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

Charles J. Dorman

Department of Microbiology, Moyne Institute of Preventive Medicine, School of Genetics and Microbiology, Trinity College Dublin, Dublin 2, Ireland.

*Corresponding author. Email address: cjdorman@tcd.ie

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ABSTRACT

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

48 **Contents**

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1. Introduction

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

If the genes acquired by HGT become part of the chromosome they will be 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

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

2.2. H-NS paralogues and orthologues

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

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

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

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

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

The H-NST protein of EPEC was shown originally to reverse H-NS-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

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

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

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

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

The Hfp protein can form heterodimers with H-NS and with StpA, raising the possibility that it can modulate the activities of these proteins in some way. An important role has been identified for Hfp and StpA in maintaining normal growth at low or high temperatures. Interestingly, the genes encoding Hfp and StpA have opposing expression patterns as a function of growth phase in batch culture: the *stpA* gene is expressed transiently in logarithmic growth whereas the *hfp* gene is expressed maximally at the onset of stationary phase and thereafter (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 *chrysanthemi* (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

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

5. Plasmid conjugation control by H-NS

Plasmid R27 and its relatives are capable of self-transmission by conjugation. However, this process is temperature sensitive and R27 transfers best at low temperatures (below 30˚C) with conjugation rates declining precipitously with increasing temperature (Maher and Taylor, 1993). The plasmid transfer apparatus is expressed only at the permissive temperatures, indicating that thermoregulation may lie at the level of *tra* gene expression (Gilmour et al., 2001). Given the many links between H-NS-like proteins and thermoregulation of transcription and in light of the presence on R27 of a gene encoding a member of this family of proteins, the possibility that H-NS_{R27} might control conjugation in response to temperature has been investigated (Forns et al., 2005). The same study also considered the possible contribution of the chromosomally encoded orthologue (H-NS). H-NS_{R27} was found to interact in complex ways with the *oriT* region of R27 and with several of the *tra* genes; furthermore, the chromosomally-encoded H-NS also interacts physically with these regions of the 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_{R27} 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_{R27}

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_{R27} 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

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

8. Concluding remarks

This article has focused on NAPs that are related to the H-NS protein and the relationships of these NAPs with mobile genetic elements, including plasmids. It is important to point out that orthologues of other NAPs, such as Fis and HU, have also been detected on plasmids (Takeda et al., 2011) but in most cases little is known about their physiological roles. The H-NS family displays a great deal of structural versatility and can be subdivided into proteins that share amino acid sequence similarity overall with H-NS (e.g. StpA, H-NSB, Sfh), proteins of a similar size but with amino acid sequence similarity restricted to just one functional domain (Ler), proteins that resemble the oligomerization domain of H-NS and have some independent DNA binding activity (H-NST) and proteins with sequence similarity to the H-NS oligomerization domain but which depend on H-NS for DNA binding (Hha) (Figs. 1 and 2). HGT has resulted in the supplementation of the core-genome-encoded H-NS and StpA proteins by orthologues expressed from plasmids or genomic islands and all of these different proteins appear to be able to interact physically, either in solution or when bound to DNA. This confers on the group enormous potential for mutual modulation of activity and the distinct expression pattern of each protein creates 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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495 **References**

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Figures

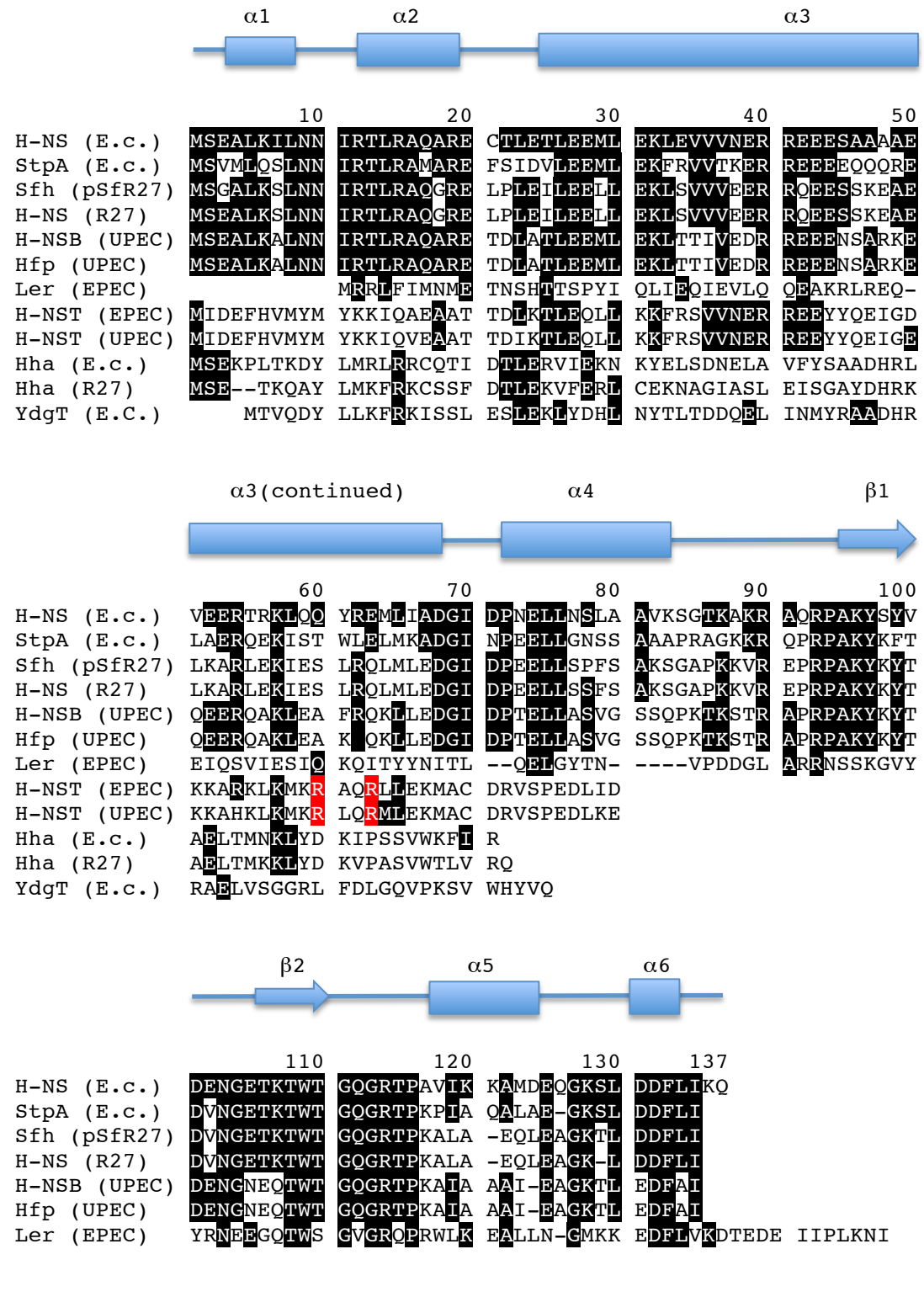

Fig. 1. Alignment of the amino acid sequences of some of the best-studied members of the H-NS-like protein family. The figure shows the chief sub-classes within the family: full-length H-NS orthologues (Hfp, H-NSB, StpA, Sfh), proteins

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 α1 to α4; the DNA
939 binding motif corresponds to beta sheet β2 and the linker that connects the
940 oligomerization and nucleic acid binding domains is located between positions
941 78 (α4) 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.
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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$_{R27}$), 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$_{R27}$ 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.