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Bacterial transcription factors involved in global regulation†

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Summary

The presence of intricate global cell regulation mechanisms may be one reason for the exceptional environmental and evolutionary success of microbes. Promoters, the *cis*-acting signals, are responsive to several stimuli related to growth, stress and substrate specificity. Their response is mediated by a wide variety of *trans*-acting regulators that sense the environment and the physiological state of the cell and adjust the transcription of specific genes. One of the main transcriptional regulation webs operates in the transition from affluent to barren conditions, with σ^S being the chief actor in a company of players that stage a competition for the sparsely available RNA polymerase molecules. In this role, σ^S may be assisted by several factors, including nucleoid-related proteins and metabolites. In addition, the levels of σ^S itself are regulated by mechanisms that include inactivation and degradation. Several transcription factors, belonging to different regulatory pathways, may operate in the same promoter. In such a case, the final transcriptional output depends both on the interplay of effectors and on the properties of the recruitment of the effector–RNA polymerase complex to the promoter. RNA polymerase itself is also capable of establishing selective interactions with activators and specific promoter regions through the carboxy-terminal domain of its alpha subunit (αCTD). Transcriptional regulation controls pervade such crucial events in the life of bacterial cells as *Escherichia coli* cell division, *Bacillus subtilis* sporulation and *Caulobacter crescentus* differentiation. These examples suggest that bacteria have been particularly inventive in adapting gene expression regulation to survive under a diversity of environments and have done so by exploiting the malleable molecular mechanisms involved in transcription, developing complexities that may match those found in eukaryotic cells.

Received 23 February, 1999; revised 23 March, 1999; accepted 29 March, 1999. †We dedicate this report to the memory of Professor Eladio Viñuela whose teachings influenced the careers of many Spanish molecular biologists. *For correspondence. E-mail mvicente @cnb.uam.es; Tel. (+34) 91 585 46 99; Fax (+34) 91 585 45 06.

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Introduction

Despite, or more likely because of, their constrained dimensions, bacteria are very successful life forms, different species being able to colonize the most extreme and diverse environments. The comparative simplicity of bacterial cells is reflected in their genomic sizes: a free-living *Mycoplasma* may cope with life with just 1% of the number of genes that a human cell needs to carry. As bacterial genes generally lack introns, the economy in genetic material is even more remarkable: one human cell contains more DNA than 1000 *Escherichia coli* cells. Nevertheless, microbes manage their limited amount of genetic information in an exquisite manner, exploiting all imaginable devices at the molecular, physiological and cellular levels to survive and proliferate. However, the basic mechanisms used by microbial cells to manage their small and compact genetic information involve principles similar to those found in their eukaryotic counterparts. The description of the molecules involved in the initiation of transcription, in modifying the architecture of DNA and in monitoring the global state of the cell comprised the first part of the Juan March Workshop on 'Bacterial Transcription Factors involved in Global Regulation' (Madrid, 10–13 June, 1998). How these mechanisms operate at the cellular level during the growth and survival of bacterial populations was discussed in the second part, culminating in descriptions of the regulatory circuits operating during cell division, differentiation and sporulation.

Although 4288 potential genes have been recognized in *E. coli* (some 30% with unassigned function; Blattner *et al*., 1997), the number of RNA polymerase molecules per cell is calculated to be only about 2000. The regulatory networks of the bacterial cell manage this paucity in the amount of

transcriptional enzyme in a very efficient manner. At least one-third of the molecules in the polymerase core enzyme population are not engaged in transcription, forming a pool that can be called into action in response to environmental changes. Free core RNA polymerase molecules can be directed to transcribe specific regulons by association with an assortment of alternative sigma factors (probably seven in *E. coli*), which are themselves expressed under specific circumstances. Some of these may be sequestered by complementary antisigmas until needed. R. Burgess reported that the detailed nature of sigma–core interactions is beginning to be elucidated (Arthur and Burgess, 1998). In *E. coli*, nearly 100 transcription factors can modify the expression of specific genes by establishing intermolecular contacts with DNA and subunits of RNA polymerase. In addition to the dedicated transcription factors, most of which are present in comparatively low abundance, members of the class of abundant DNA-binding proteins (IHF, Fis, HU, H-NS . . .) contribute to transcription regulation by modifying the architecture of many regulatory regions, bringing together in three-dimensional space DNA sequences that otherwise would not be so near to each other (Pérez-Martín and de Lorenzo, 1997).

From genes back to physiology

As bacteria have been around for a minimum of 3000 million years (compared with the much younger eukaryotic cells: only 1000 million), we have every reason to believe that virtually all basic molecular mechanisms that support life were first passed through a tough test of performance in a prokaryotic host. DNA replication and recombination, RNA synthesis, transcription and translation are the basic building blocks that support all other biological processes. In this context, bacteria are not just models for understanding more complex biological problems: they are both the problem and the experimental system for finding answers. Let us consider the issue of regulation of gene expression, not only with regard to the control of specific promoters in a bacterial cell, but also as a global phenomenon. The last few years have witnessed various amazing and emerging issues in prokaryotic transcription, which include, but go much beyond, the understanding of how simple promoters work. Promoters do not function in isolation; they are subjected to various types of physiological controls, which exquisitely adjust their transcriptional output to the general environmental conditions of the cells (Cases and de Lorenzo, 1998). These mechanisms subordinate the expression of individual promoters to the overall growth status of the bacteria. After years of focusing exclusively on how promoters function, we face a growing need to understand the connection between gene expression and physiology. Unfortunately, some key tools for monitoring fine mechanisms *in vivo* are missing or not completely developed. β-Galactosidase fusions and primer extension assays are insufficient to understand promoters *in vivo* in a global context, and a considerable effort has to be invested in developing new (and preferably simple) techniques for examining promoter occupation by various factors and the interplay between them. Knowing how promoters work *in vitro* is not enough, and the literature is filled with examples of different behaviour of the same systems under different conditions (Abshire and Neidhardt, 1993). Recent developments in genomic arrays and DNA chips can contribute tremendously to addressing otherwise obstinate issues of global regulation, provided that these approaches go hand in hand with functional analysis and *in vivo* examination of the molecular mechanisms involved (Jenks, 1998).

Promoters through the growth curve

One key event in the life of bacteria in their natural habitats is the transition between feast and famine. Bacteria have to survive during periods of non-growth as well as being able to take advantage of optimal growth conditions. This involves processes in which evolution has been peculiarly inventive. *E. coli* promoters positively influenced by a rapid growth (typically those driving the expression of ribosomal rRNAs) have revealed interesting mechanisms of physiological regulation. At their highest growth rate, *E. coli* cells contain about 70 000 ribosomes (on average, each polysome contains several tens of ribosomes), and 60% of the transcription activity is dedicated to ribosomal synthesis. This changes dramatically when growth slows down: at a 5 h generation time, the number of ribosomes per cell is down to ≈2000. Amazingly, this gross reduction in ribosomal gene expression is achieved without any specific activator or repressor proteins.

Dissection of an *rrn* promoter region (Ross *et al*., 1998) has shown that its strength comes from several factors. As summarized by R. Gourse, it comprises two closely linked promoters, P1 and P2, each of which is preceded by short AT-rich sequences ('UP elements') that stimulate transcription (e.g. 60-fold in the case of P1 and over 325-fold in the case of the consensus UP element; Estrem *et al*., 1998) by contacting a universally conserved seven-amino-acid patch in the αCTD, the C-terminal domain of the α-subunit of RNA polymerase. Indeed, each UP element contains two subsites, one for each of the two holoenzyme subunits. Three FIS binding sites further upstream from the UP elements of *rrnP1* reinforce promoter strength. To attain the high potential promoter strength afforded by these features, the initiating nucleoside triphosphate must be very readily available – otherwise, the unusually short half-life of RNA polymerase–*rrnP* complexes (≈20 times shorter than that of RNA polymerase–*lacP* complexes) leads to their disintegration. Because of this, a reduction in the pool of the relevant NTP, such as is typically associated

with a slowing of growth rate, leads to a severe reduction in effective *rrnP* activity. This leads to a simple homeostatic model, which takes account of the consumption of ATP and GTP during translation: if the global level of translation outstrips the supply of ATP and GTP, the pools fall sharply, hence rapidly reducing *rrnP* activity to an appropriate level (Gaal *et al*., 1997). This contributes a new element to the 'stringent response' whose most well-known component is the signal molecule ppGpp. This highly phosphorylated nucleotide, whose ribosome-bound synthesis is classically activated when translation is slowed down by amino acid starvation, destabilizes RNA polymerase–promoter complexes. At promoters such as those for RNA, in which these complexes are already extremely unstable, this accounts for the inhibition of rRNA synthesis (Bartlett *et al*., 1998; Heinemann and Wagner, 1997).

Entry into stationary phase

E. coli also contains promoters that are directly controlled by growth phase or growth rate independently of the type of nutrients present in the medium. In other instances, the activity of promoters responsive to specific inducers is co-regulated by growth phase through a collection of mechanisms that have only recently started to be understood. When starvation or various other stresses cause a reduction or cessation in growth, many genes are shut down, while others are induced to help the cells to survive (Fig. 1). There is considerable overlap among starvation and stress regulons (Dukan and Nyström, 1998). In part, this is because starvation causes 'knock-on' stresses. For example, during carbon limitation, global biosynthesis ceases rapidly, whereas catabolism falls off more slowly. The resulting overflow through the respiratory chain is accompanied by an increase in the reactive oxygen species that are by-products of electron transfer. In these conditions, disulphide bonds have been detected in a cytoplasmic alkaline phosphatase, many proteins become carboxylated and fatty acids become partially oxidized. Presumably, to minimize such effects, various oxidative stress proteins, such as superoxide dismutase, catalases and peroxidases, are induced as stasis defence proteins. These belong to regulons controlled by several global regulators (including SoxRS, OxyR, FNR, FadR and ArcA). However, the most influential regulator of stasis-induced genes is a sigma factor, σ^S, encoded by *rpoS*, which directs the expression of nearly 100 genes.

The determining role of *rpoS* in the expression of enterobacterial genes involved in many cell functions during stationary phase has triggered a considerable interest in the various mechanisms involved. T. Nyström (Farewell *et al*., 1998) has shown recently that a mutation in *rpoS* (encoding σ^s) not only abolishes transcription of many stationary phase genes, but also causes elevated induction or loss of repression of some σ^D ($\equiv \sigma^{70}$, the product of *rpoD*)- and σ^H-dependent genes. These effects may occur because, during stasis, sigma factors compete for a limiting amount of core RNA polymerase, so that perturbations in the level of one sigma can significantly alter the expression of stasis-induced genes dependent on other sigma factors. Such sigma factor competition may be influenced by the stationary phase modification of a significant fraction of core RNA polymerase. Differential competition between F factors, both in stationary phase and during other stress responses, may also be affected by chromosome structure. An interesting example is the control of DNA superhelicity through the classical ATP-driven equilibrium of gyrase/topoisomerase activities and the action of various nucleoid-associated factors (H-NS, IHF, HU, Lrp, Fis and others) (Ussery *et al*., 1994). The $\approx 10^5$ molecules of H-NS help the *E. coli* cell to package over 4000 genes, each of which, on average, would span 25% of the entire length of the cell if extended! It is hard to imagine that this does not affect the transcription of many (if not

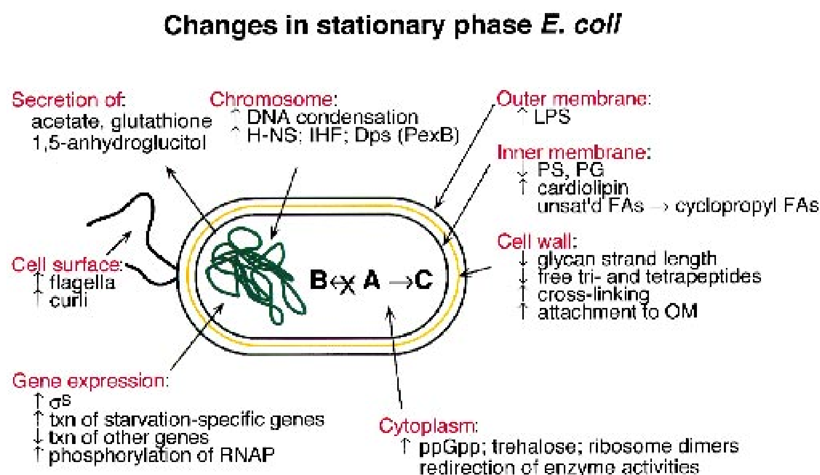

Fig. 1. Changes in *E. coli* cells entering into stationary growth phase take place at all the subcellular levels. This figure, presented by D. Siegele at the Meeting, is adapted from Huisman *et al*. (1996).

all!) genes. In spite of its importance, H-NS has not yet unveiled all its mysteries, and there is a considerable controversy about its structure, which is difficult to study because of the heterodisperse nature of the purified protein, and about whether or not it is covalently modified under different growth conditions.

Selection of promoter sequences by σ^S-RNA polymerase may not only depend on the nature or conformation of the target DNA or the assisting nucleoid-related proteins. Other factors or conditions *in vivo* can influence the selectivity of the holoenzyme or its performance, in particular intracellular solutes, such as K glutamate or acetate, trehalose, glycine-betaine, polyamines and even polyphosphate. As was discussed by C. Arraiano, different stresses in exponential phase can also induce alternative mechanisms that replace the selection of promoter sequences by σ^S-RNA polymerase (Santos *et al*., 1999). Simultaneous variations in the chemical composition of the cytoplasm and in the conformation of the nucleoid affect growth-dependent changes in transcription. Also, as mentioned above, ppGpp dramatically affects the performance of the RNA polymerase in a fashion dependent on nutrient availability (Krohn and Wagner, 1996). Furthermore, additional regulatory proteins (i.e. histone-like proteins) often bind σ^S-controlled promoters, enhancing the specificity of the holoenzyme for a given DNA sequence. In fact, RNA polymerase does not deal *in vivo* with linear, naked promoter sequences but with DNA in the context of a complex (and promoter-specific) three-dimensional nucleoprotein assembly. Marschall *et al*. (1998) have shown recently that such a complex is recognized much better by one form of the holoenzyme, even though both forms (with either σ^S or σ^D) might recognize naked promoter DNA equally well. Thus, these additional factors are crucial for generating sigma factor specificity at a given promoter (Hengge-Aronis, 1999). Owing to this wealth of mechanisms, it is not infrequent to find promoters subject to the simultaneous effects of various growth phase-related factors (such as Crp and FIS) or to a combination of growth phase and catabolite repression elements (Ishihama, 1997a; Cases and de Lorenzo, 1998; Rhodius and Busby, 1998).

But how are σ^S levels regulated? Although the expression of the protein is itself subjected to multiple levels of control, the regulation of its turnover seems to be predominant. Recent observations by R. Hengge-Aronis have unveiled the mechanism by which the response regulator named RssB is required for degradation of σ^S by the housekeeping ClpXP protease (Bouche *et al*., 1998). Systematic mutagenesis to change amino acids in σ^S that are not shared with σ^D has indicated that RssB not only promotes σ^S proteolysis, but also interferes directly with σ^S activity in the way that antisigma factors do. Furthermore, such mutagenesis has led to the identification of a proteolysis-promoting element in σ^S that is located just downstream of the promoter-recognizing region 2.4 (the turnover element). R. Hengge-Aronis also reported experiments *in vitro* showing that phosphorylated RssB and RpoS interact directly. The site of interaction in RpoS is precisely the turnover element. Moreover, RssB also interferes with σ^S activity in the way that antisigma factors do. In fact, antisigmas no longer appear to be confined to a few rare instances involving minor alternative sigmas; they may well also regulate the activity of nearly every major sigma as well (including the major housekeeping factors). A. Ishihama has reported the association of the σ^D of *E. coli* with a protein called Rsd, which could be an antisigma as well, although its physiological significance is still unclear (Jishage and Ishihama, 1998). He also suggests that DnaK, which associates with σ^N, may behave also as an antisigma to this factor. In its turn, DnaK, as discussed by R. Hengge-Aronis, seems to protect σ^S in some specific genetic backgrounds. The sequestering of sigma factors by antisigmas, and their interactions with chaperones as a widespread mechanism of physiological control, deserve more investigation. Furthermore, not every change in gene expression during the onset of stationary phase can be traced to σ^S (Schellhorn *et al*., 1998), and new mechanisms hitherto undisclosed might reveal more surprises.

Transcriptional switches of cell fate

The pivotal decision in the production of a *Bacillus subtilis* spore under adverse conditions is made when enough of the Spo0A transcription factor becomes phosphorylated to switch on its regulon of early sporulation genes: the phosphorylation results from the action of an elaborate phosphorelay system that integrates information about many different physiological parameters (Perego, 1998). Kinase–phosphatase competition regulates *Bacillus subtilis* development. As a result, different sigma factors become activated selectively in different cell compartments to execute spatially and temporally specific programmes of gene expression. As described by J. Errington, commitment to this cascade is determined by the activation of the prespore-specific factor σ^F, which is initially held inactive by an antisigma (SpoIIAB). Upon completion of the asymmetrically located sporulation septum an anti-antisigma (SpoIIAA-P) is activated by dephosphorylation by a membrane-bound phosphatase (SpoIIE), which is selectively localized in the face of the spore septum that is facing the smaller, or prespore, compartment (Wu *et al*., 1998a). It is remarkable that a transcriptional regulatory cascade that can be represented as [Spo0A-P $\rightarrow \sigma^F \rightarrow$ commitment to sporulation] actually involves perhaps 10 times as many regulatory proteins that are not themselves transcription factors.

Streptomyces coelicolor, a mycelial organism, exhibits a type of sporulation quite different from endospore formation in *B. subtilis*, with chains of many tens of unigenomic

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Table 1. *E. coli* transcription factors involved in the transcription of cellular genes[a].

Class	Contact subunit	Factor
I	α	Ada, AraC, CRP (class I promoters), CysB, FlhDC, Fis, Fnr (class I promoters), GalR, IHF, LacI, MarA, MelR, NusA, OmpR, OxyR, Rob, SoxS, TrpR, TyrR, nascent RNA
II	σ	CRP (class II promoters), Fnr (class II promoters), PhoB, (MerR), (NtrC)
III	β	DnaA, NusA, Rho, ppGpp, nascent RNA
IV	β'	NusA, Rho, polyphosphate, nascent RNA

a. Adapted from Ishihama (1997b).

exospores forming from the multigenomic tips of aerial hyphae (Chater, 1998). Nevertheless, the maturation of prespore compartments into spores is dependent on a homologue of the *B. subtilis* σ^F ($\sigma^F{}_{sc}$). As yet, however, there is no evidence that $\sigma^F{}_{sc}$ is regulated by partner switching of an antisigma factor and, indeed, the whole regulatory cascade leading to the formation of prespore compartments and transcription of *sigF* (the gene for $\sigma^F{}_{sc}$) is quite different from the famous phosphorelay of *B. subtilis*. In the context of this survey, it is notable that more transcriptional regulators collude in activating the *S. coelicolor sigF* gene than in its equivalent in *B. subtilis*. In *S. coelicolor*, six regulatory genes participate in at least two pathways whose convergence is necessary for sporulation septation and *sigF* expression to take place. In one of the two pathways, a sporulation-specific sigma factor transcribes genes for apparent DNA-binding proteins of classes that typically respond to physiological or environmental signals. Three other regulatory genes, all encoding proteins of novel families, are expressed independently of this mini-cascade. Current models suppose that most of these regulatory proteins sense signals associated with the orderly cessation of aerial hyphal growth, but there are not yet any clues about how they interact to activate *sigF* or sporulation septation.

αCTD: the far-reaching arm of RNA polymerase

A key discovery in the field of prokaryotic transcriptional regulation was the observation (Igarashi and Ishihama, 1991) that the α-subunit of RNA polymerase includes two separate domains. The carboxy-terminal domain (αCTD) mediates the activation by many well-known transcriptional factors, such as CRP or FnR (in type I promoters), Fis, AraC or OmpR. αCTD is connected to the αNTD (amino-terminal domain), which interacts with other components of RNA polymerase by a non-structured hinge, whose flexibility in reaching out to contact further upstream promoter regions seems to grow with every new system examined. A more recent surprise is the observation by M. Salas and her co-workers (Monsalve *et al*., 1997) that the interaction between a defined surface of protein p4 of phage Φ29 and the αCTD of *B. subtilis* RNA polymerase may either activate or repress transcription depending on the sequence of the promoter considered. Their results support the view that, while p4-αCTD-mediated recruitment of the polymerase to an otherwise suboptimal promoter sequence does activate transcription, excessive stabilization of the enzyme over a threshold level leads to repression.

Not all activators have αCTD as their target in the transcriptional machinery. There seems to be a consensus to name activators type I and type II to those contacting, respectively, only the α-subunit of RNA polymerase or other surfaces of the enzyme (i.e. the sigma factor). In a step further, A. Ishihama has proposed a classification of transcriptional activators into four types, depending on their specific interactions with distinct components of RNA polymerase (α, type I; σ, type II; β, type III; β', type IV) (Table 1). Some small effectors (e.g. ppGpp, polyphosphate) can alter the activity or the specificity of the polymerase and, in a broad sense, they could also be considered transcriptional regulators (Ishihama, 1997b). Despite being quite convenient, this classification is flawed, however, by the fact that some activators can contact more than one surface of RNA polymerase and that the contacted sites depend on the organization of the promoter (e.g. with CRP).

It is not unusual that bacterial promoters integrate signals by carrying arrays of upstream binding sites for factors that respond to different environmental signals, while contacting distinct polymerase sites. In cases in which the promoter is co-dependent on various transcriptional activators, the DNA sequence elements for them and for the RNA polymerase are arranged in order to permit the two activators to make independent contacts with the enzyme (Belyaeva *et al*., 1998; Rhodius and Busby, 1998). This might be the case in the regulation of the microcin C7 production genes, which, as was discussed by J. E. González-Pastor, A. Kolb, J. L. San Millán, and F. Moreno (unpublished), depends not only on the alternative factor σ^S, but also on the upstream binding of cAMP-CRP, H-NS and IHF. All these factors are thought to transduce various environmental signals to the RNA polymerase through a constellation of protein–protein and DNA–protein interactions. A different case of co-dependence is present in the *nir* promoter of *E. coli*, which is simultaneously regulated by the redox sensor protein FNR and the nitrate/nitrite-triggered activators NarP and NarL (Wu *et al*., 1998b). In this instance, the FNR-dependent activation is suppressed by upstream factors, and

the role of NarP/NarL is to overcome this suppression. All in all, the various mechanisms for co-dependence should give the cell the maximum flexibility to mix and match different transcription factors and environmental signals. Sorting out the assembly of nucleoprotein complexes *in vivo* and *in vitro* is one of the challenges for which the development of novel techniques is required.

Activation and RNA polymerase recruitment

Promoters whose performance *in vivo* results from a concourse of various factors and effectors shed a new light on the very notion of what is transcriptional activation. Individual transcription factors are known to operate at one or more of any of the stages of the pathway leading to transcript production, including the formation of a closed promoter complex, isomerization to an open complex (or DNA strand separation), early formation of a short transcript and elongation coupled to the advance of the core RNA polymerase and the release of the sigma factor from the complex. But what happens when more than one transcription factor acts on the same promoter? What actually determines promoter output? Experts in eukaryotic promoters (notably Ptashne and Gann, 1997) have argued that it is recruitment of a basal transcription machinery *in vivo* to a given DNA sequence that produces activation. Could this also be true in prokaryotic promoters? We have discussed above that some prokaryotic repressors act precisely by recruiting the RNA polymerase to the promoter too strongly and thus gluing the enzyme to the cognate sequence and inhibiting any step beyond the formation of an open complex. But otherwise, bacterial promoters subjected to positive control generally fail to form stable closed complexes in the absence of the activator(s), thus adding to the notion that RNA polymerase recruitment is a major determinant of promoter activity as in the eukaryotic counterparts.

The major exception to this rule has been the interaction of RNA polymerase containing σ^N, which is unrelated to all other sigma factors, with its cognate promoters. On its own, σ^N-RNA polymerase forms stable closed complexes with promoters. Transcription initiation from these complexes is absolutely dependent on activator proteins that typically bind to distant (>100 bp) upstream enhancer sequences (UAS), because ATP hydrolysis mediated by the activator is required for, and coupled to, the formation of an open complex. According to the current model (Kustu *et al*., 1991) (Fig. 2), once an activator bound to the UAS receives a specific signal (e.g. deriving from nitrogen starvation, exposure to some aromatic compounds or others), an upstream nucleoprotein complex is formed (perhaps including further activator molecules captured from solution), which loops round and contacts the σ^N factor of the holoenzyme prebound to the −12/−24 sequences that are typical of this type of promoter. ATP hydrolysis is then channelled through a so far unknown mechanism into DNA strand separation and subsequent transcription initiation. This model, which has mostly been generated from observations made *in vitro*, argues that recruitment cannot be an activating step, as the σ^N-RNA polymerase is already bound to the promoter. However, this may not be true in all cases. Recent observations (Bertoni *et al*., 1998) have shown that σ^N-RNA polymerase does not by itself have much affinity for the σ^N-dependent *Pu* promoter of *Pseudomonas putida* and that IHF is required for its recruitment to the DNA via contacts with αCTD. A deficiency of IHF results in an almost inactive promoter *in vivo* and a very weak promoter *in vitro*. What is the critical, rate-limiting activation step in this case? Open complex formation mediated by the so-called XylR protein or recruitment of the σ^N-RNA polymerase mediated by IHF? Perhaps when various factors are at play in the

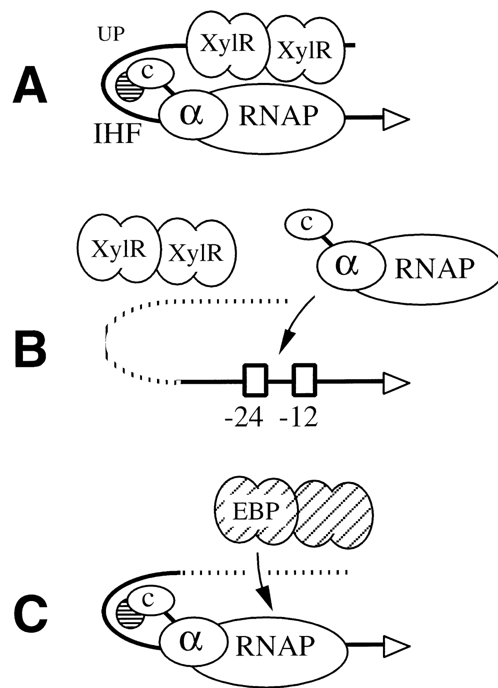

Fig. 2. Roles of IHF in the σ^N promoter *Pu*.
A. The scheme summarizes a plausible picture of how IHF can assist the interaction of σ^N-RNA polymerase holoenzyme while providing a structural aid to bring into close proximity the enzyme bound to its target sequences at −12/−24 and the activator of the system (XylR), bound to the upstream activating sequences. The main feature is that the αCTD of the polymerase interacts with a UP-like element, which partially overlaps the IHF site and extends further upstream.
B. The loss of IHF prevents the enzyme from binding the promoter sequence (Bertoni *et al*., 1998), thus inhibiting promoter activity regardless of the binding or activity of XylR to the upstream region.
C. Restrictor effect of IHF. If the promoter is occupied by the σ^N-RNA polymerase, IHF causes a configuration of the DNA region that suppresses promiscuous activation from solution by heterologous enhancer binding proteins (EBPs). See PérezMartín and de Lorenzo (1995) for further details. Protein sizes are not to scale.

same promoter, the eukaryotic-derived notion of a basal transcription machinery that becomes effective only when recruited to a given DNA sequence could be applied to this case as well.

Complex controls for essential functions

The expression of genes controlling functions essential for cell proliferation is often complex and may, for example, involve multiple promoters to ensure that expression is always appropriate to growth rate. In unicellular organisms such as *E. coli*, the cell division machinery must in any case accommodate a fall in the rate of protein synthesis when growth slows down, so that cell division continues to happen whenever the cell mass doubles. In many bacteria, the genes specific to cell division are arranged in a single cluster (in a well-known exception, *Helicobacter*, the genes are scattered among at least seven loci; Vicente *et al*., 1998). Regulation of expression in this 12-gene cluster involves a variety of molecular control devices (Fig. 3). Those found in the stretch from *ddl* to *ftsZ* have been studied more intensely and include specific kinds of promoters (one of them able to respond to σ^S; Ballesteros *et al*., 1998), modulation by ppGpp (Gentry *et al*., 1993; Navarro *et al*., 1998; Powell and Court, 1998; Joseleau-Petit *et al*., 1999), post-transcriptional cleavage of the messenger (Cam *et al*., 1996), cell cycle controls (Garrido *et al*., 1993) and even an antisense RNA (Dewar and Donachie, 1993). At least six promoters inside this *ddl* to *ftsZ* region contribute to the expression of *ftsZ*, the penultimate gene in the cluster. Moreover, recent results implicate signals contained in the whole cluster, upstream from *ddlB*, in its expression (Flärdh *et al*., 1998).

At least four of the promoters able to direct the expression of *ftsZ* yield a constant amount of transcript per cell independently of the growth rate (Flärdh *et al*., 1997). One of them, *ftsQ1p*, is a gearbox promoter (Vicente *et al*., 1991), with a dependency on σ^S and sharing structural

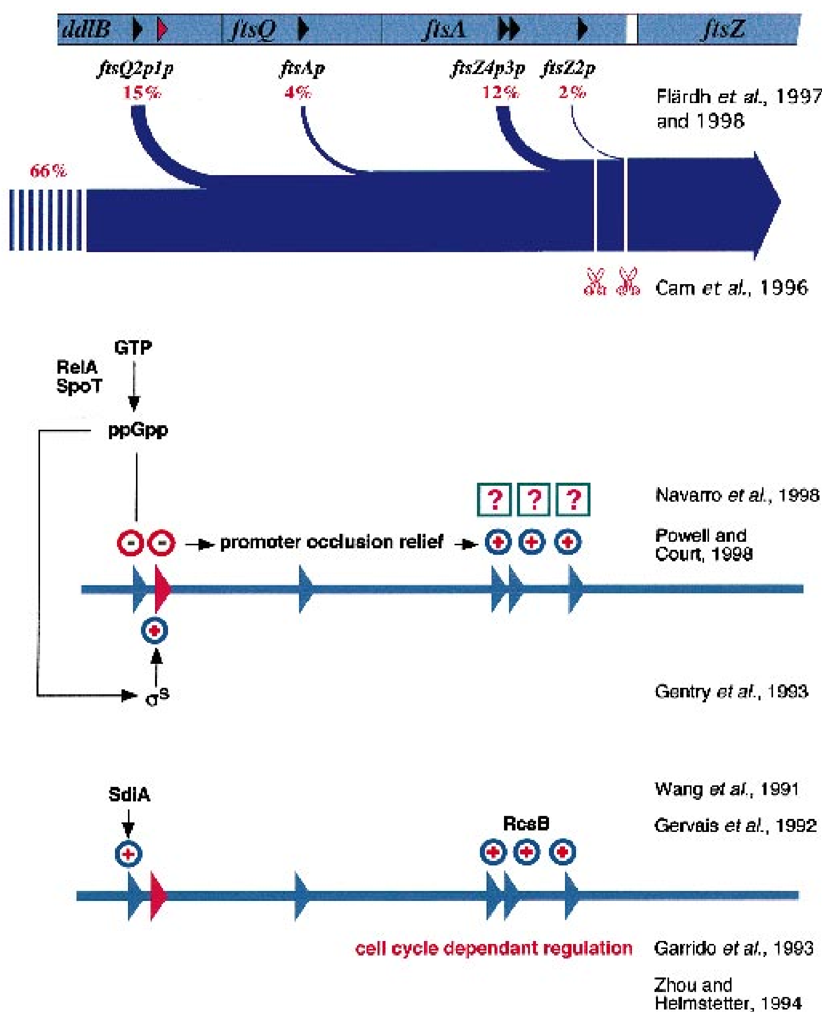

Fig. 3. Multiple controls to regulate the expression of essential cell division genes. The expression of *ftsZ*, a distal gene in the *dcw* cluster, is under the control of several promoters; those contained in the adjacent *ddlB*, *ftsQ* and *ftsA* genes are represented by arrowheads in the top row. The red arrowhead is a gearbox promoter. The second row represents the quantitative flow of transcription originating upstream of the *ddlB* gene and at each consecutive promoter or promoter group. Scissors represent mRNA processing sites. The third row depicts the connections of *ftsZ* expression with the cellular levels of the alarmone ppGpp. Minus signs indicate inhibition, while plus signs and black arrows represent induction. Question marks indicate that an additional unknown mechanism has been proposed to connect increased ppGpp levels to the relief of septation inhibition. The bottom row shows positive effectors of *ftsZ* expression, as SdiA, a part of a quorum-sensing-like pathway, and RcsB that belongs to the regulatory pathway of capsular polysaccharide. References at the right-hand side direct to the relevant publication.

and sequence similarities in −10 (extended) and upstream −35 (UP element) with the gearbox *bolA1p* (Ballesteros *et al*., 1998). However, the other three *ftsZ* promoters are not transcribed by σ^S and do not share obvious sequence homologies. This has led to the proposal to restrict the term 'gearbox promoters' to those with properties like *bolA1p* and *ftsQ1p*, while those like *ftsZ2*, *3* and *4p* are described as exhibiting a gearbox mode of expression (Ballesteros *et al*., 1998).

An instance of similar complexity in the expression of essential genes is found in the *dnaA*, *dnaN* (*dnaN**), *recF* cluster, encoding the *oriC* initiation protein DnaA, the β- and β*-subunits of DNA polymerase III, RecF (a protein involved in the resumption of replication at disrupted forks) and *gyrB*, which codes for the β-subunit of DNA gyrase. During exponential phase, the expression of *dnaN*, *N** and *recF* is directed by the *dnaA* promoters. As summarized by M. Villarroya, in starved cells, σ^S controls the transcription of the *dnaN* and *dnaN** promoters and, indirectly, the expression of *recF*, on account of the increased rate of transcription termination of the transcripts initiated at *recFp*. The expression of *dnaN*, *N** and *recF* then becomes independent of *dnaA* regulation, ensuring that the integrity of DNA can be maintained under stress conditions (Villarroya *et al*., 1998).

A different form of sequential cell division gene activation and inactivation, coupled with compartmentalization of gene expression and gene product accumulation, is found in bacteria with more complex cell division cycles, as exemplified by the obligatory dimorphic organism *Caulobacter crescentus*. Y. Brun described work from his laboratory that shows that the cellular concentration of the essential cell division protein FtsZ is subject to both transcriptional and proteolytic controls (Kelly *et al*., 1998). After division to form a stalked (sessile) and a swarmer (motile) cell, a two-component cell cycle-dependent response regulator, CtrA, represses both DNA replication and the transcription of *ftsZ* in the swarmer cell. In addition, the FtsZ protein is degraded in the swarmer cell preventing further cell division. However, once the swarmer cell differentiates into a stalked cell, CtrA is degraded, allowing DNA to replicate and *ftsZ* to be expressed in preparation for the next cell division.

Conclusion

Within the perspective addressed at this meeting, bacterial gene expression is evidently a rather elaborate web of interactions, comprising various regulatory circuits controlled by an assortment of molecules. While the relative simplicity of the *lac* operon served in the past as a model to stimulate research in molecular genetics, it may no longer be considered as a typical bacterial expression unit. Perhaps we should not be surprised to find that the intense and prolonged evolution of free-living bacteria has generated a complexity of gene regulation that rivals or, in some respects, even surpasses that of eukaryotic cells.

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