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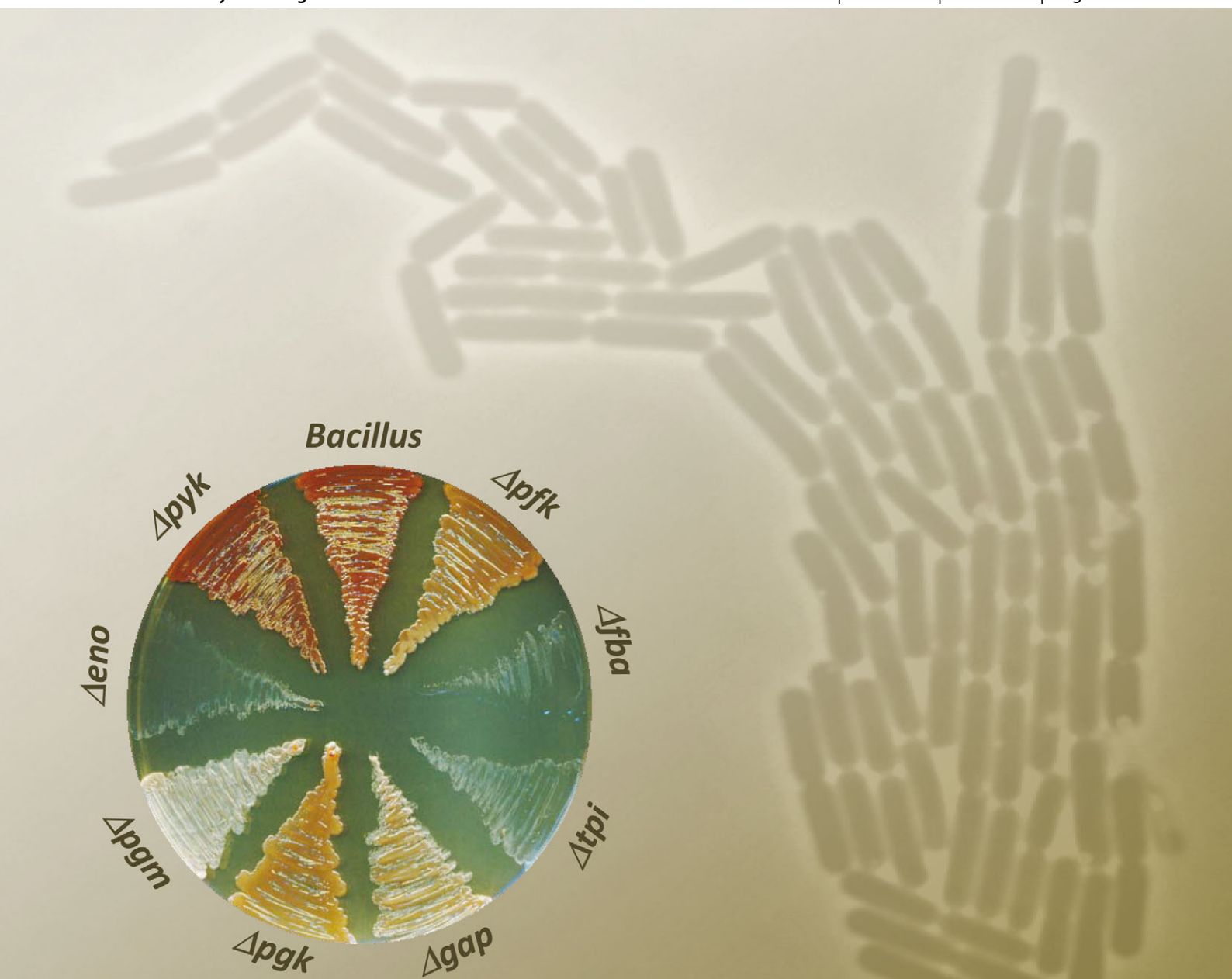

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Jörg Stülke *et al.*

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REVIEW

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Essential genes in *Bacillus subtilis*: a re-evaluation after ten years

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In 2003, an initial study on essential genes in the Gram-positive model bacterium described 271 genes as essential. In the past decade, the functions of many unknown genes and their encoded proteins have been elucidated. Moreover, detailed analyses have revealed that 31 genes that were thought to be essential are in fact non-essential whereas 20 novel essential genes have been described. Thus, 261 genes coding for 259 proteins and two functional RNAs are regarded essential as of January 2013. Among the essential proteins, the largest group is involved in protein synthesis, secretion and protein quality control. Other large sets of essential proteins are involved in lipid biosynthesis, cell wall metabolism and cell division, and DNA replication. Another interesting group of essential proteins protects the cell against endogenous toxic proteins, metabolites, or other intermediates. There are only six essential proteins in *B. subtilis*, for which no function is known. The functional analysis of these important proteins is predicted to be a key issue in the research on this model organism in the coming years.

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1 Introduction

A major issue in our understanding of life is the analysis of the essential set of genes and proteins that make up a living cell and a living organism. Such analyses reveal the most important components of any given organism; and by comparing the sets of essential genes of different organisms it will be possible to conclude about the minimal equipment needed to sustain life. This knowledge is of prime importance for the emerging discipline of synthetic biology that aims at creating artificially designed self-sustaining cells and organisms.[1] Moreover, essential proteins might be novel attractive targets for antimicrobial drugs that are urgently needed to fight several infectious diseases.[2]

Global analyses revealed between 270 and 650 essential genes in bacteria and about 900 essential genes in yeast.[3] One of the first comprehensive studies of the essential gene set for any organism was performed for the Gram-positive model organism *Bacillus subtilis*.[4] With 271 essential genes identified in this work, *B. subtilis* has one of the smallest known essential gene sets. However, the issue of identifying the essential gene set is complicated by the fact that an essential function may be carried out by redundant pairs of homologous genes and by the fact that essential functions such as the acquisition of an important metabolite can be achieved in different ways, either by uptake or *de novo* synthesis.

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When the set of essential genes of an organism is known, the next obvious question is why these genes are essential and which important functions the encoded proteins may fulfil.

Here, we review the knowledge about essential genes in *B. subtilis* with a special emphasis on the developments in the field since the first global study was published. In the past ten years, several additional genes of *B. subtilis* were found to be essential whereas others that were regarded as essential in 2003 have now successfully been deleted demonstrating the dispensability of such genes. Importantly, for several essential proteins with unknown functions, these functions were assigned in the past few years.

2 Obligatory and facultative essential genes

Essential genes are defined as genes that cannot be inactivated under specified optimal growth conditions. For *B. subtilis*, these conditions were defined as growth on LB medium at 37 °C.[4,5]

Several analyses of essential genes in bacteria revealed a substantial overlap of the essential gene sets. These genes can be regarded as obligatory essential. The obligatory essential genes encode proteins that fulfil the most important housekeeping functions, *e.g.* in the flow of genetic information. Indeed, these proteins involved in DNA replication, RNA synthesis and protein biosynthesis are conserved and essential in all bacteria. Other important groups of essential genes encode proteins involved in cell wall biosynthesis and in central metabolic functions, particularly in lipid biosynthesis (see Fig. 1).

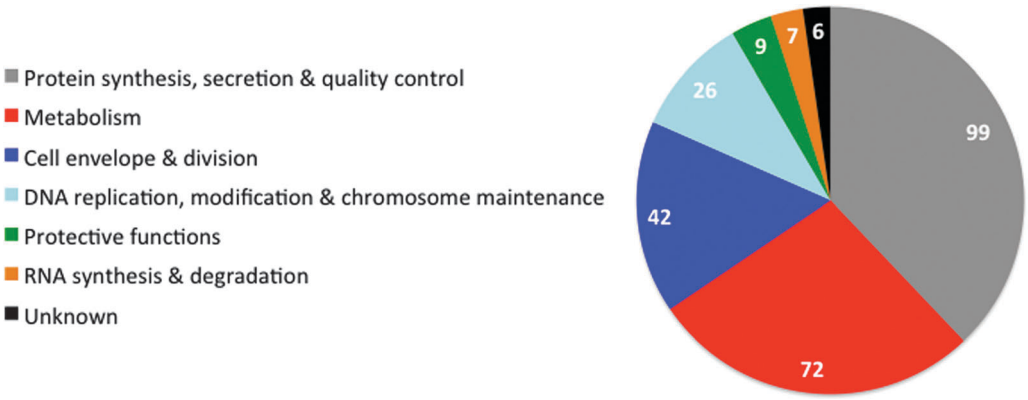

Fig. 1 Functional distribution of the 260 essential *B. subtilis* genes.

In contrast, genes that are essential in one organism but non-essential or even absent in other, even related bacteria, are called facultative essential. These genes do often encode proteins that protect the cell from toxic metabolites or other products (see below). It has been proposed that facultative essential genes can be suppressed more often than the obligatory essential genes.[6] Attempts to suppress the lack of essential genes have revealed that some essential genes become dispensable upon overexpression of other, functionally somehow related genes whereas those obligatory essential genes that encode the basic constituents of the cell can usually not be replaced.[6] Moreover, essential genes that counteract toxic activities can be deleted if the cause of the toxic effect has also been inactivated (see below).[7,8] Finally, the target of an essential enzyme may be replaced in a way that allows overcoming essentiality as it has been shown for the $tRNA^{Ile2}$-lysidine synthetase TilS from *B. subtilis*. In this case, TilS is normally required to modify the anticodon of the isoleucine-specific $tRNA^{Ile2}$, but it becomes dispensable when a mutation in the $tRNA^{Ile1}$ generates the TAT anticodon that recognizes the isoleucine-specifying AUA triplet.[9]

3 The essential gene set of *B. subtilis* – 2003 and today

A joint European–Japanese effort to systematically inactivate all unknown *B. subtilis* genes resulted in the first genome-scale identification of essential genes in this organism. Kobayashi *et al.* (2003) described 271 protein-coding genes as essential.[4] Among the essential proteins were all vegetative ribosomal proteins, many proteins involved in DNA replication, transcription and translation (such as aminoacyl tRNA synthetases and tRNA/rRNA modifying enzymes), enzymes of cell wall and lipid biosynthesis and, unexpectedly, most glycolytic enzymes. Among the essential proteins, eleven were of unknown function in 2003.

In the past few years, the composition of the set of essential genes has substantially changed. Of the original 271 genes, 31 were shown to be non-essential in recent studies. Moreover, 21 new genes (19 protein-coding genes and two RNA-coding genes) were added to the list. Thus, 261 genes encoding 259 proteins and two RNAs are regarded as being essential today. The complete updated list of essential genes in *B. subtilis* can be found in SubtiWiki (http://www.subtiwiki.uni-goettingen.de/wiki/index.php/Essential_genes).[10] Among the newly identified essential genes five genes each are involved in protective functions or encode proteins of unknown function, respectively, four genes are involved in metabolism, and two genes encode the functional RNAs *rnpB* (RNA component of RNase P) and *scr* (RNA component of the signal recognition particle). It should, however, be noted that several genes encoding antitoxins or antisense RNAs that control toxin expression may still have escaped their discovery as essential genes.

Of the eleven previously unknown essential proteins, four were found to be non-essential, whereas functions could be assigned to six of these proteins: they encode RNases (*rnjA*, RNase J1, formerly *ykqC*; *rny*, RNase Y, *ymdA*; *rnz*, RNase Z, *yqjK*),[11–14] enzymes involved in tRNA modification (*tilS*, $tRNA^{Ile}$-lysidine synthetase, *yacA*; *tsaB*, threonyl carbamoyl adenosine (t6A) modification of tRNAs, *ydiC*)[15,16] and an enzyme of lipid biosynthesis (*plsY*, acylphosphate: glycerol-phosphate acyltransferase, *yneS*).[17] Thus, of the originally unknown essential proteins the function of only one protein could not be identified. However, for this protein (YlaN), it was suggested that it may be involved in the control of cell shape.[18,19] Together with the newly identified five unknown essential proteins, six essential proteins of *B. subtilis* do still expect the elucidation of their function (see below).

Among the 261 essential genes of *B. subtilis*, the largest group (99) is involved in functions related to protein biosynthesis, protein secretion and protein quality control. Moreover, 72 essential genes encode enzymes involved in metabolism, and 42 genes are required for cell wall biosynthesis and cell division. Finally, a rather large set of essential genes is implicated in several aspects of DNA metabolism (replication, DNA modification, chromosome maintenance). The remaining essential genes encode RNases or proteins that have a protective function (see below) (see Fig. 1).

4 Essential functions

Many aims of a cell can be achieved in different ways: there may be redundant enzymes that have the same or overlapping activity, or

completely different proteins may serve the same purpose. In *B. subtilis*, the first case is exemplified by the threonyl-tRNA synthetases ThrS and ThrZ; either of the two proteins has to be functional for the viability of the cell. A recent analysis of gene pairs in *B. subtilis* identified six essential functions that are encoded by such pairs. In addition to ThrS/ThrZ, these are the membrane protein translocases SpoIIIJ/YidC2, the DNA topoisomerases TopA/TopB, the fatty acid biosynthetic enzyme pairs FabI/FabL and FabHA/FabHB, and the DNA polymerases I PolA and YpcP.[5,20–22] As for the obligatory essential proteins, the enzyme pairs are required for key housekeeping functions.

Alternatively, synthetic essentiality can result from convergent metabolic functions of non-homologous proteins. This is the case for the biosynthesis and acquisition of important metabolites such as nucleotides or amino acids: either the biosynthetic enzymes or the specific transport systems are necessary to provide the cell with these essential building blocks even though the individual enzymes or transporters never show up in screens for essential genes. These limitations in the screens for essential genes have to be taken into consideration in all attempts to artificially create new genomes or to reduce existing genomes.[23–25]

Recently, the emerging signalling nucleotide cyclic di-AMP was found to be essential in *B. subtilis*.[26,27] This second messenger is produced by either of two vegetative diadenylate cyclases DisA and CdaA, and by the sporulation-specific enzyme CdaS. The presence of one of these enzymes in growing cells is required to support growth of *B. subtilis*. Thus, lack of the vegetative enzymes DisA and CdaA is synthetically lethal. c-di-AMP is unique since it is the only known essential signalling nucleotide. It is thought to be required for cell wall biosynthesis and cell division,[26,27] and essentiality was also discovered for the single diadenylate cyclases in other Firmicutes such as *Listeria monocytogenes*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*.[28–30]

5 Re-evaluation of essentiality of glycolytic enzymes

When the essential gene set was originally identified, the authors were surprised to find that nearly all glycolytic enzymes were essential.[4] This was unexpected since the glycolytic flux does not seem to be required on a rich medium such as LB. During growth in this medium, the bacteria can derive both sugars for cell wall and nucleic acid syntheses as well as amino acids from the medium. In the case of the glyceraldehyde 3-phosphate dehydrogenase, essentiality of the glycolytic *gapA* gene was confirmed by a study on conserved gene pairs.[5] While the glycolytic gene *gapA* was found to be essential, its gluconeogenic counterpart *gapB* is dispensable.[5]

We have initiated two follow-up studies related to the essentiality of glycolytic enzymes: first, we re-evaluated gene essentiality by individually deleting all glycolytic genes, and second, interaction partners for the essential glycolytic enzymes were identified. To our surprise, deletion mutants were obtained for each of the glycolytic genes when the bacteria were selected on a complex medium containing the two preferred carbon sources, glucose and malate. Moreover, all glycolytic mutants do even grow on a minimal medium with glucose and malate as the carbon sources and ammonium as the single nitrogen source (see Fig. 2). This finding implies that glycolysis is indeed non-essential if the bacteria grow in the presence of substrates that enter metabolism up- and downstream of glycolysis, respectively. In agreement with the previous studies, the *gapA*, *pgm*, and *eno* mutants are unable to grow in LB medium (see Table 1). The reason why these genes are dispensable during growth on minimal medium supplemented with glucose and malate but not on LB is so far unknown. In contrast, the *pfkA*, *fbaA*, *tpiA*, and *pgk* mutants do grow on LB medium and have therefore been deleted from the list of essential genes (see above). The deletion of the *eno*, *pgm*, and *pfkA* genes has been reported before,[31,32] thus supporting our conclusion that these genes are not essential under suitable conditions.

Interestingly, the *pfkA* and *gapA* mutant strains are able to grow even if either glucose or malate is the single carbon source (see Fig. 2). For the *pfkA* mutant lacking phosphofructokinase this can be explained by the flux through the pentose phosphate pathway under glycolytic conditions, and by the circumvention of phosphofructokinase by the fructose-1.6-bisphosphatases Fbp and GlpX under gluconeogenic conditions (growth with malate). The glyceraldehyde-3-phosphate dehydrogenase is normally only

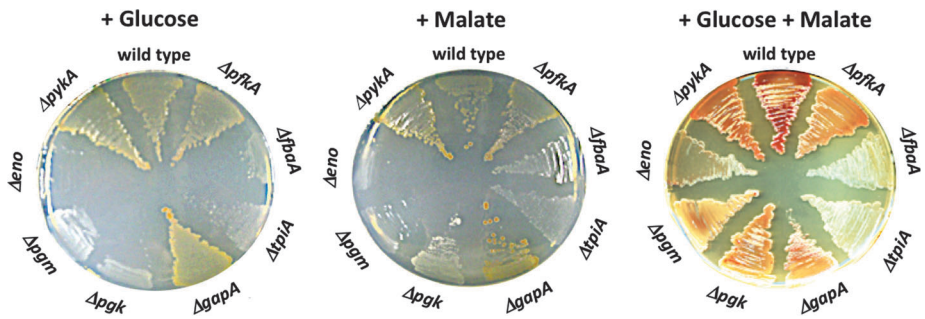

Fig. 2 Growth of *B. subtilis* mutants with inactivated glycolytic genes on minimal medium. Deletion of the glycolytic genes was achieved by transformation with PCR products constructed using oligonucleotides to amplify DNA fragments flanking the target genes and the chloramphenicol antibiotic resistance cassette, as described previously.[61,62] The wild type strain 168 and the isogenic mutant strains were propagated on C minimal medium agar plates that were supplemented either with 0.5% (w/v) glucose or 0.5% (w/v) malate, or with 0.5% (w/v) glucose and malate.[63] The plates were incubated for 3 days at 42 °C.

Table 1 Growth of *B. subtilis* mutants with inactivated glycolytic genes on rich media

		Medium		
Strain	Genotype	LB	SP	SP + glucose + malate
168	Wild type	+++	+++	+++
GP590	Δ*pfkA*	++	+	++
GP591	Δ*fbaA*	+	n. g.	+
GP700	Δ*tpiA*	+	−	+
GP592	Δ*gapA*	n. g.	n. g.	+
GP699	Δ*pgk*	+	−	+
GP593	Δ*pgm*	n. g.	n. g.	+
GP594	Δ*eno*	n. g.	n. g.	+
GP589	Δ*pyk*	+++	+	+++

n. g., No growth; −, poor growth; +, growth; ++, good growth; +++, very good growth; the plates were incubated for 24 h at 42 °C.

active under glycolytic conditions, whereas the homologous enzyme GapB takes over the function under gluconeogenic conditions. Thus, it is surprising to see that GapA is not essential for growth with glucose as a single carbon source. It is possible that metabolism is inefficient under these conditions resulting in partial derepression of *gapB* expression, thus allowing GapB to catalyze the oxidation of glyceraldehyde-3-phosphate. This is indeed the case, as supported by the inability of a *gapA gapB* double mutant to grow with glucose or malate as a single carbon source whereas this strain is viable when both glucose and malate are available.

The results presented above left the question of why *gapA*, *eno* and *pgm* are essential under defined standard conditions. It was hypothesized that the essentiality of the glycolytic enzymes might result from their putative contribution to essential interactions in the cell. Therefore, interaction partners of glycolytic enzymes were identified, and indeed, several of these enzymes were found to interact with essential proteins. Among these essential interaction partners are enzymes involved in RNA processing and degradation, *i.e.* RNases J1 and Y (encoded by *rnjA* and *rny*, respectively). *In vivo* interaction and two hybrid analyses suggest that the glycolytic enzymes phosphofructokinase and enolase are part of a RNA-degrading complex, the RNA degradosome.[12] In addition to the two glycolytic enzymes this complex consists of the essential endoribonuclease RNase Y, the essential exoribonuclease RNase J1 and its non-essential paralog RNase J2, the polynucleotide phosphorylase and the RNA helicase CshA.[33,34] The participation of glycolytic enzymes in RNA degradation is not unprecedented: in *E. coli* enolase is part of the RNA degradosome that is assembled around the essential RNase E.[35] It should be noted, however, that the precise contribution of glycolytic enzymes to RNA degradation has not yet been elucidated.

6 A big surprise: one third of the ribosomal proteins are dispensable

In the original list of essential genes all genes encoding vegetatively expressed ribosomal proteins were regarded to be essential.[4] However, this conclusion was not derived from experimental analysis; the essential role of ribosomal proteins simply appeared obvious. Recent experimental studies of essentiality of ribosomal proteins demonstrate that several ribosomal proteins are dispensable in *B. subtilis*. This resulted in the reduction of the number of essential ribosomal proteins from 52 to only 35. Among the non-essential ribosomal proteins are those that are only expressed under specific conditions (either stress or zinc limitation). Moreover, about one third of the vegetative ribosomal proteins (L1, L9, L11, L15, L22, L23, L28, L29, L31, L32, L33.1, L33.2, L34, L35, L36, S6, S20, and S21) are non-essential.[36–38] For several genes encoding ribosomal proteins, their deletion does not result in any detectable phenotype during vegetative growth. In contrast, many mutants resulted in slower growth and a reduced number of ribosomes. Moreover, the ribosomal proteins L1 and S21 were impaired in sporulation and motility, respectively.[36] The results on the essentiality or dispensability of ribosomal proteins in *B. subtilis* are supported by the observation that there are 22 non-essential ribosomal proteins (out of 54) in *E. coli*.[39]

The non-essentiality of such large sets of ribosomal proteins supports the concept of the ribosome as a ribozyme in which the proteins contribute to the structural organisation of the complex; to translation fidelity and efficiency.[40] It will be interesting to check whether combinations of deletions of ribosomal protein genes are tolerated by the bacteria.

7 A special case: the unique essential two-component system WalK–WalR

While essentiality is obvious for many genes involved in protein synthesis or DNA replication, there are other essential genes that are less easy to understand. This is especially the case for genes encoding regulators. An example is the essential WalKR two-component system of *B. subtilis*. It has to be noted that this is the only known essential two-component system in bacteria suggesting that it is required for the expression of essential genes. The essential role of the WalKR system has been under investigation for several years. Eventually, microarray analyses revealed that the system is required for the expression of essential genes of cell wall metabolism. Among the members of the WalR regulon that depend on activation by WalR for their expression are the genes encoding the essential cell division proteins FtsA and FtsZ and the genes for the teichoic acid biosynthetic enzymes TagB, TagD, and TagF. Moreover, the autolysine-encoding *cwlO* and *lytE* genes are activated by WalR. While each of the latter genes is individually dispensable, *B. subtilis* requires either CwlO or LytE for growth.[41] Thus, the essential role of WalR is caused by its role in the expression of several essential genes and a co-essential gene couple.[42,43]

The WalKR system is not only essential in *B. subtilis*, but also in many other Firmicutes, among them important pathogens such as *S. aureus*. Since targeting of enzymes or structural proteins by antibiotics results in the rapid development of

resistance mechanisms, it was suggested that essential regulatory systems might present novel, alternative targets that do not readily develop resistance. Indeed, compounds addressing the essential WalKR system have been identified.[44,45]

8 Essential proteins with protective functions

Some essential genes derive their essentiality not from the important function of the encoded protein in basic cellular activities; instead they are essential due to their role in the protection against toxic cellular components. This has been studied or proposed for eight essential genes of *B. subtilis* (see Fig. 3). The SknR repressor encoded on the skin element, a cryptic prophage, is essential because expression of two of its target genes is toxic for *B. subtilis*. The encoded proteins, YqaH and YqaM interact with the DNA replication initiator DnaA and the DNA helicase DnaC, respectively, and inhibit their activities. If the *yqaH* and *yqaM* genes are deleted, *sknR* is no longer essential.[7] The YhdL protein acts as an antagonist for the alternative sigma factor σ^M suggesting that constitutive activity of σ^M may result in the expression of one or more proteins that are detrimental for growth. Indeed, *yhdL* becomes non-essential upon simultaneous deletion of the *sigM* gene.[46] Unfortunately, the σ^M regulon member(s) that are responsible for the toxic effect have not yet been identified. Similarly, the antagonist of the alternative sigma factor σ^Y, YxlC, is essential. As described for the essentiality of the anti-sigma factor for σ^M, the precise reason for the toxicity of high σ^Y activity is unknown.[47] Very recently, an essential role for the nuclease-like protein HlpB was reported: it is required for the resolution of toxic recombination intermediates generated by the ATP-dependent DNase AddAB. As for the other essential proteins with a protective function, *hlpB* is dispensable in the absence of the cause of toxicity, *addA* or *addB*.[48] Three essential enzymes are required for the disposal of toxic metabolic intermediates: PncB, DgkB, and YtbE prevent the accumulation of nicotinate, diacylglycerol and yet unidentified toxic aldehydes, respectively (see Fig. 3).[8,49,50] The SunI protein provides *B. subtilis* with immunity to the endogenous bacteriocin sublancin (SunA). Accordingly, the *sunI* gene is essential as long as the cells produce sublancin (*i.e.* in the presence of a functional *sunA* gene), whereas deletion mutants of the complete *sunA–sunI* region can easily be obtained.[51] An interesting example of a facultative essential enzyme is RNase III: the corresponding *rnc* gene is essential in *B. subtilis* but not in other closely related species.[52] The possibility to isolate suppressor mutations that bypass RNase III essentiality is in good agreement with facultative essentiality.[53] Very recently, the reason for the exclusive essentiality of RNase III in *B. subtilis* has been uncovered: the enzyme is required for the degradation of duplexes formed between toxin-coding mRNAs (*txpA* and *yonT*) and their cognate antisense RNAs (*ratA* and as-*yonT*, respectively) (see Fig. 3).[54] Indeed toxin-encoding mRNAs accumulate upon depletion of RNase III in *B. subtilis*.[55] Since both Type 1 toxin–antitoxin systems are encoded on prophages,[56] it is obvious that RNase III is not essential in a *B. subtilis* strain cured of the prophages. It is likely that the detailed study of newly identified toxin–antitoxin systems will reveal novel essential genes specifying protective functions that have escaped previous studies.

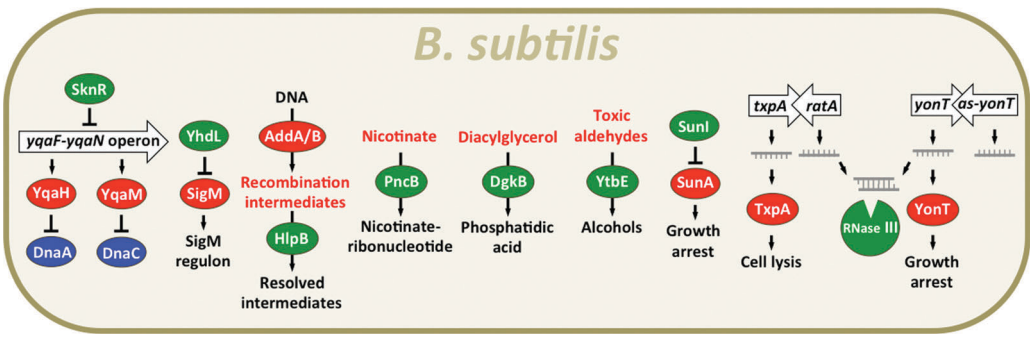

Fig. 3 Protective essential proteins in *B. subtilis*. The essential proteins are shown in green, and the toxic proteins or products are shown in red. As shown for YhdL/SigM, YxlC is essential since it prevents the toxic expression of SigY regulon members. For details, see text.

Table 2 Essential proteins with unknown functions in *B. subtilis*

Protein	Domains	Paralogue	Regulon	Expression	Mass (kDa)	Localization
YcgG	—	YqcI	SigE, SpoIIID	Sporulation	29	—
YddT	Signal peptide (1–28 aa)	YomL	AbrB	—	25	—
YomL	Signal peptide (1–28 aa)	YddT	—	—	25	—
YloU	—	YqhY	—	Constitutive	13	Cytosolic
YqhY	—	YloU	—	Constitutive	14	Cytosolic
YlaN	—	—	—	Constitutive	10	Cytosolic

aa, Amino acids.

9 Essential genes encoding proteins of unknown function

As stated above, there are currently six essential genes encoding proteins of unknown function (see Fig. 1). Given the importance of essential genes and the encoded proteins for the biology of the cell, the identification of the function of these six proteins is a major challenge for the future research on *B. subtilis*.

Interestingly, five of the six unknown essential genes have paralogous genes in *B. subtilis* (see Table 2). The YcgG protein is similar to the non-essential YqcI protein (67% identity). The *ycgG* gene was reported to be expressed during sporulation in the mother cell compartment,[57] however, some basal expression occurs throughout growth.[58] The YddT–YomL couple is extremely interesting since the two proteins differ in only 4 out of 228 amino acids. Nevertheless, each of the corresponding *yddT* and *yomL* genes was reported to be essential.[5] The *yomL* gene is part of the SPβ prophage, and the viability of *B. subtilis* cured of this prophage suggests a protective function for YomL. Interestingly, the genes located upstream of *yddT* and *yomL* encode proteins with identical amino acid sequences. It is tempting to speculate that YddT and YomL are parts of very similar yet specific and independent toxin–antitoxin systems. The last essential gene pair encodes the unknown proteins YloU and YqhY (31% identity). These proteins are constitutively expressed and were found as intracellular proteins in recent genome-scale studies with *B. subtilis*.[58,59] The rather high constitutive expression of both the *yloU* and the *yqhY* genes suggests an important function throughout growth of *B. subtilis*.

The *ylaN* gene encodes a constitutively expressed cytosolic protein that was suggested to be involved in the control of cell shape.[19,58,59] The determination of the crystal structure of the homologous protein from *S. aureus* revealed the presence of an unidentified ligand, which is likely to be a cofactor.[18] As for YloU and YqhY, the high constitutive expression of YlaN suggests a crucial role for this protein.

10 Future directions of research

For the complete understanding of the biology of the *B. subtilis* cell, the elucidation of the function of all essential genes is of key priority. This knowledge will be important for the generation of minimal cells, but also for the creation of tailor-made cells for biotechnological purposes. Thus, the six remaining unknown essential proteins of *B. subtilis* require intensive investigation. However, the comprehensive understanding of essential functions and essential proteins is hampered by the emerging moonlighting of many proteins.[60] In some cases, the yet to be discovered second (moonlighting) activity might cause essentiality. Moreover, proteins may be synthetically essential as shown in a recent study for the nitrogen regulator GlnR and the glutamine synthetase GlnA.[25] The functional identification of the remaining unknown essential genes would make *B. subtilis* the very first organism in which the functions of all essential proteins are understood.

11 Further information

Acknowledgements

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References

1 D. G. Gibson, J. I. Glass, C. Lartigue, V. N. Noskov, R. Y. Chuang, M. A. Algire, G. A. Benders, M. G. Montague, L. Ma, M. M. Moodie, C. Merryman, S. Vashee, R. Krishnakumar, N. Assad-Garcia, C. Andrews-Pfannkoch, E. A. Denisova, L. Young, Z. Q. Qi, T. H. Segall-Shapiro, C. H. Calvey, P. P. Parmar, C. A. Hutchison, H. O. Smith and J. C. Venter, *Science*, 2010, **329**, 52–56.
2 M. Juhas, L. Eberl and G. M. Church, *Trends Biotechnol.*, 2012, **30**, 601–607.
3 M. Juhas, L. Eberl and J. I. Glass, *Trends Cell Biol.*, 2011, **21**, 562–568.
4 K. Kobayashi, S. D. Ehrlich, A. Albertini, G. Amati, K. K. Andersen, M. Arnaud, K. Asai, S. Ashikaga, S. Aymerich, P. Bessieres, F. Boland, S. C. Brignell, S. Bron, K. Bunai, J. Chapuis, L. C. Christiansen, A. Danchin, M. Débarbouillé, E. Dervyn, E. Deuerling, K. Devine, S. K. Devine, O. Dreesen, J. Errington, S. Fillinger, S. J. Foster, Y. Fujita, A. Galizzi, R. Gardan, C. Eschevins, T. Fukushima, K. Haga, C. R. Harwood, M. Hecker, D. Hosoya, M. F. Hullo, H. Kakeshita, D. Karamata, Y. Kasahara, F. Kawamura, K. Koga, P. Koski, R. Kuwana, D. Imamura, M. Ishimaru, S. Ishikawa, I. Ishio, D. Le Coq, A. Masson, C. Mauël, R. Meima, R. P. Mellado, A. Moir, S. Moriya, E. Nagakawa, H. Nanamiya, S. Nakai, P. Nygaard, M. Ogura, T. Ohanan, M. O'Reilly, M. O'Rourke, Z. Pragai, H. M. Pooley, G. Rapoport, J. P. Rawlins, L. A. Rivas, C. Rivolta, A. Sadaie, Y. Sadaie, M. Sarvas, T. Sato, H. H. Saxild, E. Scanlan, W. Schumann, J. F. M. L. Seegers, J. Sekiguchi, A. Sekowska, S. J. Séror, M. Simon, P. Stragier, R. Studer, H. Takamatsu, T. Tanaka, M. Takeuchi, H. B. Thomaides, V. Vagner, J. M. van Dijl, K. Watabe, A. Wipat, H. Yamamoto,

M. Yamamoto, Y. Yamamoto, K. Yamane, K. Yata, K. Yoshida, H. Yoshikawa, U. Zuber and N. Ogasawara, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 4678–4683.
5 H. B. Thomaides, E. J. Davison, L. Burston, H. Johnson, D. R. Brown, A. C. Hunt, J. Errington and L. Czaplewski, *J. Bacteriol.*, 2007, **189**, 591–602.
6 T. Bergmiller, M. Ackermann and O. K. Silander, *PLoS Genet.*, 2012, **8**, e1002803.
7 T. Kimura, Y. Amaya, K. Kobayashi, N. Ogasawara and T. Sato, *J. Bacteriol.*, 2010, **192**, 6209–6216.
8 S. Matsuoka, M. Hashimoto, Y. Kamiya, T. Miyazawa, K. Ishikawa, H. Hara and K. Matsumoto, *Genes Genet. Syst.*, 2011, **86**, 365–376.
9 C. Fabret, E. Dervyn, B. Dalmais, A. Guillot, C. Marck, H. Grosjean and P. Noirot, *Mol. Microbiol.*, 2011, **80**, 1062–1074.
10 U. Mäder, A. G. Schmeisky, L. A. Flórez and J. Stülke, *Nucleic Acids Res.*, 2012, **40**, D1278–D1287.
11 S. Even, O. Pellegrini, L. Zig, V. Labas, J. Vinh, D. Bréchemmier-Baey and H. Putzer, *Nucleic Acids Res.*, 2005, **33**, 2141–2152.
12 F. M. Commichau, F. M. Rothe, C. Herzberg, E. Wagner, D. Hellwig, M. Lehnik-Habrink, E. Hammer, U. Völker and J. Stülke, *Mol. Cell. Proteomics*, 2009, **8**, 1350–1360.
13 K. Shababian, A. Jamalli, L. Zig and H. Putzer, *EMBO J.*, 2009, **28**, 3523–3533.
14 O. Pellegrini, J. Nezzar, A. Marchfelder, H. Putzer and C. Condon, *EMBO J.*, 2003, **22**, 4534–4543.
15 S. P. Salowe, J. Wiltsie, J. C. Hawkins and L. M. Sonatore, *J. Biol. Chem.*, 2009, **284**, 9656–9662.
16 C. T. Lauhon, *Biochemistry*, 2012, **51**, 8950–8963.
17 L. Paoletti, Y. J. Lu, G. E. Schujman, D. de Mendoza and C. O. Rock, *J. Bacteriol.*, 2007, **189**, 5816–5824.
18 L. Xu, S. E. Sedelnikova, P. J. Baker, A. Hunt, J. Errington and D. W. Rice, *Proteins*, 2007, **68**, 438–445.
19 A. Hunt, J. P. Rawlins, H. B. Thomaides and J. Errington, *Microbiology*, 2006, **152**, 2895–2907.
20 T. Murakami, K. Haga, M. Takeuchi and T. Sato, *J. Bacteriol.*, 2002, **184**, 1998–2004.
21 R. J. Heath, N. Su, C. K. Murphy and C. O. Rock, *J. Biol. Chem.*, 2000, **275**, 40128–40133.
22 S. Fukushima, M. Itaya, H. Kato, N. Ogasawara and H. Yoshikawa, *J. Bacteriol.*, 2007, **189**, 8575–8583.
23 C. Henry, R. Overbeek and R. L. Stevens, *Biotechnol. J.*, 2010, **5**, 695–704.
24 Y. Azuma and M. Ota, *BMC Syst. Biol.*, 2009, **3**, 111.
25 K. Tanaka, C. S. Henry, J. F. Zinner, E. Jolivet, M. P. Cohoon, F. Xia, V. Bidnenko, S. D. Ehrlich, R. L. Stevens and P. Noirot, *Nucleic Acids Res.*, 2013, **41**, 687–699.
26 Y. Luo and J. D. Helmann, *Mol. Microbiol.*, 2012, **83**, 623–639.
27 F. M. P. Mehne, K. Gunka, H. Eilers, C. Herzberg, V. Kaever and J. Stülke, *J. Biol. Chem.*, 2013, **288**, 2004–2017.
28 R. R. Chaudhuri, A. G. Allen, P. J. Owen, G. Shalom, K. Stone, M. Harrison, T. A. Burgis, M. Lockyer, J. Garcia-Lara, S. J. Foster, S. J. Pleasance, S. E. Peters, D. J. Maskell and I. G. Charles, *BMC Genomics*, 2009, **10**, 291.
29 H. J. Song, K. S. Ko, J. Y. Lee, J. Y. Baek, W. S. Oh, H. S. Yoon, J. Y. Jeong and J. Chun, *Mol. Cells*, 2005, **19**, 365–374.
30 J. J. Woodward, A. T. Iavarone and D. A. Portnoy, *Science*, 2010, **328**, 1703–1705.
31 M. A. Leyva-Vazquez and P. Setlow, *J. Bacteriol.*, 1994, **176**, 3903–3910.
32 M. E. Muñoz-Márquez and E. Ponce-Rivas, *J. Basic Microbiol.*, 2010, **50**, 232–240.
33 M. Lehnik-Habrink, H. Pförtner, L. Rempeters, N. Pietack, C. Herzberg and J. Stülke, *Mol. Microbiol.*, 2010, **77**, 958–971.
34 M. Lehnik-Habrink, R. J. Lewis, U. Mäder and J. Stülke, *Mol. Microbiol.*, 2012, **84**, 1005–1017.
35 A. J. Carpousis, *Annu. Rev. Microbiol.*, 2007, **61**, 71–87.
36 G. Akanuma, H. Nanamiya, Y. Natori, K. Yano, S. Suzuki, S. Omata, M. Ishizuka, Y. Sekine and F. Kawamura, *J. Bacteriol.*, 2012, **194**, 6282–6291.
37 B. Wienen, R. Ehrlich, M. Stöfffler-Meilicke, G. Stöffler, I. Smith, D. Weiss, R. Vince and S. Pestka, *J. Biol. Chem.*, 1979, **254**, 8031–8041.
38 H. Nanamiya, G. Akanuma, Y. Natori, R. Muruyama, S. Kosono, T. Kudo, K. Kobayashi, N. Ogasawara, S. M. Park, K. Ochi and F. Kawamura, *Mol. Microbiol.*, 2004, **52**, 273–283.
39 S. Shoji, C. M. Dambacher, Z. Shajani, J. R. Williamson and P. G. Schultz, *J. Mol. Biol.*, 2011, **413**, 751–761.
40 E. K. Leung, N. Suslov, N. Tuttle, R. Sengupta and J. A. Piccirilli, *Annu. Rev. Biochem.*, 2011, **80**, 527–555.
41 P. Bisicchia, D. Noone, E. Lioliou, A. Howell, S. Quigley, T. Jensen, H. Jarmer and K. M. Devine, *Mol. Microbiol.*, 2007, **65**, 180–200.
42 M. E. Winkler and J. A. Hoch, *J. Bacteriol.*, 2008, **190**, 2645–2648.
43 S. Dubrac, P. Bisicchia, K. M. Devine and T. Msadek, *Mol. Microbiol.*, 2008, **70**, 1307–1322.
44 Z. Qin, J. Zhang, B. Xu, L. Chen, Y. Wu, X. Yang, X. Shen, S. Molin, A. Danchin, H. Jiang and D. Qu, *BMC Microbiol.*, 2006, **6**, 96.
45 K. Stephenson and J. A. Hoch, *Curr. Med. Chem.*, 2004, **11**, 765–773.
46 M. J. Horsburgh and A. Moir, *Mol. Microbiol.*, 1999, **32**, 41–50.
47 R. Mendez, A. Gutierrez, J. Reyes and L. Márquez-Magaña, *DNA Cell Biol.*, 2012, **31**, 946–955.
48 M. Pediaditakis, M. Kaufenstein and P. L. Graumann, *J. Bacteriol.*, 2012, **194**, 6184–6194.
49 P. Rossolillo, I. Marinoni, E. Galli, A. Colosimo and A. M. Albertini, *J. Bacteriol.*, 2005, **187**, 7155–7160.
50 J. Lei, Y. F. Zhou, L. F. Li and X. D. Sun, *Protein Sci.*, 2009, **18**, 1792–1800.
51 J.-Y. F. Dubois, T. R. H. M. Kouwen, A. K. C. Schurich, C. R. Reis, H. T. Ensing, E. N. Trip, J. C. Zweers and J. M. van Dijl, *Antimicrob. Agents Chemother.*, 2009, **53**, 651–661.
52 C. Chevalier, E. E. Huntzinger, P. Fechter, S. Boisset, F. Vandenesch, P. Romby and T. Geissmann, *Methods Enzymol.*, 2008, **447**, 309–327.
53 M. A. Herskovitz and D. Bechhofer, *Mol. Microbiol.*, 2000, **38**, 1027–1033.

54 S. Durand, L. Gilet and C. Condon, *PLoS Genet.*, 2012, **8**, e1003181.
55 S. Durand, L. Gilet, P. Bessières, P. Nicolas and C. Condon, *PLoS Genet.*, 2012, **8**, e1002520.
56 S. Durand, N. Jahn, C. Condon and S. Brantl, *RNA Biol.*, 2012, **9**, 1491–1497.
57 P. Eichenberger, M. Fujita, S. T. Jensen, E. M. Conlon, D. Z. Rudner, S. T. Wang, C. Ferguson, K. Haga, T. Sato, J. S. Liu and R. Losick, *PLoS Biol.*, 2004, **2**, e328.
58 P. Nicolas, U. Mäder, E. Dervyn, T. Rochat, A. Leduc, N. Pigeonneau, E. Bidnenko, E. Marchadier, M. Hoebeke, S. Aymerich, D. Becher, P. Bisicchia, E. Botella, O. Delumeau, G. Doherty, E. L. Denham, M. J. Fogg, V. Fromion, A. Goelzer, A. Hansen, E. Härtig, C. R. Harwood, G. Homuth, H. Jarmer, M. Jules, E. Klipp, L. Le Chat, F. Lecointe, P. Lewis, W. Liebermeister, A. March, R. A. T. Mars, P. Nannapaneni, D. Noone, S. Pohl, B. Rinn, F. Rügheimer, P. K. Sappa, F. Samson, M. Schaffer, B. Schwikowski, L. Steil, J. Stülke, T. Wiegert, K. M. Devine, A. J. Wilkinson, J. M. van Dijl, M. Hecker, U. Völker, P. Bessières and P. Noirot, *Science*, 2012, **335**, 1103–1106.
59 A. Otto, J. Bernhardt, H. Meyer, M. Schaffer, F. A. Herbst, J. Siebourg, U. Mäder, M. Lalk, M. Hecker and D. Becher, *Nat. Commun.*, 2010, **1**, 137.
60 S. D. Copley, *Bioessays*, 2012, **34**, 578–588.
61 A. M. Guérout-Fleury, K. Shazand, N. Frandsen and P. Stragier, *Gene*, 1995, **167**, 335–336.
62 A. Wach, *Yeast*, 1996, **12**, 259–265.
63 F. M. Commichau, C. Herzberg, P. Tripal, O. Valerius and J. Stülke, *Mol. Microbiol.*, 2007, **65**, 642–654.