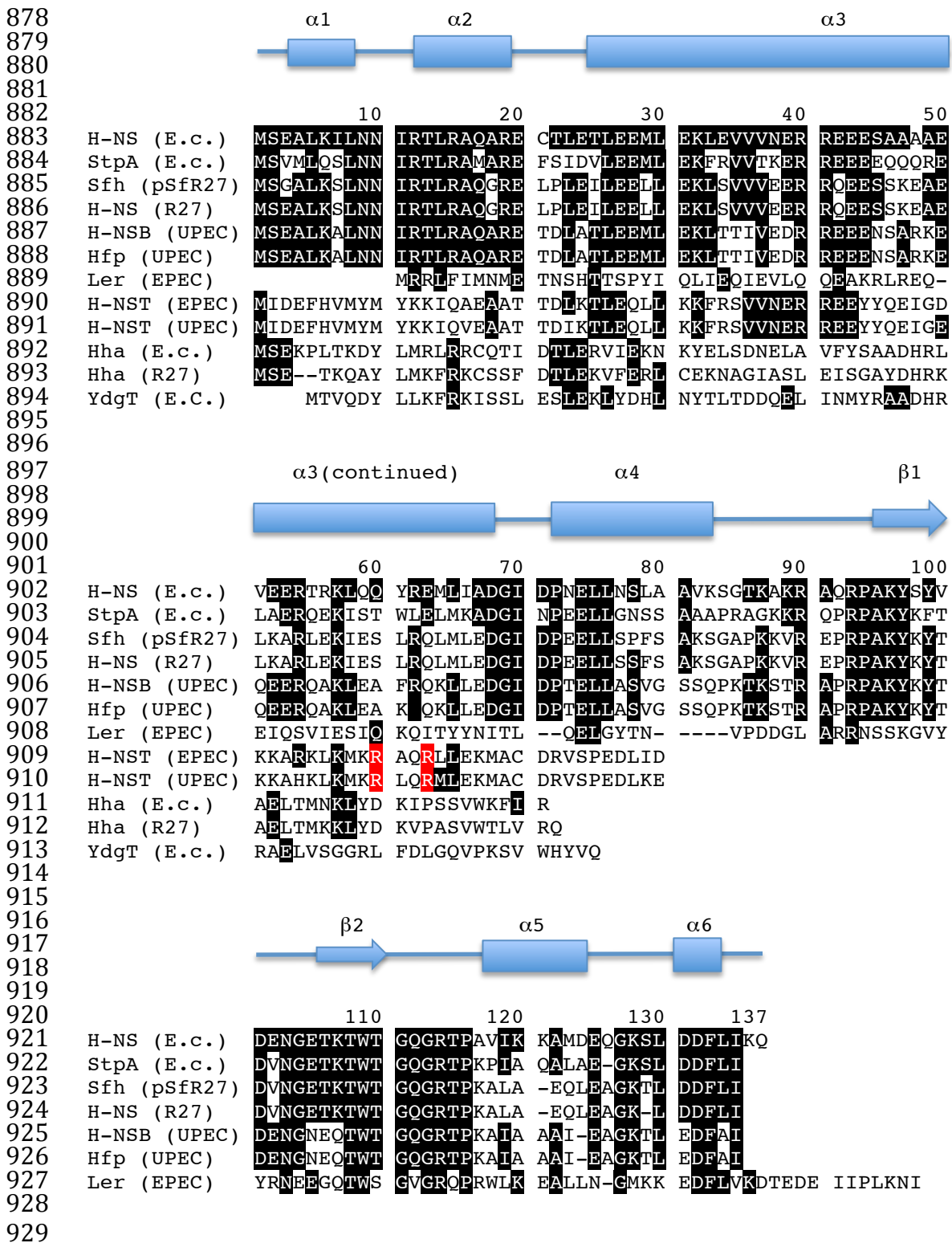
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877 **Figures**



930 **Fig. 1.** Alignment of the amino acid sequences of some of the best-studied  
 931 members of the H-NS-like protein family. The figure shows the chief sub-classes  
 932 within the family: full-length H-NS orthologues (Hfp, H-NSB, StpA, Sfh), proteins

933 with length and partial amino acid sequence similarity to H-NS (Ler), proteins  
934 with similarity to the oligomerization domain that have independent DNA  
935 binding activity (H-NST) and proteins with similarity to the oligomerization  
936 domain that lack independent DNA binding activity (Hha, YdgT). A summary of  
937 the main structural features of H-NS is also provided (adapted from Arold et al.,  
938 2010). The oligomerization domain consists of alpha helices  $\alpha 1$  to  $\alpha 4$ ; the DNA  
939 binding motif corresponds to beta sheet  $\beta 2$  and the linker that connects the  
940 oligomerization and nucleic acid binding domains is located between positions  
941 78 ( $\alpha 4$ ) and 90 (Dorman et al., 1999). The linker region contains the fewest  
942 conserved amino acids in the aligned sequences of the proteins with lengths  
943 similar to that of H-NS. The two arginines in H-NST that have been implicated in  
944 DNA binding (Levine et al., 2014) are highlighted in red.  
945



Protein	DNA binding activity?	RNA binding activity?	DNA bridging activity?	Heterodimer with H-NS?
 H-NS	Yes	Yes	Yes	not applicable
 StpA	Yes	Yes	Yes	Yes
 H-NS <sub>R27</sub>	Yes	?	?	Yes
 Hha	No	?	?	Yes
 H-NST	Yes	?	?	Yes
 Ler	Yes	?	?	No

946

947

948 **Fig. 2.** Properties of selected members of the H-NS-like protein family.

949 Representatives of proteins that are encoded by plasmids (H-NS<sub>R27</sub>), genomic

950 islands (Ler and H-NST) and the core genome (H-NS, StpA and Hha) are

951 illustrated. The domain structure of each protein is summarized with reference

952 to that of H-NS where OD = oligomerization domain, L = linker and DBD = DNA

953 binding domain. The straight linker domain of H-NS<sub>R27</sub> represents its relative

954 rigidity, in contrast to the flexible linkers of H-NS and StpA. Each protein is

955 shown as a homodimer and its ability to bind and bridge DNA, bind RNA and

956 form heterodimers with H-NS is summarized. Unknown properties are indicated

957 by the question marks.