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DNA topology and adaptation of *Salmonella typhimurium* to an intracellular environment

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The expression of genes coding for determinants of DNA topology in the facultative intracellular pathogen *Salmonella typhimurium* was studied during adaptation by the bacteria to the intracellular environment of J774A.1 macrophage-like cells. A reporter plasmid was used to monitor changes in DNA supercoiling during intracellular growth. Induction of the *dps* and *spv* genes, previously shown to be induced in the macrophage, was detected, as was expression of genes coding for DNA gyrase, integration host factor and the nucleoid-associated protein H-NS. The *topA* gene, coding for the DNA relaxing enzyme topoisomerase I, was not induced. Reporter plasmid data showed that bacterial DNA became relaxed following uptake of *S. typhimurium* cells by the macrophage. These data indicate that DNA topology in *S. typhimurium* undergoes significant changes during adaptation to the intracellular environment. A model describing how this process may operate is discussed.

Keywords: intracellular growth; DNA topology; gene expression; adaptation; *Salmonella typhimurium*

1. INTRODUCTION

Bacterial adaptation to stressful environments is the subject of intense investigation at present and studies performed *in vitro* have helped to identify many of the components used by prokaryotes to survive when under stress. Much of this work has been carried out with the facultative intracellular pathogen, *Salmonella typhimurium* (see § 5). The central importance of gene regulation to stress responses is obvious and a great deal of detailed information is available about the processes that control gene expression at the transcriptional and post-transcriptional levels. Almost all of this information has been acquired through work with the bacteria under *in vitro* conditions, although attempts have been made to extrapolate from the *in vitro* work to the *in vivo* situation. The recent development of techniques that permit stress responses to be studied directly in the *in vivo* situation has accelerated the pace of this field.

An overview of gene regulatory processes reveals controls that operate at a 'local' level (to regulate individual promoters, etc.) and others that play a more general or 'global' role. This report deals with regulatory mechanisms of the latter class. It is concerned with determinants of the topology of bacterial DNA, each of which has been shown *in vitro* to be capable of modulating the transcriptional profile of the cell. These are DNA gyrase, a type II topoisomerase that introduces negative supercoils into DNA, an activity that is unique to prokaryotes; DNA topoisomerase I, an enzyme that relaxes DNA and which acts antagonistically to gyrase; integration host factor (IHF), a sequence-specific DNA-binding protein that places 180° bends into DNA and modulates the function of many bacterial promoters, as well as influencing transposition, site-specific recombination, DNA replication and other DNA transactions; H-NS, a nucleoid-associated protein that binds DNA in a sequence-independent manner (it is thought to recognize and bind at, or close to, regions of intrinsic curvature in DNA) and can regulate transcription from a large number of promoters (almost always negatively) as well as influencing other DNA reactions such as site-specific recombination. In addition to the genes coding for these global regulators, this study also encompasses two well-characterized stress response promoters, those of the plasmid-located *spv* virulence genes and of the chromosomally linked *dps* gene that expresses a DNA protection system in starved cells.

2. DNA TOPOISOMERASES

Gyrase is composed of two subunit proteins, GyrA and GyrB, and has an A_2B_2 tetrameric structure. It requires ATP to supercoil DNA negatively (Gellert *et al.* 1976) and this requirement links gyrase activity to the physiological state of the cell. Thus, when bacteria experience certain stresses, such as an upshift in osmolarity or a transition from aerobic to anaerobic growth, gyrase activity is altered and the result is a change in the level of supercoiling in the DNA (Dorman *et al.* 1988; Higgins *et al.* 1988; Hsieh *et al.* 1991*a*,*b*; Jensen *et al.* 1995; Van Workum *et al.* 1996). Supercoiling imparts free energy to DNA and

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this drives structural transitions in the DNA helix, including open complex formation at certain promoters (Drlica 1992). In this way, many promoters located in different parts of the genome can respond simultaneously to an alteration in the external environment. The promoters of the gyrase genes are supercoiling responsive. They are inhibited by increases in negative superhelicity but become induced when DNA is relaxed (Menzel & Gellert 1983, 1987*a*,*b*). Thus, gyrase expression can respond to changes in supercoiling at the level of transcription and gyrase activity can respond to fluctuations in the [ATP]:[ADP] ratio at the level of topoisomerase activity.

Another gene with a supercoiling-sensitive promoter is *topA*, which codes for topoisomerase I (Tse-Dinh 1985). This monomeric type I topoisomerase relaxes DNA that has become supercoiled past a critical point (Wang 1971). It is thought that the countervailing activities of topoisomerase I and DNA gyrase establish a homeostatic supercoiling balance in the cell (DiNardo *et al.* 1982; Menzel & Gellert 1983). Topoisomerase I does not have an ATP requirement and uses energy stored in the negatively supercoiled DNA to drive the DNA relaxation reaction (Drlica 1992). The *topA* promoter is activated when DNA supercoiling increases and is inhibited by relaxation (the opposite of the *gyr* gene promoters) (Tse-Dinh 1985).

3. INTEGRATION HOST FACTOR

The IHF is encoded by two unlinked genes, *ihfA* and *ihfB*. The protein has a heterodimeric AB structure and binds to the consensus sequence WATCAANNNNTTR (where W is a pyrimidine, R is a purine and N is any base). IHF binding introduces a bend of up to 180° at the binding site and this is thought to be central to its biological role (Ellenberger & Landy 1997; Rice *et al.* 1996; Travers 1997). It promotes long-range interactions in DNA and between proteins bound at distant sites on the same DNA molecule (Goosen & Van de Putte 1995; Nash 1996). IHF is required for the formation of nucleoprotein complexes such as the lambda intasome (Goodman & Nash 1989; Snyder *et al.* 1989) and the type 1 fimbrial invertasome (Blomfield *et al.* 1997; Dorman & Higgins 1987; Eisenstein *et al.* 1987), both in *Escherichia coli*. Expression of the *ihf* genes is subject to complex control; they respond to growth phase, RpoS, guanosine tetraphosphate and are subject to autoregulation (Aviv *et al.* 1994). (RpoS is an alternative sigma factor used by RNA polymerase when bacteria undergo stress (Hengge-Aronis 1996); guanosine tetraphosphate is an alarmone that is synthesized by bacteria during starvation (Cashel *et al.* 1996).) Furthermore, the *ihfA* gene is part of an operon with the phenylalanine tRNA synthetase genes, *pheST*. Although *ihfA* has its own promoter, it is also subject to coregulation with *pheST* (Mechulam *et al.* 1987; Miller 1984).

4. PROTEIN H-NS

Protein H-NS is a component of the bacterial nucleoid. It binds to DNA and can constrain supercoils. Its oligomeric structure is a matter of disagreement in the literature, although it is probably at least tetrameric (Spurio *et al.* 1997). H-NS can form heteromeric complexes with a closely related paralogue, StpA (Cusick & Belfort 1998), and this may be important for the biological roles of both proteins (Dorman *et al.* 1999; Zhang *et al.* 1996). H-NS influences the transcription of many genes. Usually it acts as a repressor and the genes under its control usually have other, specific regulators (Atlung & Ingmer 1997; Bertin *et al.* 1999; Ussery *et al.* 1994; Williams & Rimsky 1997). Some of these are transcription activators, which directly oppose the negative influence of H-NS (Jordi *et al.* 1992). H-NS-responsive genes have little in common, apart from a general contribution to the ability of the bacterium to adapt to environmental stress. The mechanism of action of H-NS is a matter of controversy, and it is likely that more than one mechanism of gene regulation is employed. In several cases an association of H-NS binding with a region of DNA curvature has been reported (Yamada *et al.* 1991), but data from *in vitro* experiments have caused the importance of curvature in H-NS binding to be questioned (Jordi *et al.* 1997).

5. *SALMONELLA TYPHIMURIUM*

S. typhimurium is a facultative intracellular pathogen and is a useful model for studying gene expression during *in vivo* growth (Finlay & Falkow 1997; Gulig 1996; Jones 1997). A great deal of research has been conducted *in vitro* into the response of *S. typhimurium* to stress at the level of gene expression (Alpuche-Aranda *et al.* 1992; Foster & Spector 1995; Groisman & Saier 1990). Recently, this work has been extended by a number of *in vivo* studies using reporter gene fusions to stress-regulated promoters. These investigations have identified several genes as being activated during *in vivo* growth or as coding for products that are essential for survival while the bacterium is within the host (Heitoff *et al.* 1997; Hensel *et al.* 1995; Valdivia & Falkow 1997).

Investigations performed with bacteria grown *in vitro* have illustrated the contributions made to stress responses by genes whose products modulate DNA topology (Dorman 1995). We wished to study the responses of these genes to intracellular growth as a first step in elucidating their involvement in bacterial adaptation to *in vivo* growth. The work was carried out in the murine macrophage-like J774A.1 cell line (American Type Culture Collection, Manassas, VA, USA), which has been used extensively for the study of intracellular growth (Buchmeier *et al.* 1993; Francis & Gallagher 1993; Rhen *et al.* 1993; Uchiya *et al.* 1999; Wilson *et al.* 1997). Survival in macrophage requires the bacteria to survive several environmental assaults, including oxidative stress, acid stress and cationic peptides (Foster & Spector 1995; Francis & Gallagher 1993; Groisman 1994; Para-Lopez *et al.* 1994). The virulent SL1344 strain of *S. typhimurium* was used because it was fully virulent and was therefore capable of expressing all of the factors required for macrophage survival (Hoiseth & Stocker 1981). The response of *S. typhimurium* to signals encountered in macrophage includes a role for the pleiotropic regulatory proteins PhoP/PhoQ. This two-component signal transduction system controls members of a large regulon of genes negatively or positively in response to intracellular signals (García Véscovi *et al.* 1996, 1997; Groisman 1994; Groisman *et al.* 1997; Gunn & Miller

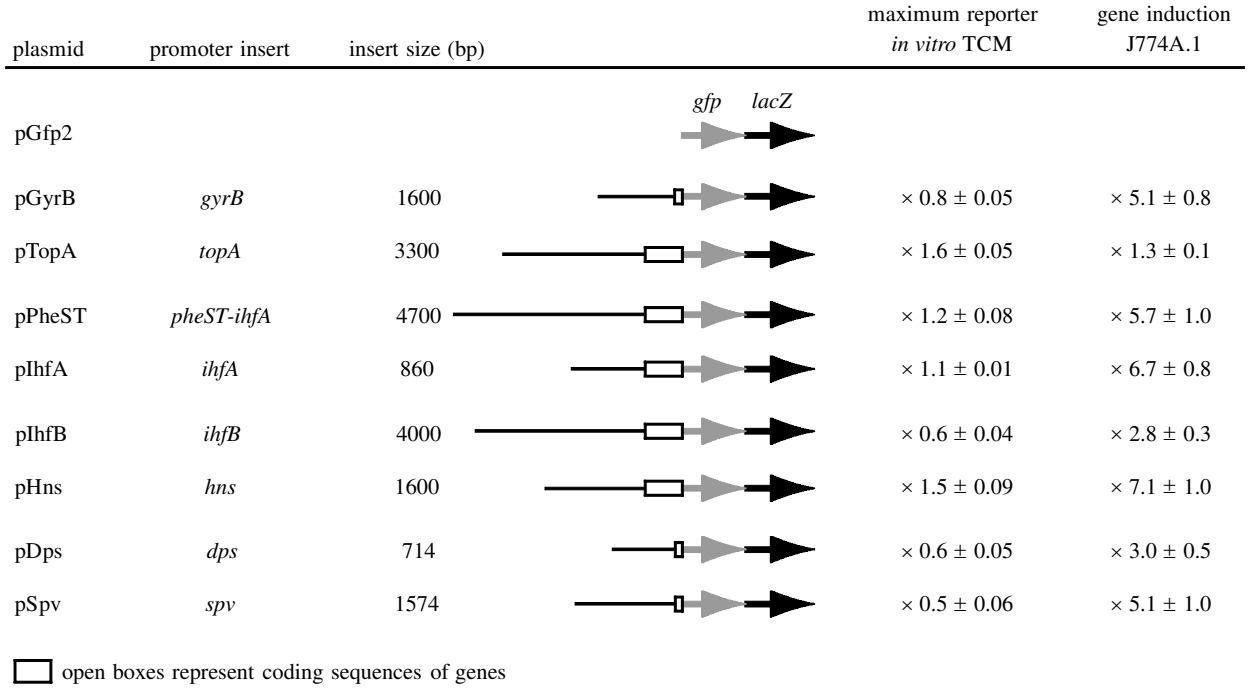

Figure 1. A schematic representation of the different promoters assessed for macrophage gene expression in plasmid pGfp2 is shown (not to scale). Plasmid pGfp2 contains contiguous *lacZ* and *gfp* reporter genes downstream of a multiple cloning site (Marshall *et al.* 2000). Promoter fragments were amplified with the primer combinations detailed in table 1 (sites engineered into primers are underlined and named in parentheses). Following cleavage with restriction endonucleases the fragments were cloned into similarly cleaved pGfp2 in a direction driving reporter gene expression. Open reading frames of genes assessed are indicated by open boxes. Maximal induction levels are shown as fold increase in LacZ expression, similar induction profiles were recovered when assaying for GFP intensity (data not shown). The LacZ levels were assessed using a chemiluminesence assay as previously described (Marshall *et al.* 2000). GFP intensity levels were determined using a FACScan (Becton Dickenson, Oxford, UK) with argon lasers emitting at 488 nm and bacteria were detected by side scatter as previously described (Valdiva *et al.* 1996). The mean fold induction and standard deviations were calculated from a minimum of three independent experiments.

1996; Soncini & Groisman 1996; Waldburger & Sauer 1996). Under *in vitro* growth conditions, specific regulators of this type cooperate with the more global influences of DNA topology to modulate the transcriptional profile of the cell (Dorman 1995). Therefore, we wished to ascertain if determinants of DNA topology formed part of the bacterium's response to intracellular growth.

6. *spv* AND *dps* GENES

In addition to the genes involved in the regulation of DNA topology, this study included two *S. typhimurium* promoters shown previously to be activated in J774A.1 cells. These were from the *spv* and the *dps* genes, each of which has been studied in detail previously *in vitro* and *in vivo* (Marshall *et al.* 2000). The *spv* genes are located on a 90 kb virulence plasmid in the non-typhoid serovars of *Salmonella* (Guiney *et al.* 1995; Gulig 1996; Libby *et al.* 1997). They are required for the establishment of a systemic infection in the host and have been shown by signature-tagged mutagenesis and *in vivo* expression technology to be required for full virulence and to be expressed during infection (Heitoff *et al.* 1997; Hensel *et al.* 1995). The *spv* locus is composed of a regulatory gene, *spvR*, coding for a LysR-like transcription activator that positively regulates both its own gene and the *spvABCD* operon of structural genes (Fang *et al.* 1991, 1992; Krause *et al.* 1992; Guiney *et al.* 1995; Pullinger *et al.* 1989; Sheehan & Dorman 1998). Activation of *spv* transcription occurs during stationary phase *in vitro* and during *in vivo* growth (Chen *et al.* 1995; Kowartz *et al.* 1994). It requires the RpoS stress-response sigma factor, and is modulated by IHF, the leucine-responsive regulatory protein (Lrp), L-leucine, H-NS and cAMP-Crp (the latter probably acting indirectly through its effect on RpoS expression) (Marshall *et al.* 1999; O'Byrne & Dorman 1994*a*,*b*; Robbe-Saule *et al.* 1997). In addition, *spv* requires a negatively supercoiled DNA template for transcription *in vitro* (O'Byrne & Dorman 1994*b*; Marshall *et al.* 1999).

The *dps* gene is located on the bacterial chromosome, and like *spv*, it requires the RpoS sigma factor and IHF for full expression; unlike *spv*, *dps* is under the control of the OxyR regulator, a redox-sensitive DNA-binding protein (Altuvia *et al.* 1994). Dps protein is produced by starving bacteria, it co-crystallizes with the bacterial DNA and this is thought to protect the nucleic acid from damage while the bacteria are in stationary phase (Wolf *et al.* 1999). The *dps* promoter has a similar induction profile to that of *spv* when *S. typhimurium* is growing intracellularly and both promoters have been used successfully to express heterologous antigens in live attenuated vaccine strains (Marshall *et al.* 2000).

7. EXPERIMENTAL RESULTS AND DISCUSSION

Each promoter was cloned from the *S. typhimurium* genome by the polymerase chain reaction using standard methods, it was sequenced to ensure its structural integrity,

Table 1. *Primers (5′–3′) used for the PCR amplification of plasmids*

plasmid	primer name	primer sequence	restriction enzyme recognition site[a]
pDps	dps up	CGCGGATCCTATATATTCTTACCGG	(*Bam*HI)
	dps start	CGCGGATCCAATCTCATATCCTCTTGATG	(*Bam*HI)
pTopA	topA cds	CGGGGTACCGGATCAATCCCCATACG	(*KpnI*)
	topA up2	CGCGGATCCTTTGCCGGTATGTACGACGCC	(*Bam*HI)
pGyrB	gyrB up	ACATGCATGCTGCTTTCACAACGAAGCC	(*Sph*I)
	gyrB start	CGGGGATCCCTTGTCGAAGCGCGCTTTCTCG	(*Bam*HI)
pHns	hns up	CGCGGATCCACTGTCTGAAGATGCCTTCG	(*Bam*HI)
	hns cds	CGCGGATCCCTTCAACGCTTTCCAGAGTAC	(*Bam*HI)
pPheST	ihfA cds	CGCGGATCCAACAGATATTCTGACATTTCAGC	(*Bam*HI)
	ihfC	ACATGCATGCAGGCCGTCAGATGATCATGG	(*Sph*I)
pIhfB	ihfB cds	CGCGGATCCCGTCTTGGCGGGAATGTGC	(*Bam*HI)
	ihfB up	CGCGGATCCTGGCACGTACAACGACCACC	(*Bam*HI)
pIhfA	ihfA2 val up	CGCGGATCCGTTTACGTTCCAGTTCAGGG	(*Bam*HI)
	ihfA2 val cds	CGCGGATCCAACAGATATTCTGACATTTCAGC	(*Bam*HI)
pSpv	spv up	CGCGGATCCAACAGGTCAATTAAATCC	(*Bam*HI)
	spv cds	CCCGGATCCCCTGAAAATAAACAGAATGAAATCC	(*Bam*HI)

[a] The location of the site in the primer sequence is shown by underlining.

and then fused to promoterless copies of the *gfp*-M2 gene from *Aequorea victoria* (Cormack *et al*. 1996) and the *lacZ* reporter gene from *E. coli*, carried in the low copy number plasmid pQF50 (Farinha & Kropinski 1990) derivative, pGfp2 (figure 1). This plasmid offered two reporters, green fluoresence encoded by *gfp* and β-galactosidase encoded by *lacZ*. In the experiments described here, data obtained from monitoring *lacZ* expression are presented (figure 2), although all of the results were validated by *gfp* expression (data not shown). *S. typhimurium* is naturally deficient in the *lacZ* gene so it was possible to monitor the levels of its product, β-galactosidase, from pGfp2 derivatives harboured in SL1344 strains while these were growing *in vitro* or *in vivo*. β-galactosidase expression was measured using the chemiluminescent substrate Galacton Star (Clontech, Basingstoke, UK). The cloning strategy and the standard β-galactosidase assay employed have been described elsewhere (Marshall *et al*. 2000). Details of individual plasmid constructions are summarized in figure 1.

J774A.1 macrophage-like cells grown in tissue culture medium (TCM) were infected with SL1344 bacteria at a ratio of ten bacteria per macrophage. Following infection, treatment with gentamycin ($20\,\mu g\,ml^{-1}$) was used to kill bacteria that had not been engulfed by the J774A.1 cells. Procedures for the growth of J774A.1 and SL1344 cells and the monitoring of *in vivo* gene expression have been described elsewhere (Marshall *et al*. 2000). The bacteria were also inoculated into macrophage-free TCM to determine background levels of gene expression.

Topoisomer analysis of reporter plasmid supercoiling was used to determine the effects of intracellular growth on DNA topology. The multicopy plasmid pUC18 was introduced into strain SL1344 and this strain was used to infect J774A.1 macrophage-like cells. The pUC18 DNA was recovered from the infected cells and examined electrophoretically for changes in supercoiling. Due to the low yield of plasmid DNA from the intracellular bacteria, the chloroquine–agarose gels were hybridized with a digoxygenin-labelled pUC18 probe using the labelling and detection procedures of Free & Dorman (1997). For electrophoresis in one dimension, gels contained $2.5\,\mu g\,ml^{-1}$ chloroquine; under these conditions those topoisomers that had been more supercoiled in the bacteria migrated fastest in the gel (figure 3). For electrophoresis in a second dimension, the topoisomers were passed through a gel containing $20\,\mu g\,ml^{-1}$ chloroquine. Here, topoisomers that were more relaxed in the first dimension formed an arc of positively supercoiled topoisomers migrating above the arc of negatively supercoiled topoisomers (Higgins *et al*. 1988; Wu *et al*. 1988) (figure 3).

Plasmid DNA isolated from *S. typhimurium* SL1344 cells that had been incubated in TCM alone showed a broad distribution of topoisomers similar to that seen in extracts from bacteria grown in standard laboratory media (Dorman *et al*. 1988; Higgins *et al*. 1988) and this distribution varied by just one or two topoisomers over time (figure 3*b*). In contrast, plasmid DNA removed from SL1344 that had infected J774A.1 macrophage-like cells showed a distribution that became progressively relaxed with time and included a novel fast-migrating species that increased in intensity with time spent in the macrophage (figure 3*a*). Electrophoresis with different concentrations of the chloroquine intercalator and two-dimensional electrophoresis (figure 3*c*) showed that this species was composed of positively supercoiled DNA, i.e. plasmid DNA that had been highly relaxed in the bacteria.

The discovery that *in vivo* growth led to the production of a population of highly relaxed plasmids raised the possibility that bacterial DNA lost negative supercoils as a result of life within the macrophage. This finding is analogous to the effect of starvation on bacteria grown *in vitro* (Balke & Gralla 1987) and normally elicits a response at the level of gene transcription. In particular, genes required to compensate for the change in DNA topology might have been expected to have been induced, whereas genes whose products are unhelpful would not. We studied the response to intracellular growth of a panel of *S. typhimurium* promoters likely to be involved in adaptation to DNA relaxation.

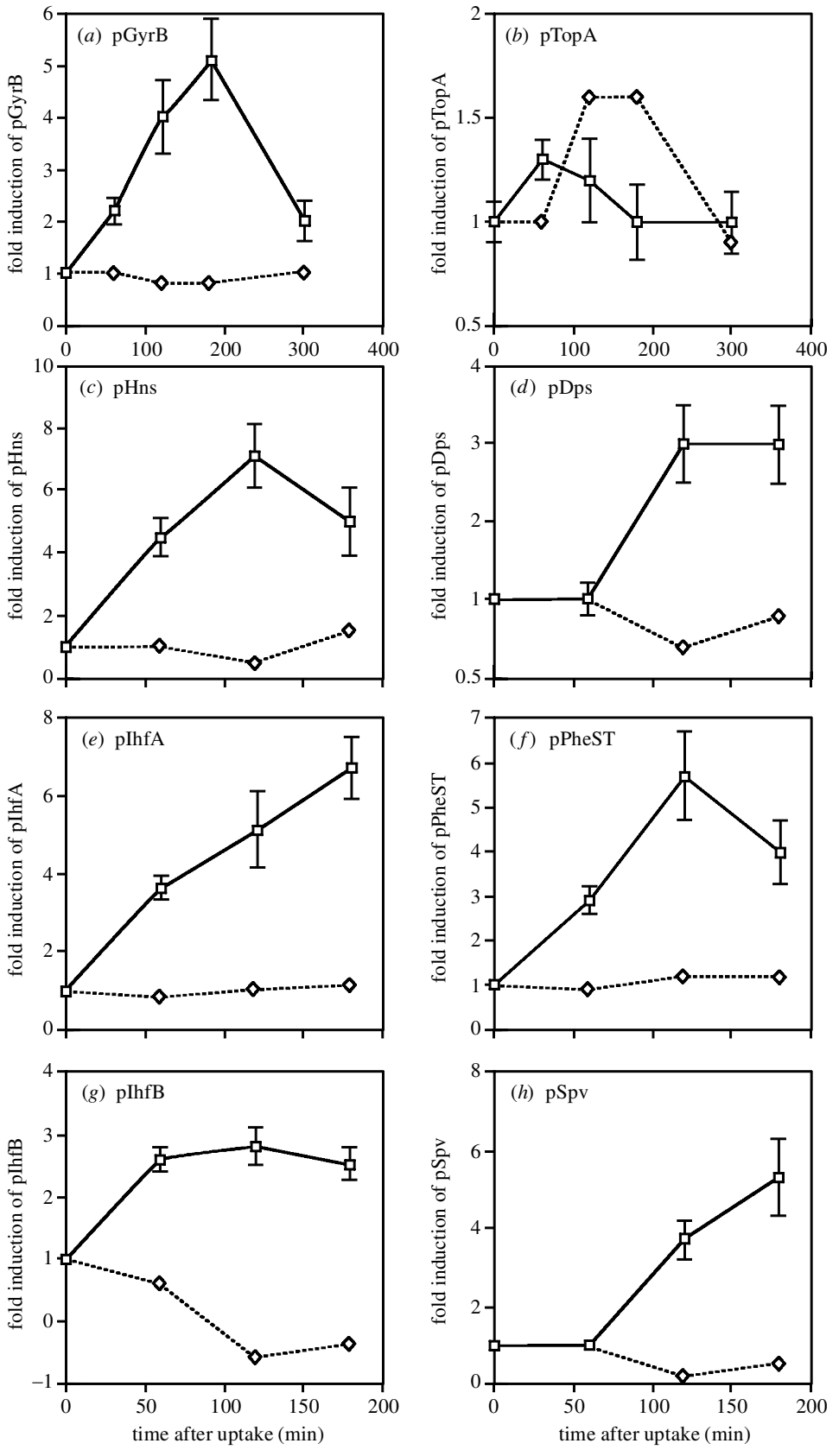

Figure 2. Induction kinetics for *S. typhimurium* promoters studied *in vivo* following uptake by J774A.1 macrophages. Macrophage infection and LacZ assays were performed as described previously (Marshall *et al*. 2000). β-galactosidse activity was expressed as light units released per viable bacterium (light units per colony forming unit) following infection relative to the activity in the inoculum sample, and is the mean of at least three independent experiments for studies with J774A.1 cells. Squares, data points for intracellular bacteria; diamonds, data points for bacteria in TCM alone.

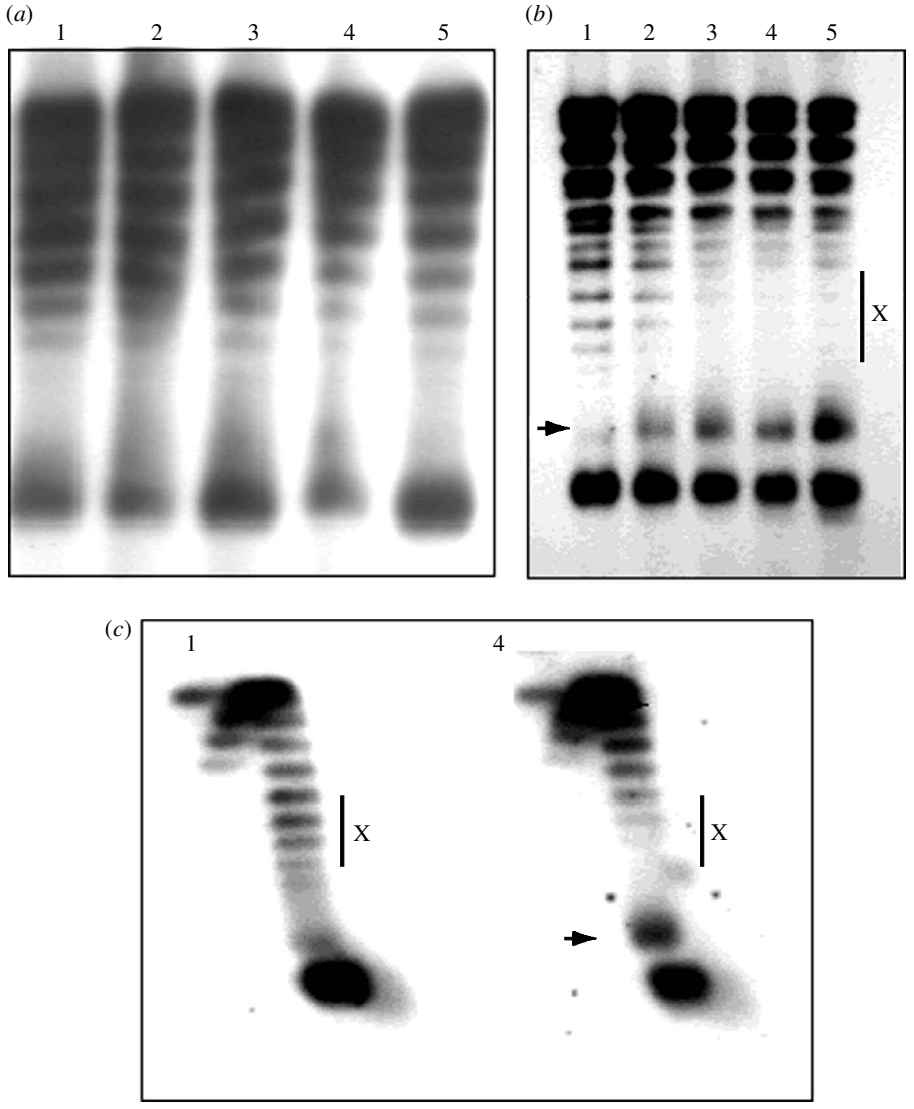

Figure 3. DNA supercoiling in intracellularly grown *S. typhimurium*. (*a*, *b*) One-dimensional agarose gel electrophoresis through 1% (w/v) agarose gel in 1 × tris borate (TBE) containing 2.5 mg chloroquine ml^{-1}. Topoisomer distribution in inoculum sample (lanes 1); after 40 min (lanes 2); after 80 min (lanes 3); after 120 min (lanes 4); after 180 min (lanes 5). (*a*) Topoisomer distribution of pUC18 following incubation of SL1344(pUC18) in TCM in 5% CO_2 at 37 °C. (*b*) Topoisomer distribution of plasmid pUC18 before and following infection of macrophages with SL1344(pUC18). (*c*) Two-dimensional agarose gel electrophoresis through 1% (w/v) agarose gel in 1 × TBE, containing 2.5 µg chloroquine ml^{-1} in the first dimension and 20 µg chloroquine ml^{-1} in the second dimension. Sample 1, pUC18 topoisomer distribution in *S. typhimurium* inoculum sample (corresponds to lane 1 of *b*); Sample 4, pUC18 topoisomer distribution in *S. typhimurium* following infection of J774A.1 macrophages for 120 min (corresponds to lane 4 of *b*). The distribution of more negatively supercoiled topoisomers present in the inoculum but absent in the intracellular bacteria is highlighted (X) in *b* and *c*. The novel fast migrating species unique to the intracellularly recovered bacteria is arrowed in *b* and *c*.

DNA gyrase would be expected to play a prominent role in the restoration of lost negative supercoiling. To do this it would require an upward shift in the [ATP]:[ADP] ratio. Even in the absence of sufficient ATP (as in starving cells) to allow gyrase to function in negative supercoiling, the *gyr* gene promoters would be expected to be induced directly by DNA relaxation. In TCM alone, the *gyrB* promoter showed no induction during a 180 min incubation. In contrast, it was induced by approximately fivefold when the bacteria were inside macrophage for the same period (figure 1). This was in agreement with the known behaviour of the equivalent promoter from *E. coli* when studied *in vitro* (Menzel & Gellert 1983, 1987*a*,*b*). In contrast, the promoter for the *topA* gene (encoding the DNA relaxing enzyme topoisomerase I) showed no induction in TCM or in macrophage (figure 1). Since the *topA* promoter from *E. coli* is known to be inhibited by DNA relaxation, this finding was in keeping with the plasmid topoisomer data described above.

Other studies have suggested that the IHF may play a role in maintaining DNA topology in a form favourable to certain promoters under conditions where a general DNA relaxation takes place (Porter & Dorman 1997). *In vitro*, this relaxation occurs in stationary phase, and IHF levels have been shown to increase during this period of the growth curve, at least in *E. coli* (Ditto *et al.* 1994). An earlier study had shown that one of the genes coding for IHF, the *ihfA* gene (designated *mig-23* for '*m*acrophage-*i*nducible

*g*ene 23'), was possibly induced when *S. typhimurium* grows intracellularly (Valdivia & Falkow 1987). In that study, the recombinant plasmid tested contained just a segment of the *pheT* gene where the *ihfA* promoter was located; it had not included the complete *pheST* operon and its promoter as well as that of *ihfA*. We constructed a plasmid that included the complete *pheST-ihfA* operon and obtained an approximately fivefold induction in the J774A.1 cells with no induction in TCM alone (figure 1). To resolve the issue of the possible contribution of the *pheST* promoter, a derivative plasmid was made in which the expression of the reporter genes was under the control of the *ihfA* promoter alone. The data obtained with this plasmid showed that *ihfA* was induced approximately sixfold during intracellular growth but not in TCM alone (figure 1). This shows that the *ihfA* promoter responds to growth in macrophage and this response is not modulated by a contribution from the *pheST* promoter. The promoter from the *ihfB* gene was also tested and found to be inducible intracellularly but not in growth medium alone (figure 1). (The response of the *ihfB* promoter to intracellular growth had not been assessed previously.) These data for *ihf* gene expression are in keeping with results obtained from *in vitro* studies showing that these genes are induced when bacteria are starved or undergo environmental stress (Aviv *et al.* 1994; Ditto *et al.* 1994).

Successful establishment of an infection requires the bacterium to multiply in the intracellular state (Finlay & Falkow 1997). Previous work performed *in vitro* with *E. coli* has shown that expression of the nucleoid-associated protein H-NS is coupled to DNA synthesis such that in rapidly growing bacteria the *hns* gene is induced while in quiescent cells it is repressed (Free & Dorman 1995). The *hns* promoter was induced strongly in *S. typhimurium* cells growing in macrophage and not induced in TCM alone (figure 1). This suggests that while the bacteria may have been stressed when associated with the J774A.1 cells, they were still able to operate an efficient cell cycle, thus creating a demand for *hns* gene expression. A previous study has indicated that intracellular *S. typhimurium* cells grow rapidly and require protein synthesis for survival. As the bacteria adapt to the intracellular environment of the macrophage, they switch from this rapid growth mode to a survival state that does not require protein synthesis (Abshire & Neidhardt 1993). The kinetics of *hns* induction revealed here, with expression declining 2 h post-infection (figure 2), concurs with the findings of this earlier study.

As positive controls for the experiments described here, the *dps* and *spv* promoters were also studied. Each had been shown previously to be induced during intracellular growth (Marshall *et al.* 2000) and induction was also detected in this study (figures 1 and 2).

This study addresses a specific aspect of bacterial physiology during infection, namely what are the consequences of the infection process for the topology of bacterial DNA and for the expression of genes coding for proteins that modulate DNA topology? It appears that infection of the J774A.1 cell line causes bacterial DNA to become relaxed. As one might predict, the relaxation-inhibited promoter of the *topA* gene remains repressed under these conditions. In contrast, the *gyrB* gene, coding for one of the subunits of DNA gyrase, is induced strongly. This is consistent with the production of extra copies of gyrase to restore supercoiling levels to values favourable to most DNA-based molecular transactions. The level of *gyrB* expression declines after 3 h, suggesting that by this stage post-infection supercoiling is restored at the *gyrB* promoter (figure 2).

The *spv* promoter used in this study illustrates some of the complexities arising from our data. It is supercoiling responsive and has been shown previously to be inhibited when DNA becomes relaxed (Marshall *et al.* 1999). However, *in vitro* data show that this inhibition requires the IHF protein; in the absence of this accessory factor, *spv* expression remains high even when DNA is relaxed (Marshall *et al.* 1999). This points to a subtle interplay between IHF and gyrase in the regulation of *spv* transcription. The *in vivo* data presented here show that *spv* is induced when the global level of DNA supercoiling is low. This may point to a shortage of IHF *in vivo* (consistent with the strong induction of the genes that code for this protein) or simply point to additional complexities inherent in the *in vivo* situation. For example, the leucine-responsive regulatory protein (Lrp) also regulates *spv* expression *in vitro* (Marshall *et al.* 1999) and this may have a positive modulatory influence when the bacterium is growing in association with macrophage. Alternatively, the negative influence of H-NS on *spv* expression described previously *in vitro* (O'Byrne & Dorman 1994*b*; Robbe-Saule *et al.* 1997) may not operate under the *in vivo* conditions studied here, and this is consistent with our data on *hns* gene induction in macrophage (figures 1 and 2) suggesting that not all H-NS-binding sites in the bacterial genome are occupied.

The data presented here allow a tentative model to be advanced to describe early events in intracellular adaptation from the perspective of DNA topology. Three lines of evidence suggest that DNA becomes relaxed early in the process. These are a direct observation of reporter plasmid topology by electrophoresis in the presence of a DNA intercalator, the positive response of the DNA relaxation-activated *gyrB* promoter, and the lack of a response from the DNA relaxation-inhibited *topA* promoter. Why does the DNA become relaxed? The most likely explanation is that the negative supercoiling activity of bacterial gyrase is inhibited as a result of interactions with the mammalian cells. This could arise from an unfavourable shift in the [ATP]:[ADP] ratio. As ATP levels fall and ADP levels rise, the negative supercoiling activity of gyrase is inhibited, although it can continue to contribute to ATP-independent relaxation of DNA (Drlica 1992). DNA topoisomerase I, and to some extent the other two topoisomerases in the cell, might also be expected to contribute to the general relaxation of genomic DNA. The induction of the genes encoding IHF may be regarded as a mechanism for dealing selectively with unfavourable alterations in DNA topology. Those promoters with appropriately located IHF-binding sites (such as *spv*) may be protected from inhibition as the DNA relaxes through the creation of a nucleoprotein complex that preserves promoter function. Other IHF-dependent processes, such as DNA replication, transposition and site-specific recombination, may also continue to function. Other evidence suggesting that DNA replication continues under the conditions of our experiments comes

from the observation of *hns* gene expression. A demand for *hns* transcription has been shown *in vitro* to be created by DNA synthesis (Free & Dorman 1995) and the *in vivo* data presented here suggest that this process operates during the macrophage infection. This is consistent with the data of Abshire & Neidhardt (1993) showing that vigorous protein synthesis occurs for approximately 2 h following macrophage engulfment of *S. typhimurium*. The overall picture that emerges is one of stressed bacteria with abnormally relaxed levels of DNA supercoiling that continue to grow and express genes that are critical for adaptation to this environment. This situation is in marked contrast to that described for invasion of epithelia by *S. typhimurium*. Their increased negative supercoiling is an essential prerequisite for expression of invasion functions (Galán & Curtiss 1990). Thus, DNA topology contributes to different types of host–pathogen interaction, and it does so in a dynamic way. Understanding how transitions between the different phases of the infection affect DNA topology and gene expression will form the next phase of the work.

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