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Nucleoid-associated proteins encoded on plasmids: Occurrence and mode of function

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ABSTRACT

Nucleoid-associated proteins (NAPs) play a role in changing the shape of microbial DNA, making it more compact and affecting the regulation of transcriptional networks in host cells. Genes that encode NAPs include H-NS family proteins (H-NS, Ler, MvaT, BpH3, Bv3F, HvrA, and Lsr2), FIS, HU, IHF, Lrp, and NdpA, and are found in both microbial chromosomes and plasmid DNA. In the present study, NAP genes were distributed among 442 plasmids out of 4602 plasmid sequences, and many H-NS family proteins, and HU, IHF, Lrp, and NdpA were found in plasmids of *Alpha*-, *Beta*-, and *Gammaproteobacteria*, while HvrA, Lsr2, HU, and Lrp were found in other classes including *Actinobacteria* and *Bacilli*. Larger plasmids frequently carried multiple NAP genes. In addition, NAP genes were more frequently found in conjugative plasmids than non-transmissible plasmids. Several host cells carried the same types of H-NS family proteins on both their plasmids and chromosome(s), while this was not observed for other NAPs. Recent studies have shown that NAP genes on plasmids and chromosomes play important roles in the physical and regulatory integration of plasmids into the host cell.

1. Introduction

Plasmids (extrachromosomal replicons) are one of the most important 'vehicles' of genes for resistance to antibiotics, metabolism of natural and synthetic compounds, pathogenicity, and host symbiosis. Plasmid transfer is involved in the rapid evolution and adaptation of microbes by transferring specific traits among different host microbes. After a host cell receives a plasmid, integration of the newly acquired genetic elements into the host cell (the successful survival of a plasmid in a host cell) is required to maintain host cell fitness. Several studies have shown that carrying a plasmid is a 'burden' on the host because the replication and maintenance of plasmids perturbs host transcriptional networks (Nojiri, 2013; Shintani et al., 2014). Two mechanisms of plasmid integration into host cells have been described as 'physical integration' and 'regulatory integration' (Dorman, 2014).

Over the last 10 years, nucleoid-associated proteins (NAPs) have been shown to be involved in folding chromosomal DNA to make it more compact through their DNA-binding ability, which is important for the regulation of transcriptional networks in hosts (Dillon and Dorman, 2010; Nojiri, 2012). One of the best studied NAPs is histone-like nucleoid structuring protein (H-NS) in enterobacteria. Other NAPs have been identified, including factor for inversion stimulation (FIS), histone-like protein from *Escherichia coli*

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strain U93 (HU), integration host factor (IHF), and leucine-responsive regulatory protein (Lrp) (Dillon and Dorman, 2010). NdpA (sometimes named YejK in *E. coli*) is a functionally unknown NAP included in the nucleoid of *E. coli* and *Pseudomonas aeruginosa* (Murphy et al., 1999; Ohniwa et al., 2011). Some of these NAP genes have been found on plasmids and are thought to play an important role in the integration and adaptation of plasmids into new host cells (Takeda et al., 2011). Recently, Dorman (2014) suggested that NAPs can be used to reduce fitness costs through physical and regulatory integration of plasmids into host cells. In the present study, the distributions of NAP genes on plasmids and their host chromosome(s) were determined as a follow-up to our previous study (Takeda et al., 2011). Recent studies on NAP genes found in plasmids and their (putative) roles in host cells are described.

2. NAPs

2.1. H-NS family proteins

H-NS, one of the best-characterized NAPs, was shown in the 1970s to be a heat-resistant protein that activates transcription in bacterial cells, and was 're-discovered' as a histone-like protein in bacteria 10 years later (Lammi et al., 1984; Navarre, 2010). A large number of excellent reviews are available (Dorman, 2004, 2014; Fang and Rimsky, 2008; Navarre, 2010; Stoebel et al., 2008). H-NS is found in the genera *Escherichia* and *Salmonella*, whose amino acid sequences show 95% identity. It is one of the most abundant proteins in a cell (about 20,000 molecules in a cell) (Falconi et al., 1988). H-NS contains two domains: the N-terminal domain for dimerization and oligomerization and a C-terminal domain for DNA-binding, and these are linked through a flexible linker (Rimsky, 2004). The DNA-binding sites of H-NS are rich in AT (Grainger et al., 2006; Lucchini et al., 2006; Navarre et al., 2006; Oshima et al., 2006). It has been proposed that H-NS binds to genomic DNA through a two-step mechanism: the H-NS protein first recognizes and binds to the high-affinity 'core' DNA region, after which it forms oligomers on the DNA and binds to the flanking DNA of the core region through protein–DNA and protein–protein interactions (Bouffartigues et al., 2007; Lang et al., 2007). The resulting H-NS nucleoprotein complexes are thought to mediate gene silencing by preventing open complex formation by RNA polymerase or trapping the open complex once it has formed, thus repressing the transcription of multiple genes (Dorman, 2004; Fang and Rimsky, 2008). H-NS protein plays two roles as a nucleoid structuring protein and a global transcriptional regulator.

Escherichia and *Salmonella* contain an H-NS homolog, StpA, in their cells whose amino acid sequences show 57% and 52% identity with H-NS, respectively. StpA was thought to have similar functions as H-NS. Indeed, it can form oligomers around binding sites (Dame et al., 2005; Lim et al., 2012), and target genes for transcriptional regulation are similar to that of H-NS (Lucchini et al., 2009; Uyar et al., 2009). Therefore, StpA was thought to have backup functions for H-NS (Shi and Bennett, 1994; Sonden and Uhlin, 1996; Zhang et al., 1996). Virulent *Escherichia* possess another H-NS homologous protein, Hfp, on one of the genomic islands, whose amino acid sequence shows 58% identity with H-NS (Müller et al., 2010). Hfp complements the decreased motility of *hns*-deficient mutant cells and functions as a transcriptional repressor (Müller et al., 2010). Ler is an antagonist of H-NS in the locus of enterocyte effacement (LEE), one of the pathogenicity islands in virulent *E. coli* (Elliott et al., 2000; Mellies et al., 2007). The transcription of Ler is repressed by H-NS, and Ler functions as a master regulator of genes on the LEE (Mellies et al., 2007).

In addition to H-NS and its homologous proteins, there are H-NS-like proteins that show partial identity with H-NS (especially in the DNA-binding domain), as well as functional homologs of H-NS that show low or negligible amino acid sequence identity with H-NS but can complement its function. These proteins are collectively called the H-NS family proteins (Ali et al., 2012; Tendeng and Bertin, 2003). MvaT is a functional homolog of H-NS that was first found as a transcriptional regulator of an operon for mevalonate-metabolic genes (*mvaAB*) in *P. mevalonii* (Rosenthal and Rodwell, 1998). MvaT regulates multiple genes for biofilm formation, flagellum synthesis, and virulence (Diggle et al., 2002; Vallet et al., 2004). Although MvaT shows low identity with H-NS (18% amino acid identity between H-NS of *E. coli* and MvaT of *P. aeruginosa*), it complements the serine-sensitivity of the *E. coli hns* mutant (Tendeng et al., 2003). Functional analyses of MvaT and its homologous protein, MvaU (46% identity with MvaT at the amino acid sequence level), in *P. aeruginosa* PAO1 have been performed, and these two proteins form hetero-dimers and hetero-oligomers (Vallet-Gely et al., 2005). These two proteins bind to various positions on chromosomal DNA in *P. aeruginosa* PAO1, and more than 90% of their binding regions are shared (Castang et al., 2008).

Other H-NS family proteins have also been found in other bacterial classes. BpH3 is an H-NS-like protein in *Bordetella* (Goyard and Bertin, 1997), and Bv3F (showing 26% identity with BpH3 at the amino acid sequence level) has been found in *Burkholderia* (Bcep1808_62169, GenBank accession no. CP000616) (both are present in *Betaproteobacteria*). HvrA is an H-NS-like protein in *Rhodobacter* (*Alphaproteobacteria*) (Bertin et al., 1999). Gram-positive bacteria also contain a functional homolog of H-NS, named Lsr2, which was originally found in *Mycobacterium* (Gordon et al., 2008, 2010). The XrvA protein was found and recently characterized as an H-NS-like protein in *Xanthomonas* (Bertin et al., 1999; Feng et al., 2009). Rok, which was identified in *Bacillus* and shows 14% amino acid identity with H-NS of *E. coli*, has similar features as the H-NS protein, although whether Rok can complement H-NS remains unclear (Smits and Grossman, 2010).

2.2. Other NAPs

NAPs other than H-NS have been identified in the family *Enterobacteriaceae*. FIS, an abundant NAP in *Escherichia*, is expressed transiently during the log phase but disappears during the stationary phase (Azam et al., 1999). It forms homodimers and binds specific DNA sequences 17 bp in size (Cho et al., 2008b; Skoko et al., 2006). It is also a global transcriptional regulator (Bradley et al., 2007). Transcription of the FIS gene is activated by IHF, while FIS inhibits

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