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Controlling competence in *Bacillus subtilis*: shared use of regulators

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Bacteria have developed a wide arsenal of survival strategies to cope with the specific problems posed by their environment. These processes are carefully regulated and complex signal transduction cascades ensure proper activation of the adequate adaptive response. An intriguing observation is that generally the regulation pathways of the different adaptive processes are highly intertwined. In this review, this phenomenon is illustrated by the regulation of genetic competence development in *Bacillus subtilis*. The different regulation pathways which make up the gene regulation network that controls the development of competence are described, and their connections to other adaptive processes in *B. subtilis* are discussed.

Overview

Bacteria are ubiquitous and extremely successful life forms. Their success is mainly a consequence of their talent of adapting to a large diversity of environments. To properly regulate the various adaptations, bacteria contain dedicated sensing devices linked to complex gene regulation systems. Due to the specific conditions to which these processes are tuned, it is reasonable to assume that each specific adaptive process contains its own specific set of sensing and regulatory proteins. However, it turns out that in many cases no unique, adaptive-process-specific gene regulation system exists. Instead, bacteria use highly intertwined networks of sensing and regulation pathways which conduct several different adaptive processes simultaneously. A striking example is the regulation of competence development in the Gram-positive bacterium *Bacillus subtilis*. In this paper, we review the regulation pathways involved in competence development, and describe how these pathways are linked to other adaptive processes in this organism.

*B. subtilis* is a soil-dwelling organism. In order to cope with the strong nutritional and physical fluctuations related to such an environment, this bacterium developed a wide arsenal of survival strategies. These processes can be conveniently observed in the laboratory by growing *B. subtilis* under batch-fermentation conditions. At the end of the exponential growth phase, when nutrients become limiting for optimal growth, *B. subtilis* cells start to synthesize a complex motility and chemotaxis system, which, in a natural habitat, would enable them to search for nutrients in the environment. If nutritional limitation continues, these motile cells enter the stationary growth phase, and start to secrete degradative enzymes such as proteases to liberate nutrients from alternative resources that are normally difficult to access. In addition, cells start to produce antibiotics to fight off possible competitors. Prolonged nutritional stress results in the development of competence, and ultimately in sporulation of the bacterial population. Sporulation provides the bacterium with a way to survive extended harsh environmental conditions.

Genetically competent *B. subtilis* cells

Genetic competence is a cellular differentiation process which converts *B. subtilis* cells into naturally transformable cells. In order to incorporate DNA from the medium, *B. subtilis* cells synthesize a specific DNA-binding and uptake system, schematically presented in Fig. 1. Currently, five different loci have been identified which are involved in this DNA transport: *comC, comE, comF, comG* and *nucA*. Sequence comparisons revealed similarities between *comG* and type-IV pilins from *Pseudomonas* species and other pilin-like complexes. These similarities, together with experimental data, led to a model in which it is proposed that DNA uptake is accomplished by a pilin-like structure, composed of several *comG*-operon-encoded proteins (Chung *et al.*, 1998; reviewed by Dubnau, 1999). ComC appears to be involved in the correct assembly of this structure. The *comE* operon encodes a polytopic transmembrane protein (ComEC) which is thought to form a pore that guides the DNA into the cell interior, where it may associate with the DNA-helicase-resembling protein encoded by the *comF* operon (Londono-Vallejo & Dubnau, 1994; Provvedi & Dubnau, 1999). There is no nucleotide sequence specificity for DNA binding and DNA uptake, and competent *B. subtilis* cells can incorporate plasmid DNA, phage DNA or chromosomal DNA. DNA uptake is accompanied by endonucleolytic cleavage catalysed by the membrane localized nuclease NucA, and results in linear fragments up to 20 kb in size (Provvedi *et al.*, 2001). These fragments are taken up in single-stranded form. The complementary
transcription factor ComK (van Sinderen-recombination genes is controlled by the competence
The expression of the DNA-binding, -uptake and
integation process active in a competent B. subtilis cell (for
details see main text). The number and configuration of the pro-
tein which form the DNA translocation complex are drawn
arbitrarily (A, NucA; C, ComC; E, ComE; F, ComF; G, ComG;
CW, cell wall; CM, cell membrane; CYT, cytoplasm).

Fig. 1. Schematic presentation of the DNA-uptake and DNA-
integration process active in a competent B. subtilis cell (for
details see main text). The number and configuration of the pro-
tein which form the DNA translocation complex are drawn
arbitrarily (A, NucA; C, ComC; E, ComE; F, ComF; G, ComG;
CW, cell wall; CM, cell membrane; CYT, cytoplasm).

strands are degraded, and nucleotides are released into the
medium. After internalization, the single-stranded DNA
presumably associates with the general DNA-recombination
protein RecA, and catalyses, together with other recombina-
tion proteins such as the DNA helicase AddAB, the inte-
gration of the newly acquired DNA into the genome
(Haijema et al., 1996).

The expression of the DNA-binding, -uptake and
-recombination genes is controlled by the competence
transcription factor ComK (van Sinderen et al., 1995). The
induction of this protein is strictly regulated. ComK
expression occurs only when exponential growth ceases,
and reaches its optimum after 2 h stationary growth. A
sufficiently high cell density is a prerequisite for competence
develop optimally. Medium constituents are important
regulatory factors as well, and the highest ComK expression
is obtained in minimal medium with glucose as sole carbon
source. An intriguing aspect of competence regulation in B.
subtilis is that, even when all conditions appear to be optimal,
only about 10–20 % of the cells in a culture will produce
ComK and will develop competence (for a comprehensive
review see Dubnau, 1993).

Primimg of comK expression
ComK binds to the comK promoter and induces its own
transcription (van Sinderen & Venema, 1994). It is this
auto-stimulatory loop which is a critical step in establishing
competence (the regulation pathways described in this
review are schematically visualized in Fig. 2). ComK binds
to various promoters as a tetramer composed of two dimers.
In the comK promoter, the ComK-dimer binding sites are
separated by 31 bp. This considerable distance hampers
interaction between the dimers and the formation of a stable
ComK tetramer. However, in between the ComK-dimer
binding sites the transcription factor DegU can bind. It was
shown that the presence of this protein strongly stimulates
binding of ComK (Hamoen et al., 2000). Since over-
production of ComK bypasses the need for DegU, ComK
appears to be able to function without DegU if present
at sufficiently high concentrations (Roggiani & Dubnau,
1993). Apparently, the main function of DegU is to stimu-
late binding of ComK at the onset of competence develop-
ment, when the number of ComK molecules is insufficient
for binding to the comK promoter. Thus expression of
comK requires DegU to ‘prime’ the auto-stimulatory comK
transcription loop.

During the transition from exponential to stationary growth,
B. subtilis starts to secrete a large number of degradative
enzymes. The two-component regulatory system DegS/
DegU plays an important role in the regulation of this
process (Kunst et al., 1988). Several mutations in either the
protein kinase DegS or the DegU response regulator provoke
hyper-production of degradative enzymes, presumably as a
result of increased DegU phosphorylation (Dahl et al., 1992;
Henner et al., 1988). These so-called ‘Hy-phenotypes’ are
very pleiotropic, resulting in decreased competence, dimin-
ished motility and glucose-insensitive sporulation (Henner
et al., 1988). The unphosphorylated form of DegU binds
efficiently to the comK promoter, and several genetic experi-
ments led to the conclusion that unphosphorylated DegU
is required for competence, whereas DegU~P is essential
for the production of degradative enzymes. Thus the
DegS/DegU two-component system provides B. subtilis with
a developmental switch between different adaptive processes
(Msadek et al., 1995). The nature of the signal that activates
DegS is unknown.

Transcriptional repression of comK expression
When cells become competent, DNA replication is blocked
and cell division ceases (Haijema et al., 2001). The com-
petence transcription factor ComK appears to be respon-
sible for these phenomena, so accurate control of comK
expression is vital to B. subtilis (Hahn et al., 1995b). Due to
the auto-stimulatory transcription of comK, premature
expression of this gene must somehow be prevented. In the
early days of sporulation research, a mutation was identified,
spo0A, which, in addition to inhibiting sporulation and
production of several proteases, also affected competence.

The protein encoded by
spo0A is not activated by a single sensor protein, like most of the
two-component signal transduction systems, but is phospho-
ylated via a complex phosphorylase system, comprising
several histidine kinases (KinA, B and C), an intermediate
phosphoacceptor (Spo0F), and a phosphotransferase
(Spo0B) (Burbulys et al., 1991). The phosphorylase somehow
monitors the metabolic and DNA-replication status of the cell, as well as medium conditions, and the various signals are integrated in this cascade, ultimately resulting in the phosphorylation of Spo0A (Perego, 1998). Spo0A is a transcription factor with a dual activity: it activates transcription of several genes involved in the early stages of sporulation (e.g. spoIIG), but it can also act as a transcriptional repressor, as is the case for a gene called abrB (Bird et al., 1993; Strauch et al., 1990). AbrB has a determining role in the regulation of stationary-growth-phase processes in B. subtilis, and is often indicated as a transition state regulator (Strauch et al., 1989).

It is a small DNA-binding protein and acts as a general transcriptional repressor of various genes involved in stationary phase processes such as sporulation (e.g. spoVG), degradative enzyme production (e.g. aprE), amino acid utilization (e.g. dpp) and antibiotic production (e.g. tycA) (Ferrari et al., 1988; Robertson et al., 1989; Slack et al., 1991). During growth, expression of abrB declines as a result of transcriptional repression by activated Spo0A (O’Reilly & Devine, 1997). Disruption of spo0A results in overproduction of AbrB, as a consequence of which expression of many stationary-phase genes is inhibited, including expression of...
comK (Hahn et al., 1995a). Recently, it was found that AbrB binds to the comK promoter and occupies the transcription initiation region (L. Hamoen, unpublished data). Although an important function of AbrB in the competence development pathway is to prevent the premature expression of comK, it also has a stimulatory effect on competence development, as judged from the observation that mutations in abrB decrease competence (Hahn et al., 1995a). Why this is so is not yet known.

AbrB is not the only regulator which inhibits transcription of the comK promoter. Expression of comK is sensitive to the amino acid composition of the medium. Amino-acid-mediated repression of several B. subtilis genes has been shown to depend on a GTP-binding transcription factor, CodY (Slack et al., 1995). CodY senses the intracellular GTP concentration as an indication of the nutritional conditions in the medium, and regulates many stationary growth phase genes such as the dipeptide transport operon dpp, but also for example spo0A (Ratnayake-Lecamwasam et al., 2001). A codY mutation alleviated the amino-acid-imposed control of comK expression, and DNA-footprinting analyses revealed that CodY occupies the RNA polymerase binding site of the comK promoter as well (Serrro & Sonenshein, 1996).

In an extensive genetic analysis, Hoa et al. (2002) identified a third repressor of the comK promoter. Overexpression of the gene ykuW inhibited transcription of comK, and a knockout of ykuW resulted in ComK overproduction. ykuW was renamed rok, an acronym for ‘repressor of comK’. Rok binds and represses not only the comK promoter but also its own promoter. High levels of ComK repress rok expression as well, and it was shown that ComK binds specifically to the promoter of rok. The ComK-dependent inhibition of rok expression appears to transform the Rok control of comK transcription into a positive feedback loop. Presumably, Rok fulfils a more pleiotropic role in B. subtilis as changes in expression levels of this protein not only influence comK expression but also affect sporulation. By now a total of five different transcription factors have been identified which control the comK promoter, a rather remarkable number for a prokaryotic promoter.

Cell-density-dependent induction of competence

Bacteria are able to communicate with each other through the production of ‘quorum-sensing’ pheromones. Accumulation of pheromones in the growth medium signals the presence of a sufficient number of congeners (a quorum), and triggers various cell-density-dependent processes. In B. subtilis, two competence-stimulating pheromones have been identified. The main competence-stimulating factor, ComX, is a 9 to 10 amino acid oligopeptide with an isopenyl modification of the tryptophan residue (Ansaldi et al., 2002; Magnuson et al., 1994). comQ, the gene located upstream of comX, is required for the post-translational modifications and secretion of the ComX pheromone. Downstream of comX are located the genes of a two-component regulatory system, comA and comP (Weinrauch et al., 1990). The membrane-spanning protein kinase ComP senses the accumulation of ComX in the medium, and responds by phosphorylating ComA (Solomon et al., 1995). The transcription factor ComA is required for the expression of srfA, a locus which was shown to be essential for competence development (Hahn & Dubnau, 1991; Nakano et al., 1991; van Sinderen et al., 1990). Sequencing of the entire srfA locus revealed a huge 30 kb spanning operon, encoding a very large protein complex responsible for the non-ribosomal synthesis of the lipopeptide antibiotic surfactin (Cosmina et al., 1993). It was shown that phosphorylation of ComA stimulates the binding of ComA to the srfA promoter, and as such induces srfA transcription and surfactin production (Roggiani & Dubnau, 1993). Surprisingly, the synthesis of this antibiotic had nothing to do with competence development. Located within the second open reading frame of the srfA operon is a small gene which encodes a 46-amino-acid peptide. It was this small peptide, designated ComS, that turned out to be essential for competence to develop (D’Souza et al., 1994; Hamoen et al., 1995). By embedding comS within srfA, B. subtilis elegantly uses a single quorum-sensing pathway for two different adaptive processes. Production of surfactin, which is a potent biosurfactant, may not only give B. subtilis cells a selective advantage by eliminating competitors, but may also allow the bacterium to use the lytic action of surfactin, so that potentially valuable genetic material released by lysed micro-organisms can be incorporated.

The competence-stimulating factor (CSF) was the second competence pheromone identified. Amino acid sequence analysis indicated that this pheromone is a pentapeptide with an amino acid sequence similar to the C-terminal part of a 40 amino acid long secreted protein, PhrC (Solomon et al., 1996). How processing of PhrC to CSF occurs is unknown (for clarity we will use here the nomenclature PhrC instead of CSF). Also PhrC exerts its quorum-sensing activity at the level of srfA transcription, and requires ComP and ComA for its activity. However, the stimulatory effect of PhrC on srfA expression also requires the oligopeptide permease Spo0K, suggesting that sensing of this pheromone occurs intracellularly (Lazazzera et al., 1997). Upstream of phrC, the gene rapC is located. Genetic studies indicated that RapC is a negative regulator of srfA expression and probably dephosphorylates ComA~P (Solomon et al., 1996). On the basis of a homologous system involved in sporulation (RapA/PhrA), it is supposed that PhrC inhibits RapC activity (phr stands for phosphatase regulator). Thus when PhrC levels rise sufficiently, RapC activity is repressed, ComA~P levels accumulate, and srfA/comS expression increases (Solomon et al., 1996). So both pheromones, ComX and PhrC, stimulate expression of srfA/comS. Why B. subtilis uses a double quorum-sensing pathway for this process is not clear.

The concentration-dependent effectiveness of antibiotics explains why surfactin production depends on the accumulation of congeners. Yet quorum sensing may also have an important function in the genetic transformation process.
Due to a RecA-based homologous recombination system active during competence, the efficiency of recombination and integration with the *B. subtilis* genome largely depends upon the measure of DNA sequence homology. Consequently, competent *B. subtilis* cells are transformed most efficiently with DNA from congeners. It is as if competence has primarily evolved to exchange genetic material within the species. This is even more apparent for *Haemophilus influenzae* and *Neisseria gonorrhoeae* (Lorenz & Wackernagel, 1994). In these bacteria, DNA binding and DNA uptake depend on specific sequence motifs dispersed over the genome as a result of which only DNA from the same or closely related species can be taken up. For *Streptococcus pneumoniae* it was shown that the development of competence is accompanied by lysis and DNA release of a subfraction of the population, and both processes appeared to be stimulated by the same quorum-sensing regulation pathway (Steinmoen et al., 2002). Therefore, natural competence can be considered as the bacterial attempt at a sexual lifecycle (Redfield, 1988). As the preferred donor DNA presumably originates from dead and lysed congeners, the cell density dependence of competence development may therefore have become an evolutionarily valuable asset.

### Post-translational control

Mutations in two loci, *mecA* and *mecB*, resulted in overproduction of ComK (Kong et al., 1993). *mecB* was later renamed *clpC*, since the protein encoded by this gene resembled the heat-shock protein ClpC of *Escherichia coli* (Msadek et al., 1994). The *mec*-phenotype suggested that MecA and ClpC are negative regulators of competence. It required purification of these proteins to reveal their precise function. Biochemical analyses indicated that MecA interacts with ComK, and ClpC interacts with MecA (Turgay et al., 1997). ClpC alone does not bind ComK, but in the presence of MecA a stable ternary complex is established. ClpC is a member of the ubiquitous HSP100 chaperone-like family, and several members of this family form a complex with the conserved protease ClpP. *In vitro* experiments indicated that *B. subtilis* ClpC and ClpP interact with each other as well (Turgay et al., 1998). Moreover, the addition of purified ClpP to the ternary ComK/MecA/ClpC complex resulted in rapid degradation of ComK. Thus MecA recruits ComK to the ClpCP protease complex for degradation, and in this way prevents auto-transcriptional activation of *comK*.

Expression of neither *mecA* nor *clpC* changes dramatically during the transition to the stationary growth phase. The question of how the MecA/ClpC-mediated inactivation of ComK is alleviated remained unanswered, until it was observed that purified ComS inhibited the *in vitro* formation of a stable ternary ComK/MecA/ClpC complex (Turgay et al., 1997). It was later shown that ComS binds to MecA and stimulates the degradation of both proteins by the ClpCP protease complex (Ogura et al., 1999; Persuh et al., 1999). In conclusion, synthesis of ComS protects ComK from degradation so that auto-stimulation of *comK* expression is initiated. Thus the competence pheromones ComX and PhrC, which stimulate *comS* transcription, ultimately control *comK* expression on the post-transcriptional level, by means of regulated proteolysis of ComK.

### More research, more regulators

As the research of competence development proceeds, the list of regulators that are involved in competence is growing. For example, there is MedA, a membrane protein which decreases expression of *comK* (Ogura et al., 1997). The function of MedA is unclear; a deletion of *medA* reduces *comK* expression considerably, yet the expression of *comG* is unaffected, and cells become competent.

A regulator which does influence competence development is SinR. The *sin* locus encodes another regulatory couple of which the activity extends beyond a single adaptive process. The first protein encoded by this bicistronic operon, Sinl, represses the activity of the transcriptional regulator SinR, encoded by the second gene (Gaur et al., 1988). A *sinR* disruption results in non-motile, filamentous cells, and a strong reduction in competence, whereas overproduction of SinR blocks sporulation and related protease production. The regulation of SinR is complex, and control at both the transcriptional and (post)translational level seems to occur (Smith, 1993). Transition from exponential to stationary growth is accompanied by an increase in Sinl production. Sinl shows similarity with the C-terminal part of SinR, and inactivates SinR by forming a heteromultimer with this protein (Bai et al., 1993). The position of SinR in the competence signal transduction cascade is somewhat controversial. Liu et al. (1996) suggested that SinR is required for optimal production of ComS, whereas Hahn et al. (1996) presented evidence suggesting that SinR is directly involved in *comK* transcription. Recently, more evidence for the latter proposition has been found. It appeared that SinR acts negatively on *rok* transcription, and that inactivation of *rok* could bypass the requirement of SinR for *comK* expression (Hoa et al., 2002).

One of the latest additions to the list of known competence regulators is YlbF (Tortosa et al., 2000). A disruption in *ylbF* affects both competence and sporulation. Although the exact function of YlbF is unknown, genetic studies suggest that this protein is required for the production of sufficient amounts of ComS. Actually, the production of ComS is rather complex since it also requires polynucleotide phosphorylase (Pnp) (Luttinger et al., 1996). Pnp is involved in RNA stability, and besides stimulating the synthesis of surfactin synthetase and ComS, this RNA-binding protein is also required for adaptation to growth at low temperatures.

Controlled proteolysis plays an essential role in the regulation of many cellular developmental processes, and it is therefore not surprising that most of the newly discovered components in the competence signal transduction network are somehow involved in this process. Mutations affecting the activity of the proteases concerned show very pleiotropic phenotypes. For example, in *B. subtilis* blocking the
expression of the protease ClpP disturbs competence development, motility, degradative enzyme production and sporulation (Msadek et al., 1998). The absence of ClpP results in high levels of MecA. Since binding of MecA to ComK reduces the activity of ComK, high MecA levels will prevent development of competence, despite the absence of ClpP-dependent proteolyses of ComK. ClpP can also form an ATP-dependent protease complex with the chaperone ClpX, and a disruption of clpX interferes with the development of competence as well. In such a mutant the expression of comS appears to be insufficient to prevent the MecA-mediated degradation of ComK. A disruption in a gene called spx suppresses the adverse effects of clpP or clpX mutations (Nakano et al., 2002). Protein-binding studies indicated that Spx forms a complex with MecA and ClpCP, and enhances the binding of ComK to this protein complex. As a result, higher concentrations of ComK are required to release ComK from the complex and prevent the proteolytic degradation of ComK. The role of Spx in the competence signal transduction cascade is unclear. A disruption of spx has no consequence for the development of competence, and expression of spx is very low under wild-type conditions. It is only when ClpP or ClpX is absent that Spx reaches levels high enough to obstruct the activity of ComS.

Due to the general use of ATP-dependent proteases in so many cellular processes, substrate interference can be an important factor in gene regulation. An example which illustrates this phenomenon is the MecA paralogue YpbH. When the concentration of YpbH is disturbed in the cell, the expression of comK changes, since YpbH binds to ClpC as well (Persuh et al., 2002).

Further intertwinement of the regulation circuitry

An important regulatory mechanism in bacterial differentiation which has not been addressed so far is the use of different sigma factors to control transcription. *B. subtilis* has specific sigma factors for expression of the motility and chemotaxis apparatus (sigma-D), for the induction of the general stress response (sigma-B), and most strikingly, for sporulation, in which a succession of several sigma factors (sigma-E, F, G and K) directs the development into the mother cell and forespore (Haldenwang, 1995). In the development of competence, sigma factors play a minor role, as most proteins concerned use the major (house-keeping) sigma factor sigma-A. However, one additional sigma factor is known to be required for competence, the minor sigma factor sigma-H, encoded by sp00H. Expression of sigma-H is low, but the level increases at the end of exponential growth. Various sigma-H-controlled genes have been identified, including the phosphorelay components: kinA, spo0F and spo0A (Predich et al., 1992; Siranosian & Grossman, 1994). phrC contains a sigma-H promoter as well, and the major role of sigma-H in competence development appears to be the production of the quorum-sensing pheromone PhrC (Solomon et al., 1996).

The main pathways of the competence signal transduction cascade are depicted in Fig. 2 with black lines. However, this scheme remains a simplification, as the different regulatory pathways are not separate entities but are mutually intertwined. A few examples have already been mentioned, such as sigma-H, which is required for the expression of phosphorelay components and PhrC. Yet many more cross-links in the regulation circuitry of Fig. 2 can be drawn (grey lines). For instance, ClpX influences sigma-H activity (Liu et al., 1999), high concentrations of phosphorylated DegU reduce sfa transcription (Hahn & Dubnau, 1991), SinR is a repressor of the spo0A promoter (Mandic-Mulec et al., 1995), and also represses rok expression (Hoa et al., 2002), and CodY exerts its repressive action on both comK and sfa transcription (Serror & Sonenshein, 1996). AbrB negatively controls, apart from comK transcription (Hahn et al., 1995a), the production of PhrC (Solomon et al., 1995), and expression of spo0H (Weir et al., 1991) and rok (Hoa et al., 2002), whereas Spo0A represses abrB, but stimulates expression of sinI (Gaur et al., 1988). Finally, the quorum-sensing pathway gets increasingly complicated in view of the observation that several rap/phr operons, including rapA/phrA and rapC/phrC, are also regulated by the ComP/ComA two-component system (Lazazzera et al., 1999; Perego et al., 1996).

Conclusions

comK expression depends on the presence of SinR, DegU, ComS and a minimal amount of Abrb. However, sufficient levels of these proteins are only transiently present during growth. ComS appears when the cell density increases, levels of Abrb decrease during growth, DegU becomes phosphorylated, and SinR is ultimately inactivated by SinI. Apparently, only a restricted time window exists in which conditions are optimal for comK expression, which may explain why only a small percentage of cells in a *B. subtilis* culture become competent. Whether there is any evolutionary advantage for this numerical limitation remains speculation. From the point of view of the species, it is not necessarily an advantage to have every cell competent, as long as a sufficient number of cells is transformable and able to enrich their genetic material. In addition, it seems as if the development of competence excludes the production of degradative enzymes, since SinR represses degradative enzyme production, but is required for competence development, and phosphorylation of DegU is required for the production of degradative enzymes, but not for competence development. Possibly, the physiological status of competent cells does not support efficient expression and secretion of degradative enzymes. In this scenario, the small number of competent cells will take advantage of the degradative enzymes secreted by the majority of non-competent cells. Since this will require a reasonable concentration of degradative enzymes in the medium, this may also explain the cell-density-dependence of competence development.

It is remarkable that, for a radical differentiation process like competence, *B. subtilis* uses almost no regulatory proteins.
that are exclusively used in this process. For several years the ComS/MecA/ComK regulation pathway was considered to be solely involved in competence regulation. However, it was shown later that a mecA-null mutation inhibits expression of sigma-D, the sigma factor involved in motility and chemotaxis, and that this inhibition partially depended on ComK (Liu & Zuber, 1998; Rashid et al., 1996). Competence is not unusual in having almost no unique 'private' regulators at its disposal. In fact, the various stationary-phase processes in B. subtilis share the majority of their regulators. A strong interlock between gene regulatory pathways appears to be a common fact among prokaryotes. Looking at the regulation of general adaptive processes in bacteria, such as catabolite repression, and heat- and cold-shock response, it is apparent that the regulation cascades are composed of many general pleiotropic regulatory proteins. So is there an evolutionary advantage of interlocking gene regulatory pathways? 'Efficiency' might be the key to this question. Because various transcriptional regulators, depending on where they bind a promoter, can function positively as well as negatively, the use of shared regulators is an efficient mechanism for coordinating and discriminating between different cellular responses. It links regulatory pathways and as such ensures a tight coordination between them. In addition, cells need lesser regulatory proteins as a result of which they handle their metabolic resources more economically.

The phenomenon of interlocked gene regulatory circuits has also been related to stability and robustness of the system (Little et al., 1999). A stable network will maintain its state in the face of small perturbations (stochastic fluctuations) of input signals and network components. It has been suggested, and recently experimentally supported, that especially negative feedback loops provide such stability ( Becskei & Serrano, 2000). Negative feedback loops are commonly present in complex gene regulatory circuits. In this review, several examples have been described, yet, in case of competence development it is questionable whether stability is an important reason for the intertwine of the gene regulatory pathways. The experience with competence development in B. subtilis teaches that the fraction of cells which ultimately become competent fluctuates considerably, despite use of carefully controlled growth conditions.

Robustness of gene regulatory circuits refers to the sensitivity of systems towards changes in the biochemical parameters of the components brought about by genetic alterations of the components (Little et al., 1999). Particularly in organisms with an active and promiscuous genetic exchange system, such as genetic competence in B. subtilis, it can be envisioned that robustness of gene regulatory circuits is vital.

The full measure of complexity of gene regulation networks complicates the ascertainment of properties such as stability and robustness. The rise of whole-genome transcription measurements with DNA arrays, and developments in high-throughput two-hybrid analyses, makes it possible to study these phenomena at a new level. Several in silico modelling studies with extensive gene regulation networks, based on transcriptome and proteome data from yeast, provided evidence that 'connectivity' increases the stability and robustness of gene regulation networks ( Featherstone & Broadie, 2002; Maslov & Sneppen, 2002; Wagner, 2000). In the near future, such in silico studies will be achievable with B. subtilis and other prokaryotes. By then it will be interesting to examine whether the competence signal transduction network is more stable than gene regulation networks of bacteria which live in stable environments, or more robust than gene regulation networks of bacteria which do not become genetically competent.

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References


