STREPTOMYCES MORPHOGENESIS

What is currently (not) known about Streptomyces morphogenesis regulation

Outline

- Importance of morphogenesis studies
- What studies have demonstrated so far
- What is still not known

WHY?

Why people study
Streptomyces morphogenesis regulation?

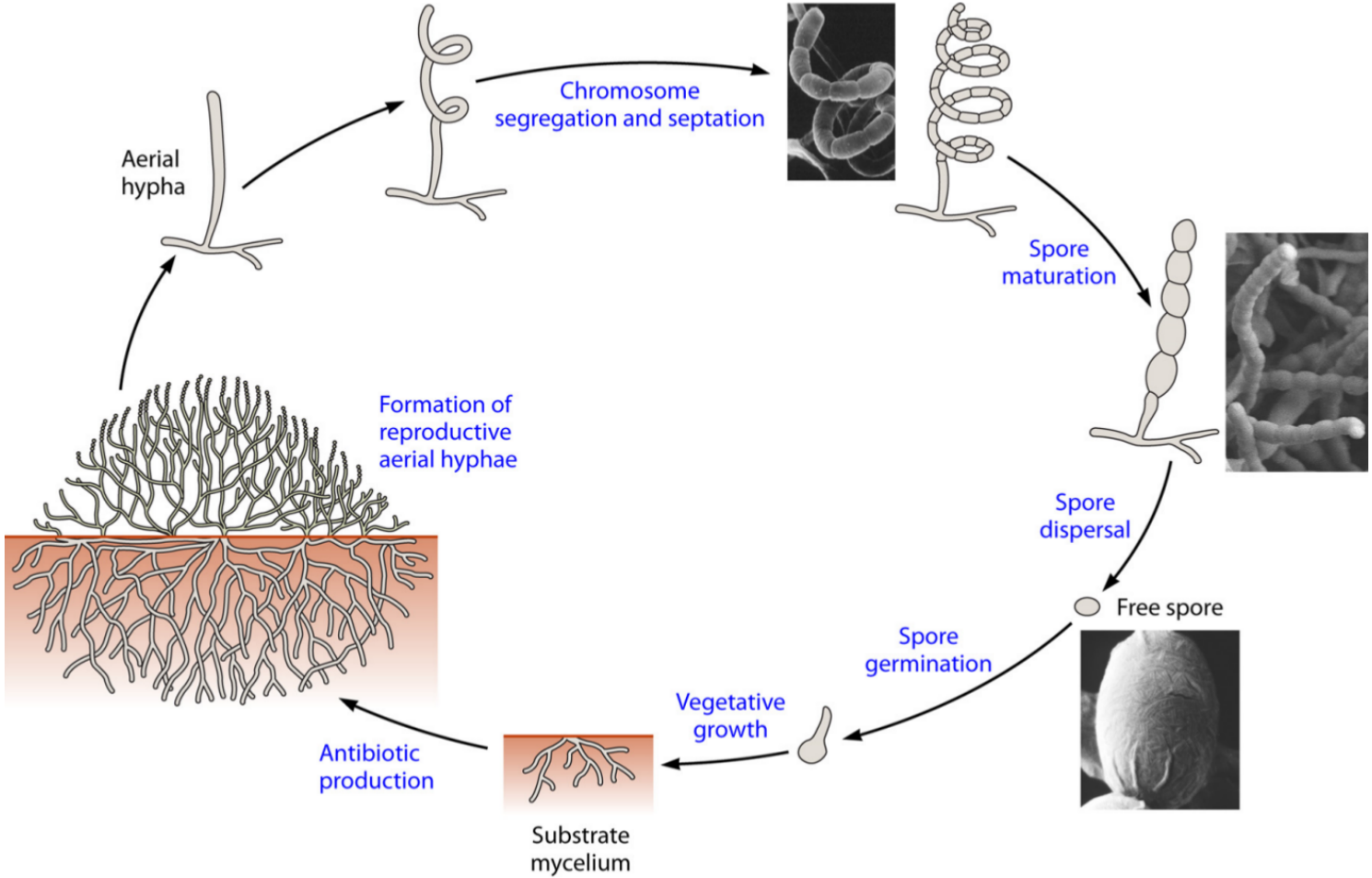
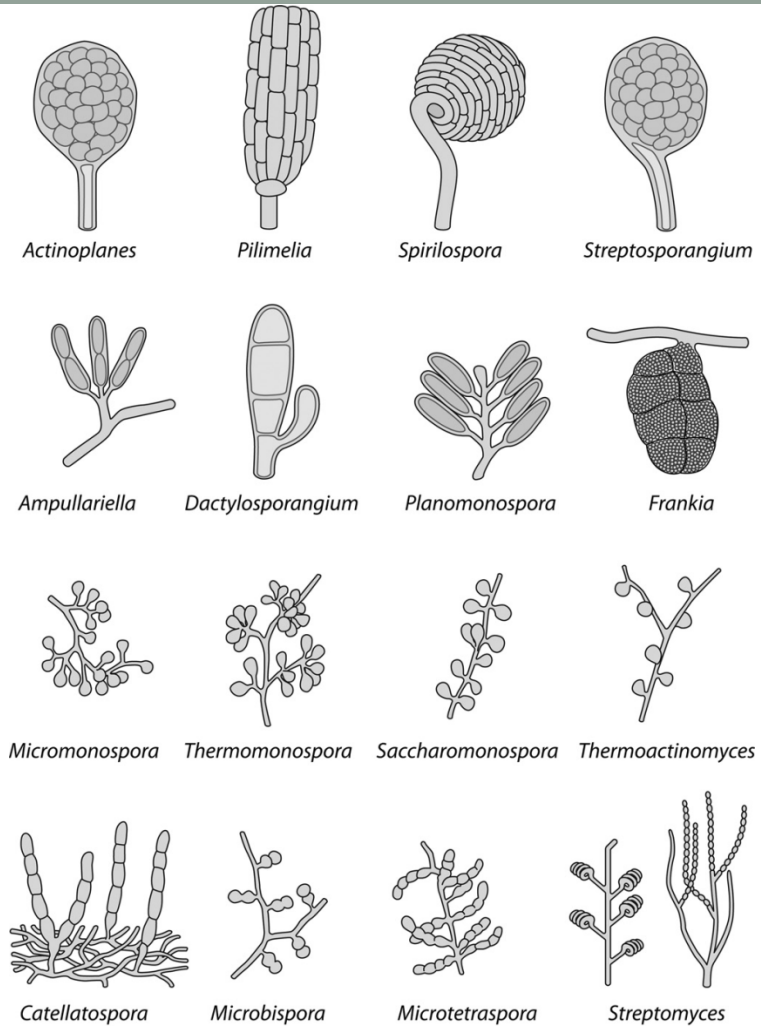
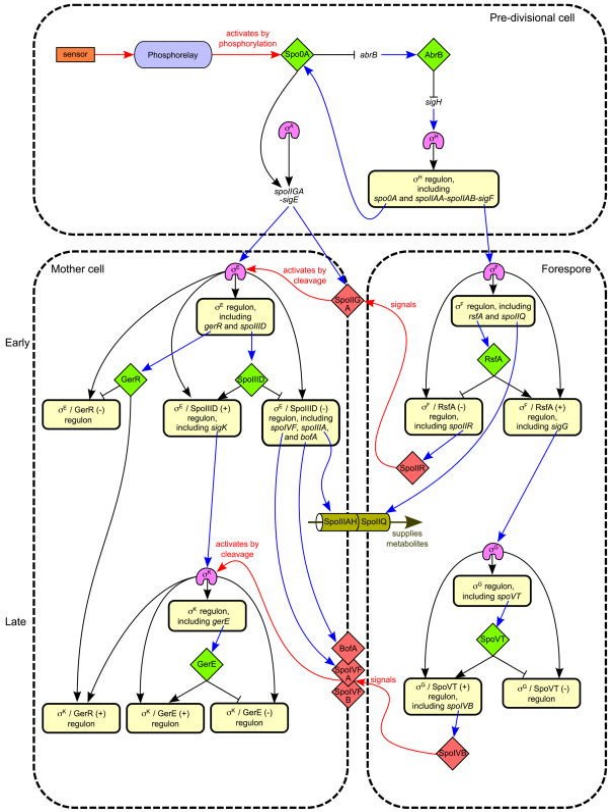


FIG 1 Schematic representation of the life cycle of sporulating actinomycetes.


Actinoplanes
Pilimelia
Spirilospora
Streptosporangium
Ampullariella
Dactylosporangium
Planomonospora
Frankia
Micromonospora
Thermomonospora
Saccharomonospora
Thermoactinomyces
Catellatospora
Microbispora
Microtetraspora
Streptomyces

Intermediate summary

- Survival strategies are induced in low-nutrient conditions (starvation).
- Sporulation is a survival strategy.
- Secondary metabolite production is also a survival strategy.
- In *Streptomyces*, both strategies seem to be co-regulated.
- *Bacillus subtilis* has the best-studied sporulation regulation →
- Sporulation differs in *Streptomyces*.



WHAT IS KNOWN

The Grand Scheme of Sporulation

Model Streptomyces

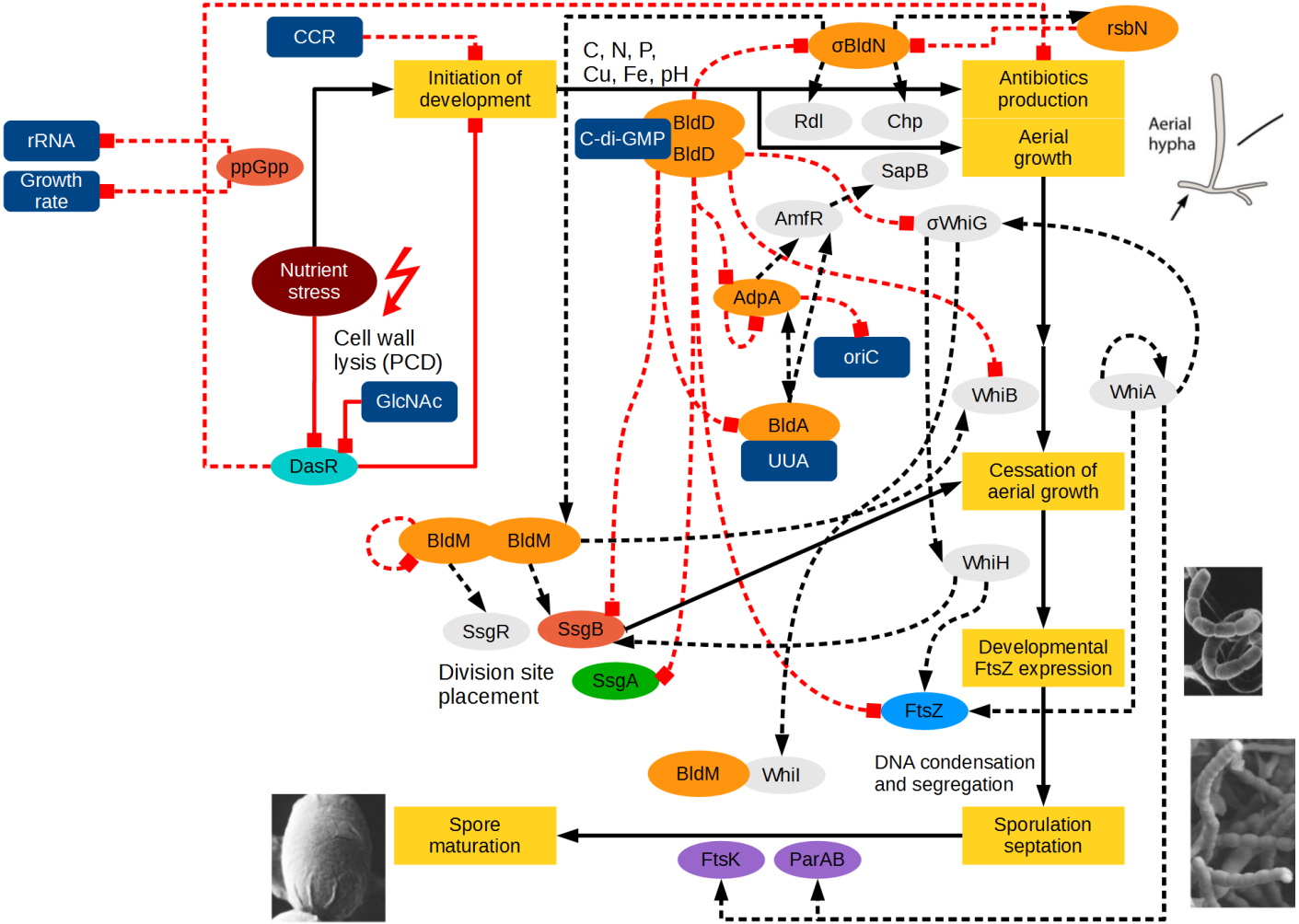
- 3 model species have provided nearly all the experimental information about morphological development of streptomycetes.
- The most widely studied is *S. coelicolor* A3(2)
- *S. griseus* has been studied for its production of a hormone-like developmental signaling molecule, A-factor.
- *S. venezuelae* sporulates rapidly, synchronously and comprehensively in submerged culture

Key regulators

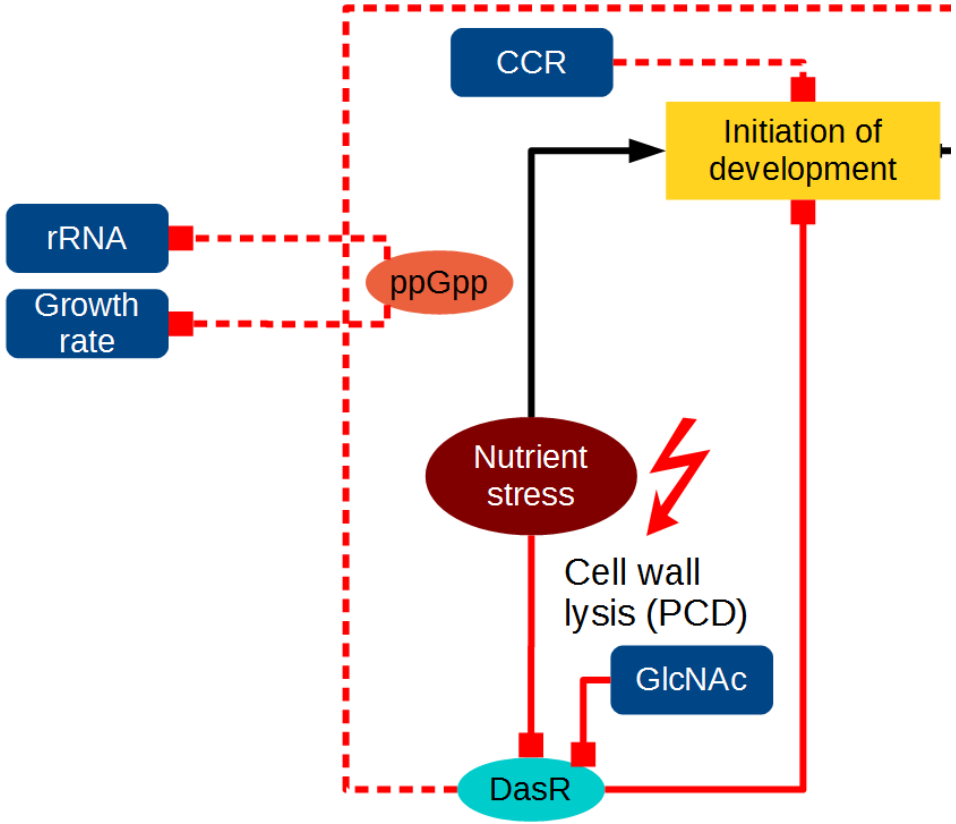
- Bald, “hairless” phenotype mutants lacking fluffy aerial hyphae: ***bld*** genes (and mutants)
- Lack of common (but not universal) gray spore pigment: ***whi*** genes (and mutants)
- Rapid aerial mycelium formation (*S. lividans*): ***ram*** genes
- Complementation-resolved *S. griseus* sporulation mutants: *ssgA*-like genes

Some of regulators and effectors

- DasR
- Bld: B, C, D, G, H, J, K, M, N
- Whi: A, B, D, G, H, I, J;
- WhiB-like (Wbl): A, C, E
- SsgA, SsgB, SsgR
- AmfR
- Chp: long (A, B, C), short (D, E, F, G, H)
- Rdl: A, B
- Sap: B, T; AmfS
- Fts: Z, K
- Par: A, B

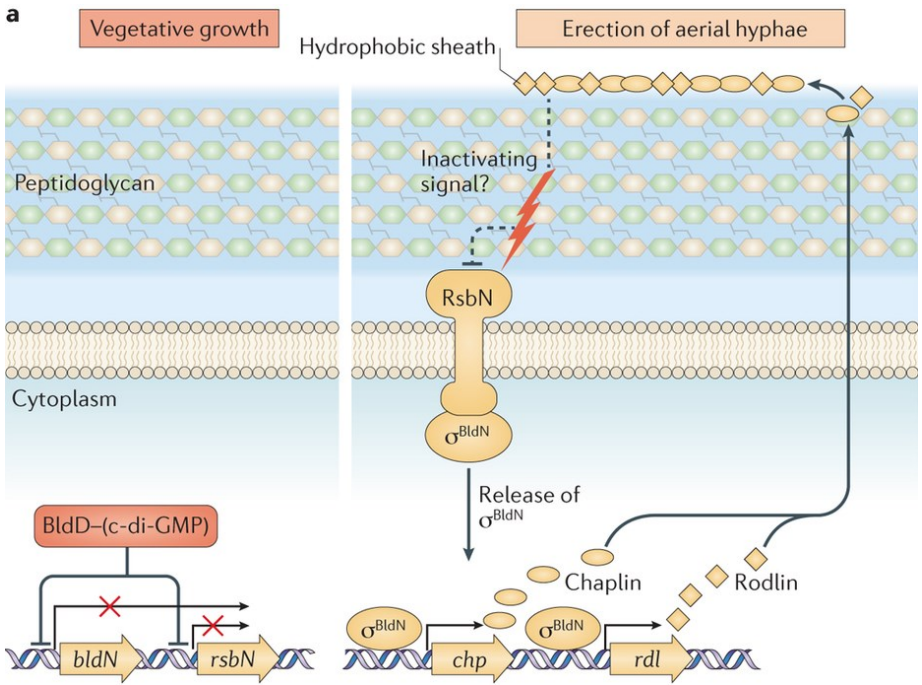

CCR
Initiation of development
C, N, P,
Cu, Fe, pH
σBldN
rsbN
Antibiotics production
rRNA
Growth rate
ppGpp
C-di-GMP
BldD
BldD
Rdl
Chp
Aerial growth
Aerial hypha
SapB
AmfR
σWhiG
Nutrient stress
Cell wall lysis (PCD)
GlcNAc
DasR
AdpA
oriC
BldA
UUA
WhiB
WhiA
Cessation of aerial growth
BldM
BldM
WhiH
SsgR
SsgB
SsgA
Division site placement
Developmental FtsZ expression
FtsZ
BldM
WhiI
DNA condensation and segregation
Spore maturation
Sporulation septation
FtsK
ParAB

Onset of development



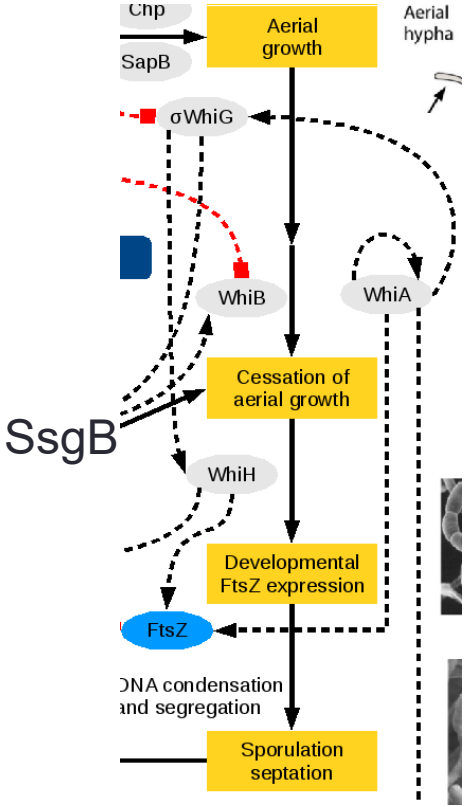
- Guanosine tetraphosphate (ppGpp), amino acids alarmone
- Cell wall derived N-acetyl-glucosamine (GlcNAc) => DasR
- Some bald mutants sporulate on minimal media: carbon catabolite repression

Early hyphae growth



- BldD is a repressor of *bldN*
- BldN is a sigma factor controlling chaplins and rodlins
- RsbN is a BldN “repressor”, BldN is RsbN activator
- SapB (*ramS*, *amfS*)

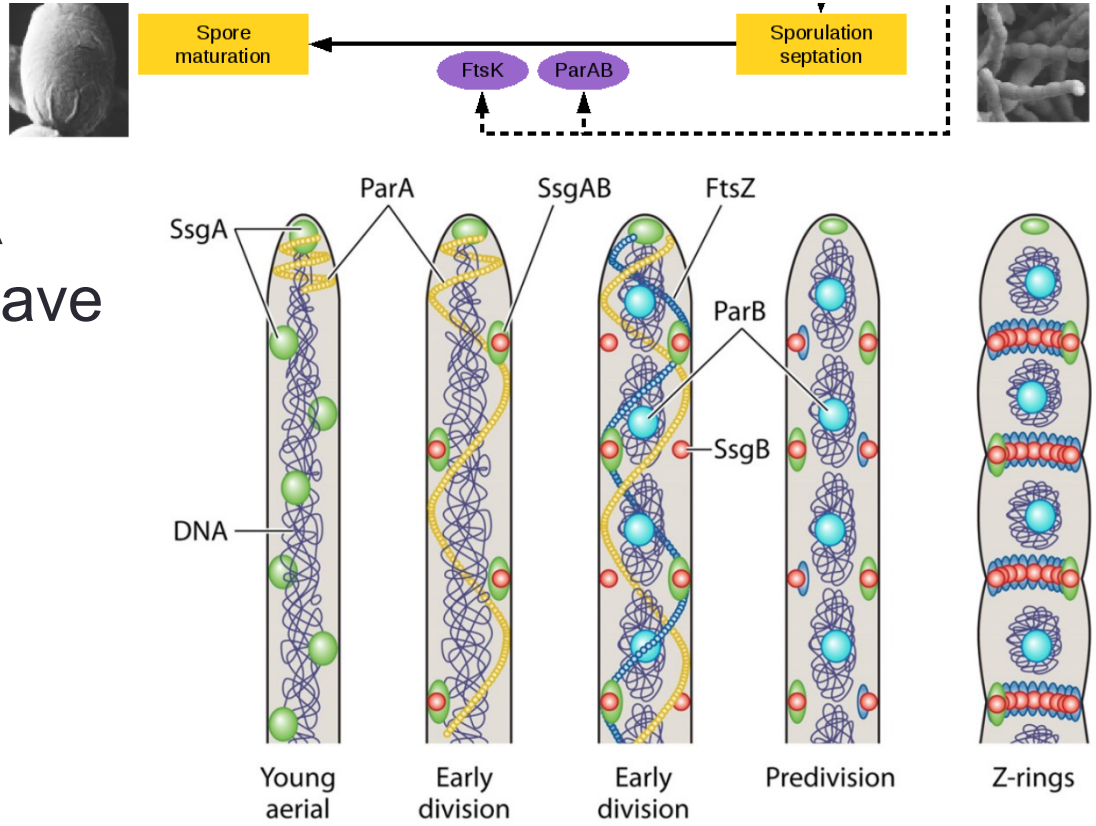
Cessation of growth and septation

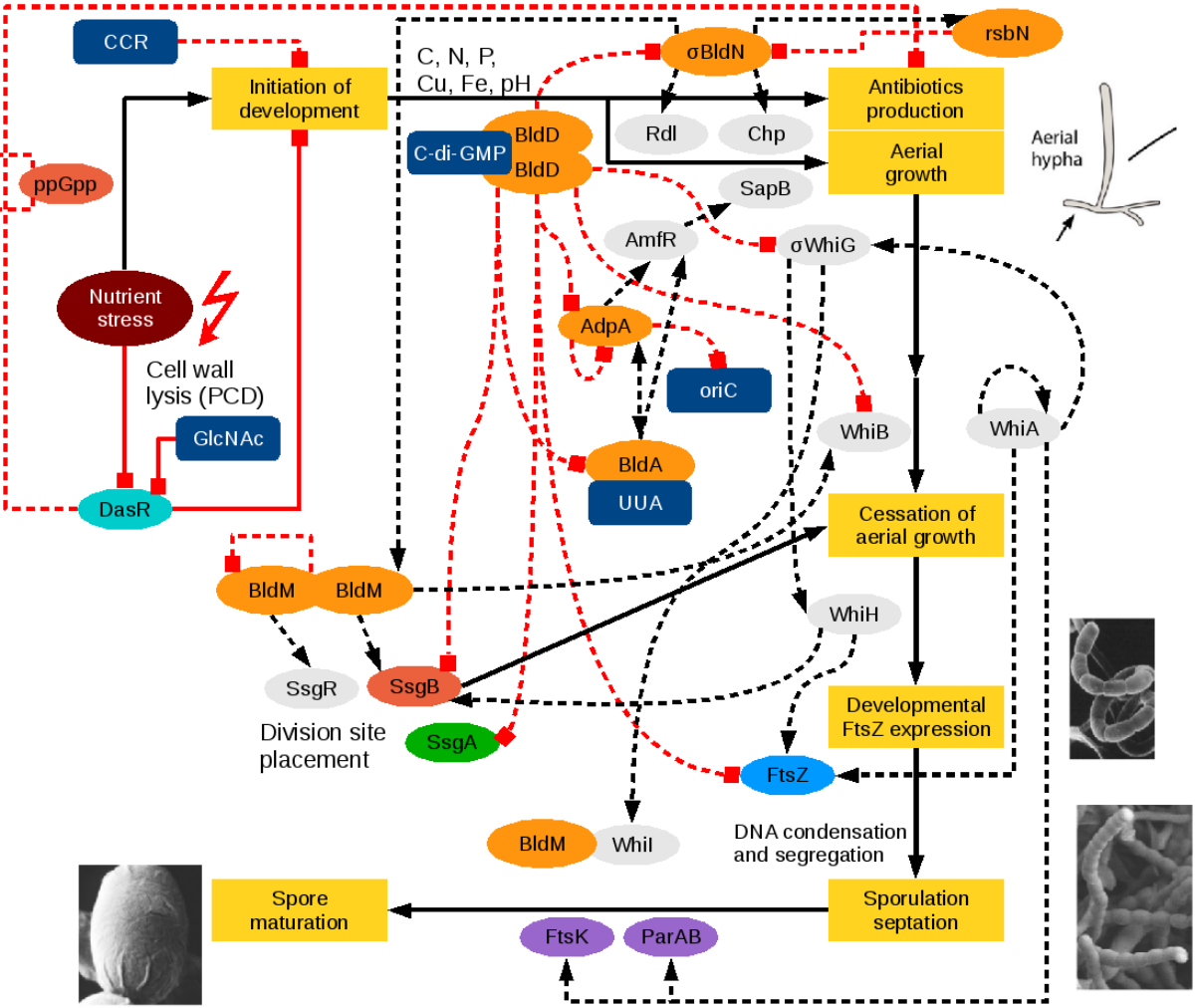


- Unknown signal => *whiA*/*whiB*; *ssgB*
- *whiA*$^{-}$/*whiB*$^{-}$: long hypercoiling hyphae
- *ssgB*$^{-}$: large aerial biomass
- *whiA*: syntenic with 3 genes of glycolysis/gluconeogenesis and 1 protein secretion gene
- Developmental FtsZ expression
- FtsZ overrides *whi* mutant phenotypes

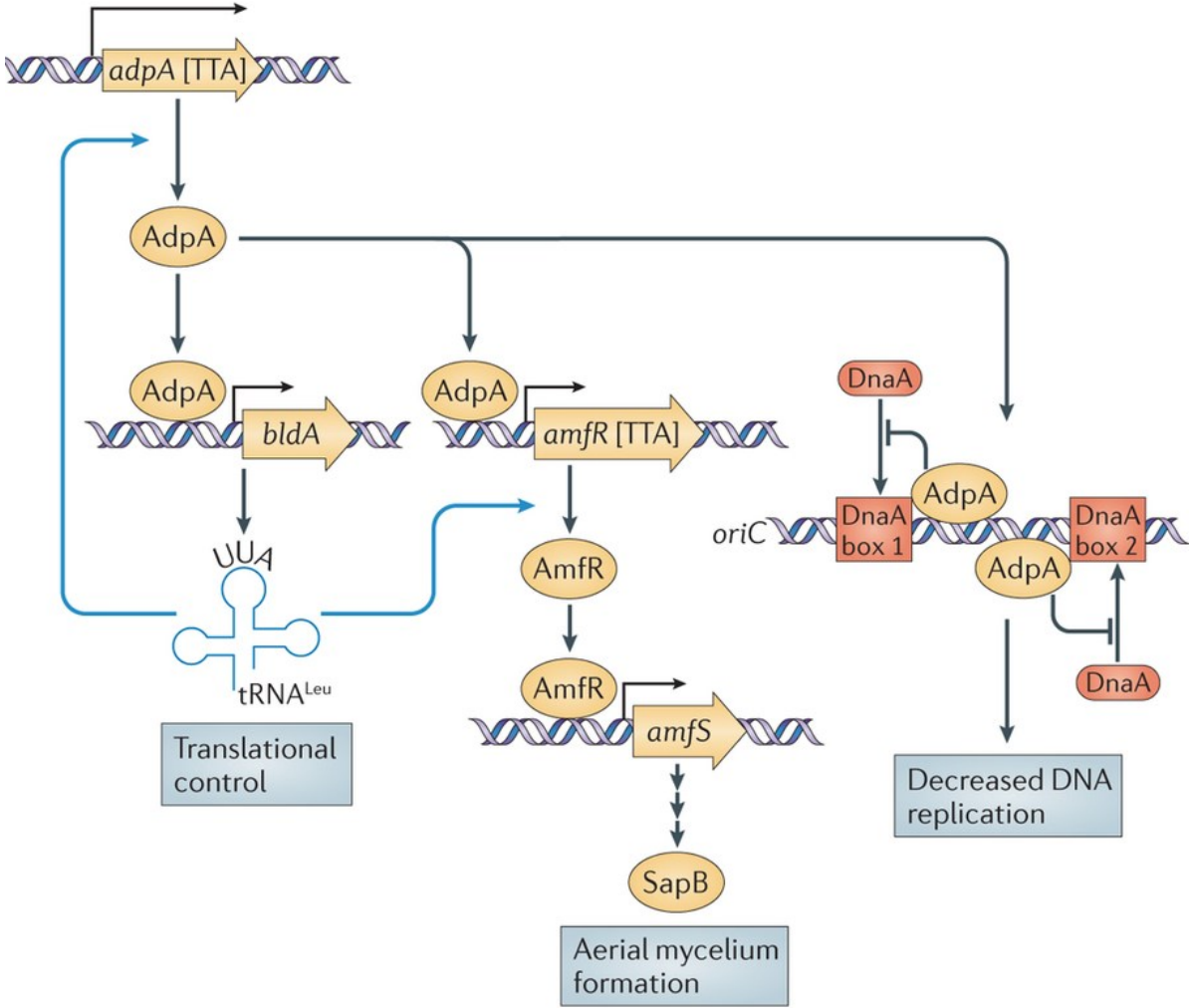
Sporulation-specific cell division

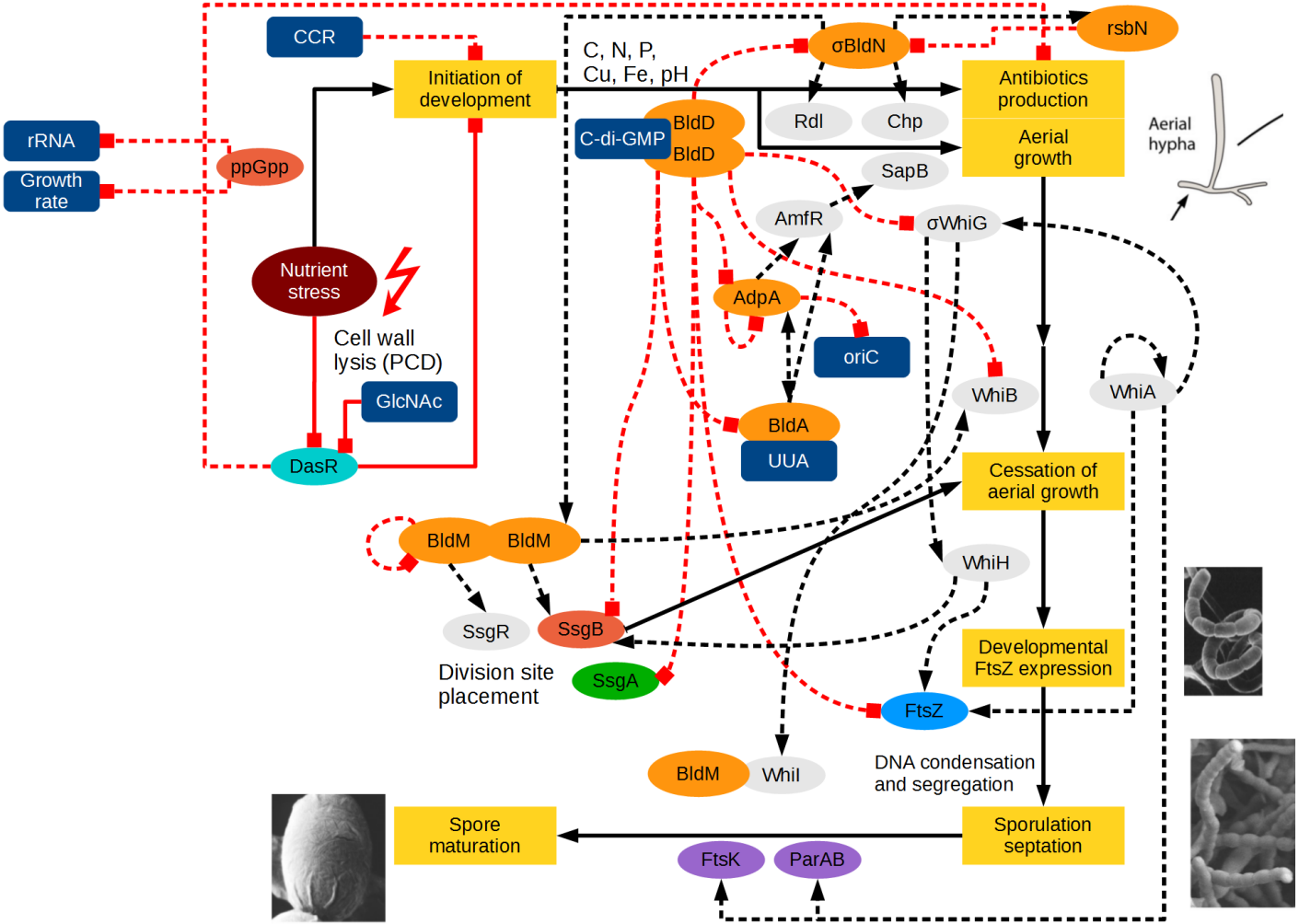
- SsgA, SsgB, FtsZ: septation sites
- ParA, ParB, FtsK: DNA segregation (mutants have incomplete spore chromosomes)
- Other proteins: SmeA, SffA, HupS, sIHF, Smc, Dps





- BldD forms a dimer with the help of tetrameric c-di-GMP
- BldD is a key repressor:
  - 147 targets, 42 of them - regulators
  - BldN (BldM, RsbN, Rdl, Chp, …)
  - WhiG (WhiA, WhiB, WhiI, WhiH, …)
  - SsgA, SsgB
  - BldH/AdpA, BldA (SapB, …)


adpA [TTA]
AdpA
AdpA
bldA
AdpA
amfR [TTA]
UUA
tRNALeu
Translational control
AmfR
AmfR
amfS
SapB
Aerial mycelium formation
DnaA
AdpA
oriC
DnaA box 1
DnaA box 2
AdpA
DnaA
Decreased DNA replication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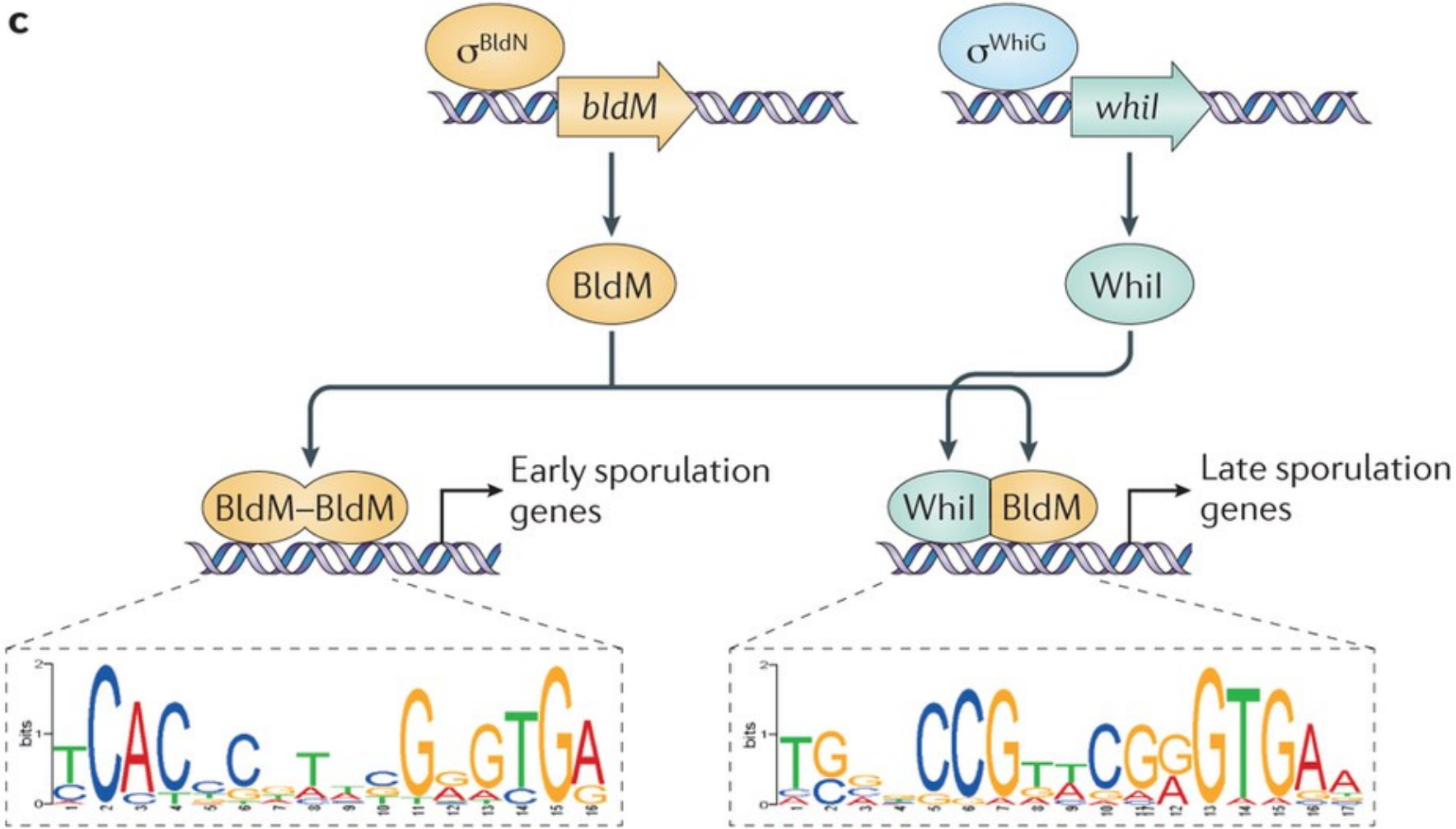
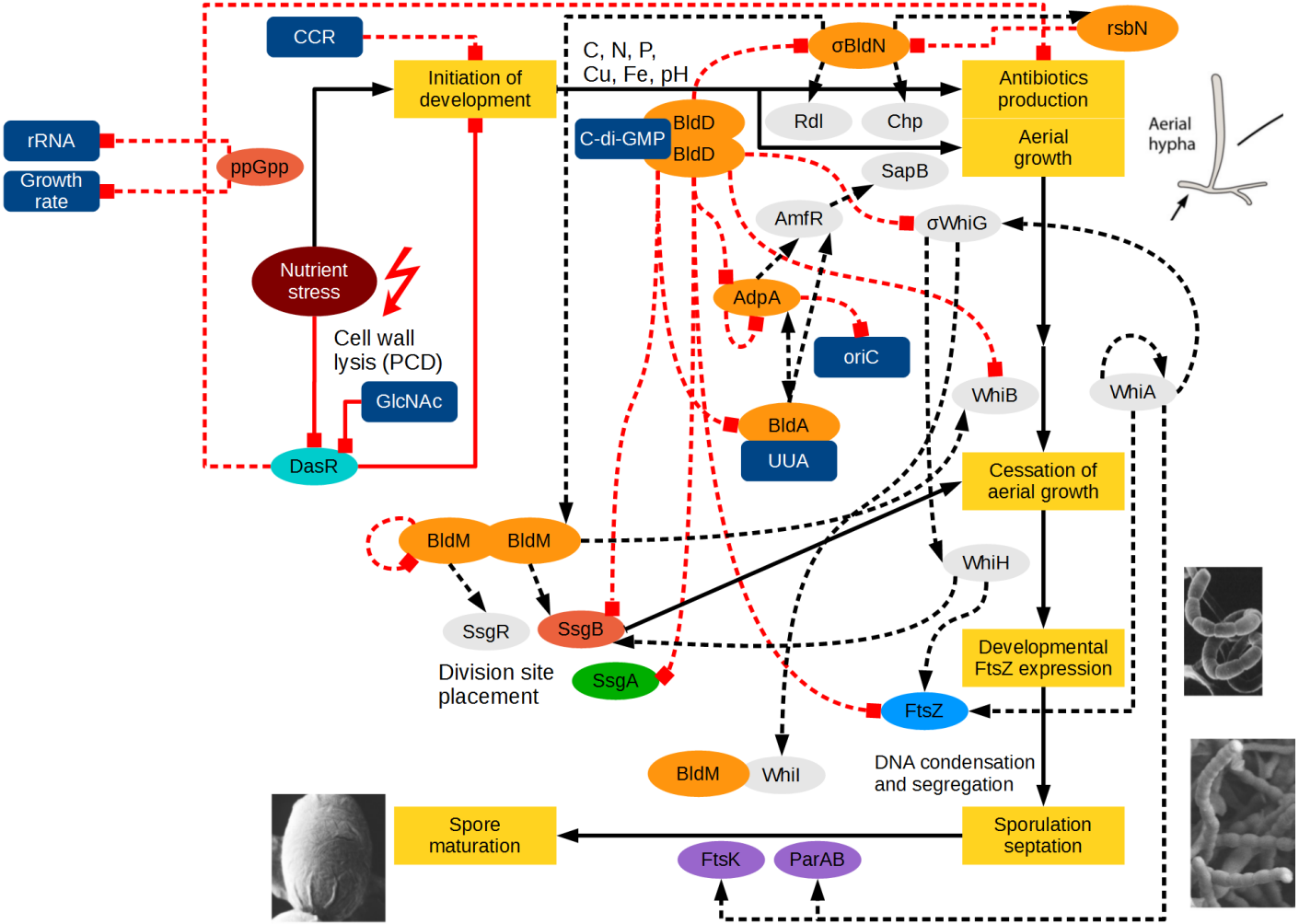

c
σBldN
bldM
σWhiG
whiI
BldM
WhiI
BldM–BldM
Early sporulation genes
WhiI
BldM
Late sporulation genes
bits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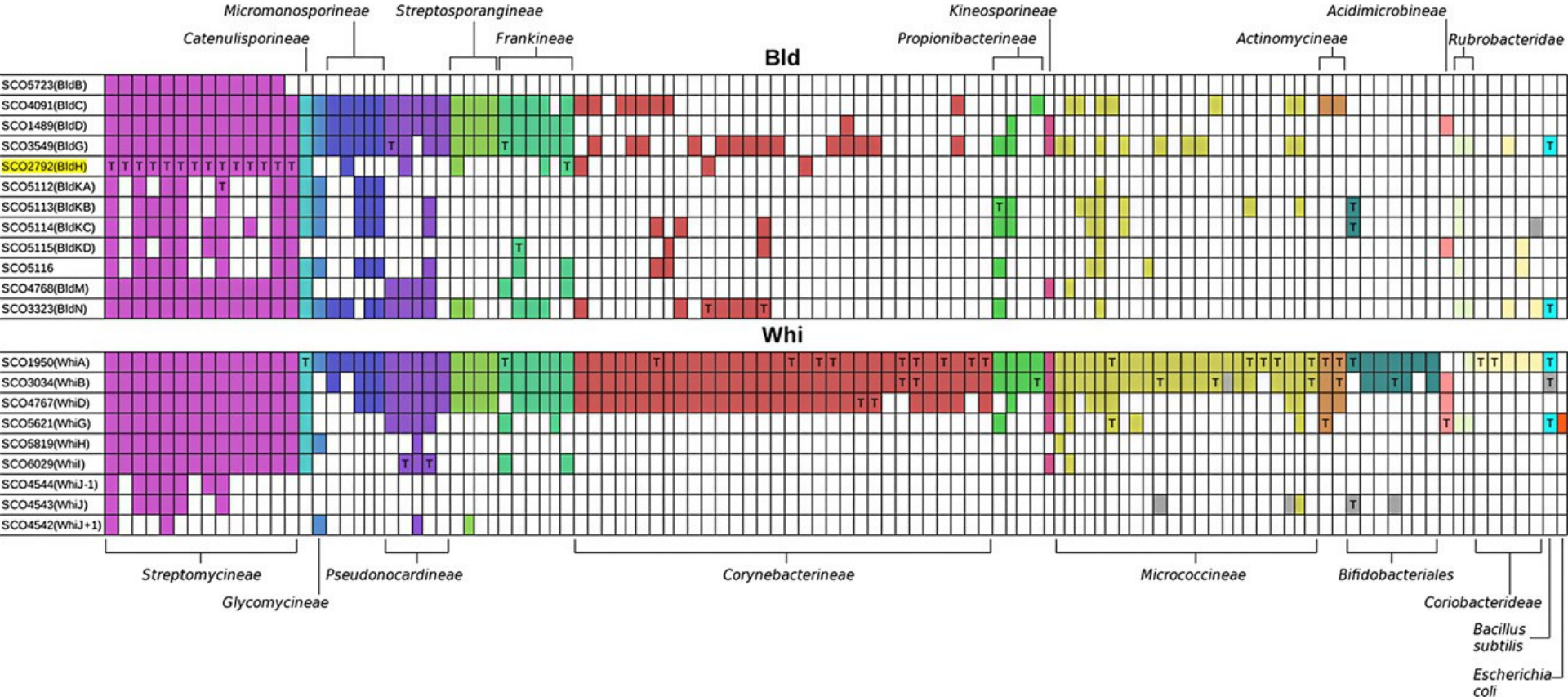
Bld and Whi conservation



WHAT IS NOT KNOWN?

Remaining unknowns

- Many gaps in the grand scheme
- Lack of coherence and unresolved complexity
- Signals controlling BldD? (other than c-di-GMP)
- BldD is a repressor…
  - but bldD^{-} mutant is… bald!
  - Possibly because of RbsN-BldN cascade?

References

- Taxonomy, Physiology, and Natural Products of Actinobacteria
- Synthesis of the spore envelope in the developmental life cycle of Streptomyces coelicolor
- Developmental biology of Streptomyces from the perspective of 100 actinobacterial genome sequences
- c-di-GMP signalling and the regulation of developmental transitions in streptomycetes

THANK YOU FOR YOUR ATTENTION

Additional material

bldC

- bldC encodes an apparently single-domain small protein with a helix-turn-helix of the MerR type (Hunt et al., 2005)
- implied to control the Whi-genes cascade

bldD

- highly pleiotropic transcription factor that controls hundreds of development-related genes
- bldD encodes a protein distantly related to SinR, a transition state regulator of B. subtilis (Elliot et al., 1998)
- BldD orthologues always show high conservation and local synteny
- implied to control the Whi-genes cascade

bldD

- The BldD regulon has been subjected to detailed analysis by immunoprecipitation of in vivo BldD–DNA complexes, which showed that BldD directly targets about 147 transcription units in vegetative, liquid-grown S. coelicolor (den Hengst et al., 2010).
- These include 42 regulatory genes, several of which are developmental (bldA, bldC, bldD, bldH, bldM, bldN, whiB, whiG). These are all repressed by BldD.

bldD

- Despite the extensive characterisation of BldD and its regulon, it is not understood why, if BldD represses developmental functions, bldD mutants are bald rather than hypersporulating (but see the slides on BldN below)
- there is no information about any signals that BldD might respond to (an initial search for possible proteins interacting with BldD was reported to have had negative results: den Hengst et al., 2010)

bldD

- BldD represses the sporulation-specific promoter of ftsZ (den Hengst et al., 2010).

bldG

- A member of the highly-paralogous family of anti-anti-sigma factors.
- Indeed, BldG influences the activity of the stress-responsive sigma factor SigH in S. Coelicolor (Sevcikova et al., 2010; Takano et al., 2011), and the anti-anti-sigma/antisigma/sigma interactions of this general type have considerable potential for promiscuity in Streptomyces (Kim et al., 2008b; Sevcikova et al., 2010; Takano et al., 2011).

bldN

- RNA polymerase sigma factor
- which control early events during sporulation (although bldN is also strongly transcribed during aerial growth)
- One of about 50 S. coelicolor ECF sigma factors (Bibb et al., 2000; den Hengst et al., 2010).
- A direct target of BldD.
- At least in S. venezuelae, BldN is a direct activator of the genes for chaplins (and their associated rodlins)
- This emergence into the air has been suggested as a trigger for the sporulation pathway controlled by the whi genes (Claessen et al., 2006).

rsbN

- in S. venezuelae, an anti-sigma factor controlling BldN is encoded by the adjacent gene, termed rsbN (= SCO3324 in S.coelicolor; Bibb et al., 2012).
- In BLASTP analysis, a reciprocal best hit to rsbN is found next to nearly all bldN orthologues in actinomycete genomes;
- but, strikingly, the RsbN-like proteins are much more divergent than their BldN target or most other families of orthologous proteins of actinobacteria

rsbN

- The rsbN gene of S. venezuelae has its own promoter, which is BldN-dependent, and is also a BldD target (den Hengst et al., 2010; Bibb et al., 2012). As a bldD mutant might therefore be expected to overexpress rsbN, the resulting increase in anti-BldN activity might interfere with the expression of BldN-dependent genes and contribute significantly to the bald phenotype of bldD mutants.

bldM

- Encodes an orphan response regulator (Molle & Buttner, 2000)
- Target of BldN.
- The distribution of convincing reciprocal hits to bldM is closely similar to that of bldN hits, suggesting that the BldN to BldM regulatory step was established very early in the evolution of actinomycete complexity.

whiG

- RNA polymerase sigma factor, which controls early events during sporulation
- an orthologue of an ancient sigma factor, regulates more recently acquired regulatory genes specific to aerial sporulation
- none is more widespread across the bacterial kingdom than whiG
- WhiG protein is a sigma factor critically involved in the decision of aerial hyphae to sporulate, and in its absence, colonies develop long, thin aerial hyphae and entirely fail to sporulate

whiG targets

- RNA polymerase containing WhiG sigma directly activates two regulatory genes involved in slightly later stages in sporulation (whiH, Ryding et al., 1998; whiI, Ainsa et al., 1999).
- The other WhiG target regulatory gene, whiH, encodes an autoregulating GntR-like protein (Ryding et al., 1998; Persson et al., 2013) confined to streptomycetes and their closest relatives (Catenulispora and Kitasatospora).

whiA

- WhiA orthologues are not confined to actinobacteria: one is present in most Gram-positive bacteria, including all actinobacteria except Acidimicrobium ferrooxidans.
- WhiA showed in vitro DNA binding to its own promoter and to a sporulation-activated promoter of the parAB operon (Kaiser & Stoddard, 2011), both of which are also WhiA-dependent in vivo (Jakimowicz et al., 2006).

whiA

- The whiA sporulation-specific promoter could be transcribed in vitro by WhiG-containing RNA polymerase (Kaiser & Stoddard, 2011), in contradiction of an earlier result (Ainsa et al., 2000).
- WhiA exerted a modest inhibitory effect on this transcription and showed some evidence of direct interaction with WhiG in a pull-down experiment involving the two purified proteins (Kaiser & Stoddard, 2011). Although not conclusive, this is the first suggestion of direct interplay between the WhiG- and WhiA-dependent parts of the sporulation regulatory cascade, previously thought to be separate (Chater, 1998; Fl€ ardh et al., 1999).

whiA

- whiA and the upstream three genes form a cluster that is highly conserved in actinobacteria and even in B. subtilis. This putative operon is probably responsible for a low level of whiA (SCO1950) expression during growth (Ainsa et al., 2000). The three upstream genes encode apparently unrelated deduced functions

whiB

- A phenotype identical to that of whiA mutants results from mutations in whiB (SCO3034)
- Mutation of the whiB orthologue (whmD) of Mycobacterium smegmatis indicated a likely role in cell division that could represent its core activity (Gomez & Bishai, 2000).
- There are strong two-way transcriptional influences (not necessarily direct) between whiA and whiB (Jakimowicz et al., 2006), but little is known about other possible WhiB targets.

whiH

- which controls the onset of sporulation-specific cell division

wblX (whiB-like)

- Orthologues of four other Wbl proteins (WblA, WblC, WhiD and WblE) occur in most actinomycetes (Figs 3 and 4), even though WblA and WhiD have developmental roles in S. coelicolor: WblA plays a key part in the transition of aerial hyphal initial branches to a sporulation-directed fate (wblA mutants have thin aerial hyphae often embedded in an extracellular matrix, with only occasional spore chains: Fowler-Goldsworthy et al., 2011); and mutants lacking WhiD have defects at a later stage, having thin-walled spores and uncontrolled sporulation septation (McVittie, 1974; Molle et al., 2000).

whiJ

- SCO4543
- a deduced DNA-binding protein (Gehring et al., 2000; Ainsa et al., 2010).
- whiJ-like genes are widely present in complex actinobacteria, but they are absent from morphologically simple ones (corynebacteria, mycobacteria, rhodococci, propionibacteria and micrococci except Beutenbergia and Intrasporangium) and from nonactinobacterial bacteria.

whiJ-associated

- Two kinds of immediately neighbouring genes:
- one kind encoding very small DNA-binding proteins (i.e. like SCO4542), and the other
- encoding proteins with features like antisigma factors (e.g. SCO4544) (Gehring et al.,2000; Ainsa et al., 2010).

whiJ

- Certain mutations in whiJ gave rise to a white-colony appearance caused by a deficiency in sporulation, although the complete deletion of whiJ had no obvious phenotypic consequences (Ainsa et al., 2010).
- WhiJ acts mainly to repress reproductive development until a suitable signal has been perceived via the SCO4542 DNA-binding protein, which would then directly interact with WhiJ to relieve repression (Ainsa et al., 2010).

bldB

- encodes a diverged member of the SCO4542 family, but is an ‘orphan’ lacking neighbouring whiJ- or SCO4544-like genes.
- bldB is the only classical bld gene to be confined to, yet universal among, streptomycetes (Fig. 3).
- We speculate that the bald phenotype of bldB mutants could imply a promiscuous interaction of BldB with WhiJ-like proteins encoded elsewhere in the genome and that this may be connected with the large numbers of such proteins found in streptomycetes.

bldH

- A major target of bldA-mediated translational control is bldH (adpA), which encodes an important global regulator of development and antibiotic production (212–215). Transcription of adpA is activated in response to the gamma-butyrolactone A-factor in S. griseus and to the related molecule SCB1 in S. Coelicolor (216–220).
- An interesting feedback loop exists whereby the translation of the adpA mRNA depends on BldA (221, 222), while AdpA in turn controls bldA transcription (223).

bldH

- Target of BldD (den Hengst et al., 2010).
- In S. griseus it is the agent of the effects of the hormone-like A-factor (Horinouchi, 2002).
- It comprises a structurally characterised C-terminal AraC/XylS-like DNA-binding domain (Yao et al., 2012) and an N-terminal domain that may sense adenine nucleotides (Wolanski et al., 2012; Liu et al., 2013a).

bldH

- The regulation of adpA in streptomycetes is remarkably complex (reviewed in detail in Liu et al., 2013a). It involves at least three levels of control:
- transcriptional [autorepression (Kato et al., 2005), repression by BldD (den Hengst et al., 2010), repression by gamma-butyrolactone-binding proteins (Horinouchi, 2007; Xu et al., 2009)];
- mRNA processing by RNaseE (Xu et al., 2010); and
- mRNA translation (Nguyen et al., 2003; Takano et al., 2003).

TTA bldH

- The TTA codon in adpA was shown by mutagenesis to be the main (but not entire) cause of the Bld phenotype of bldA mutants of S. coelicolor (Nguyen et al., 2003; Takano et al., 2003).
- A study of S. griseus and S. coelicolor has shown that the abundance of bldA tRNA is important in determining whether AdpA reaches levels sufficient to activate development and, remarkably, that there is a mutual feedforward mechanism in which AdpA activates bldA transcription (Higo et al., 2011).

TTA

- The broader developmental significance of bldA may not extend beyond Streptomycineae, as in non-Streptomycineae genomes TTA codons do not show the positional bias towards the start of genes that is observed in streptomycetes, and sometimes occur in conserved growth-associated genes (Chater & Chandra, 2008).
- Interestingly, there is a strong target for BldD binding within bldA (den Hengst et al., 2010).

TTA

- As 27/29 of the TTA-containing genes/clusters were found only in Streptomycineae, we infer that these genes and their TTA codons have adaptive value to streptomycetes and not to other actinobacteria.

ftsZ

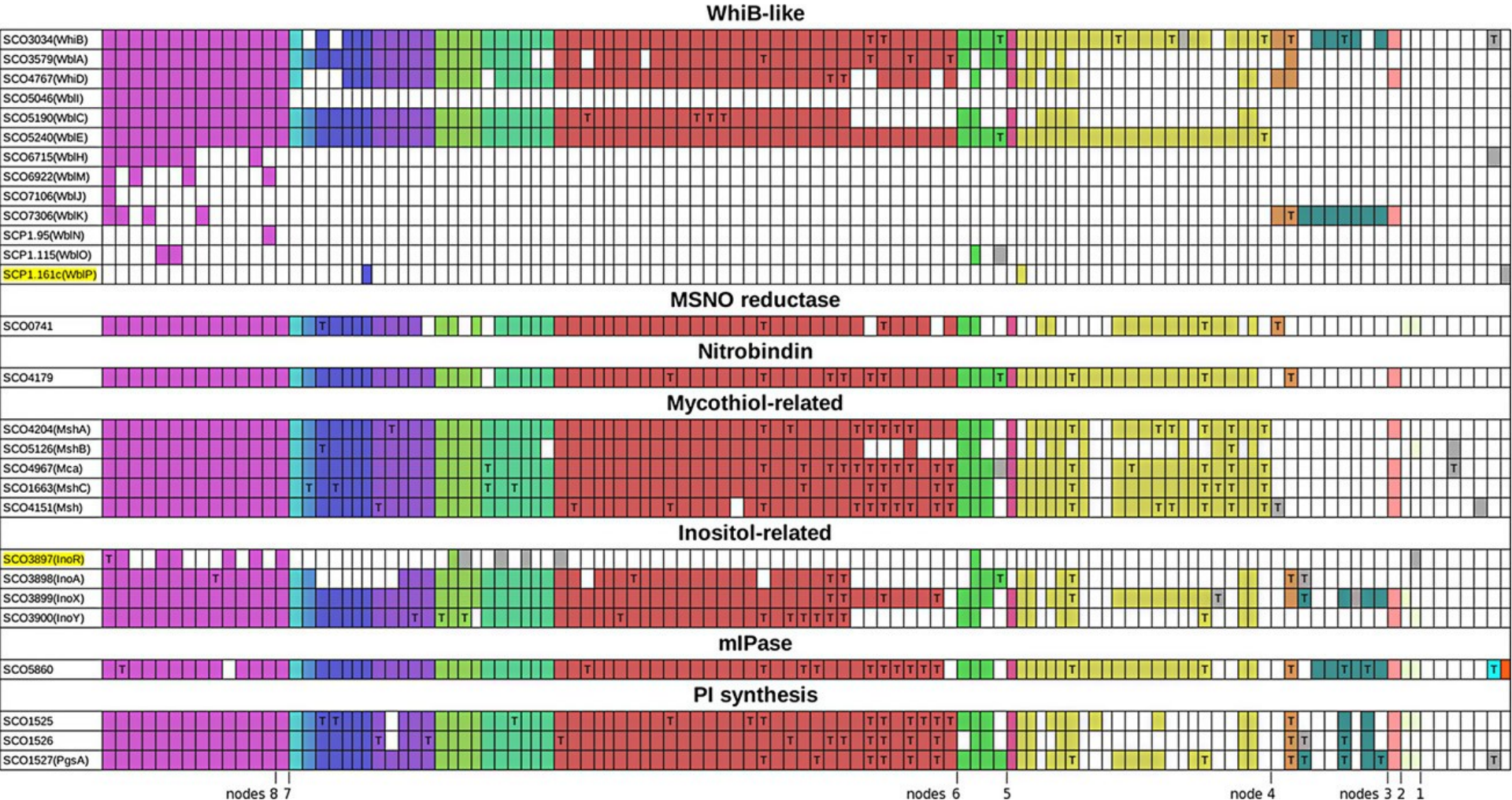
- Sufficient accumulation of FtsZ is required to support sporulation, and developmental ftsZ transcription is largely dependent on the “early” whi regulatory genes whiA, whiB, whiG, whiH, whiI, whiJ. Consistent with the notion that the control of ftsZ transcription may be a key event, at least in S. coelicolor, the non-sporulating phenotype of many of these early whi mutants could be overruled by constitutive expression of ftsZ during development. This also suggests that no other genes that are required for sporulation completely depend on these whi genes, at least not when FtsZ is overexpressed.

ssgA

- In terms of septum site localization, a key role is played by the SsgA-like proteins (SALPs), which only occur in sporulating actinobacteria (310, 311). SsgA activates sporulation-specific cell division (312, 313), and both ssgA and ssgB are required for sporulation (275, 314, 315).

ssgB

- The membrane associated SALP, SsgB, plays a prominent role in recruiting the tubulin homolog FtsZ to the division planes (Willemse et al., 2011). Formation of unigenomic spores from multi-genomic aerial hyphae requires the accurate and synchronized segregation of tens of chromosomes into pre-spore compartments. This process involves the partitioning system ParAB, the segregation protein FtsK and its homologue SffA together with the small SmeA protein which localizes SffA to the sporulation septa.


WhiB-like
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SCO3579(WblA)
SCO4767(WhiD)
SCO5046(WblI)
SCO5190(WblC)
SCO5240(WblE)
SCO6715(WblH)
SCO6922(WblM)
SCO7106(WblJ)
SCO7306(WblK)
SCP1.95(WblN)
SCP1.115(WblO)
SCP1.161c(WblP)
MSNO reductase
SCO0741
Nitrobindin
SCO4179
Mycothiol-related
SCO4204(MshA)
SCO5126(MshB)
SCO4967(Mca)
SCO1663(MshC)
SCO4151(Msh)
Inositol-related
SCO3897(InoR)
SCO3898(InoA)
SCO3899(InoX)
SCO3900(InoY)
mIPase
SCO5860
PI synthesis
SCO1525
SCO1526
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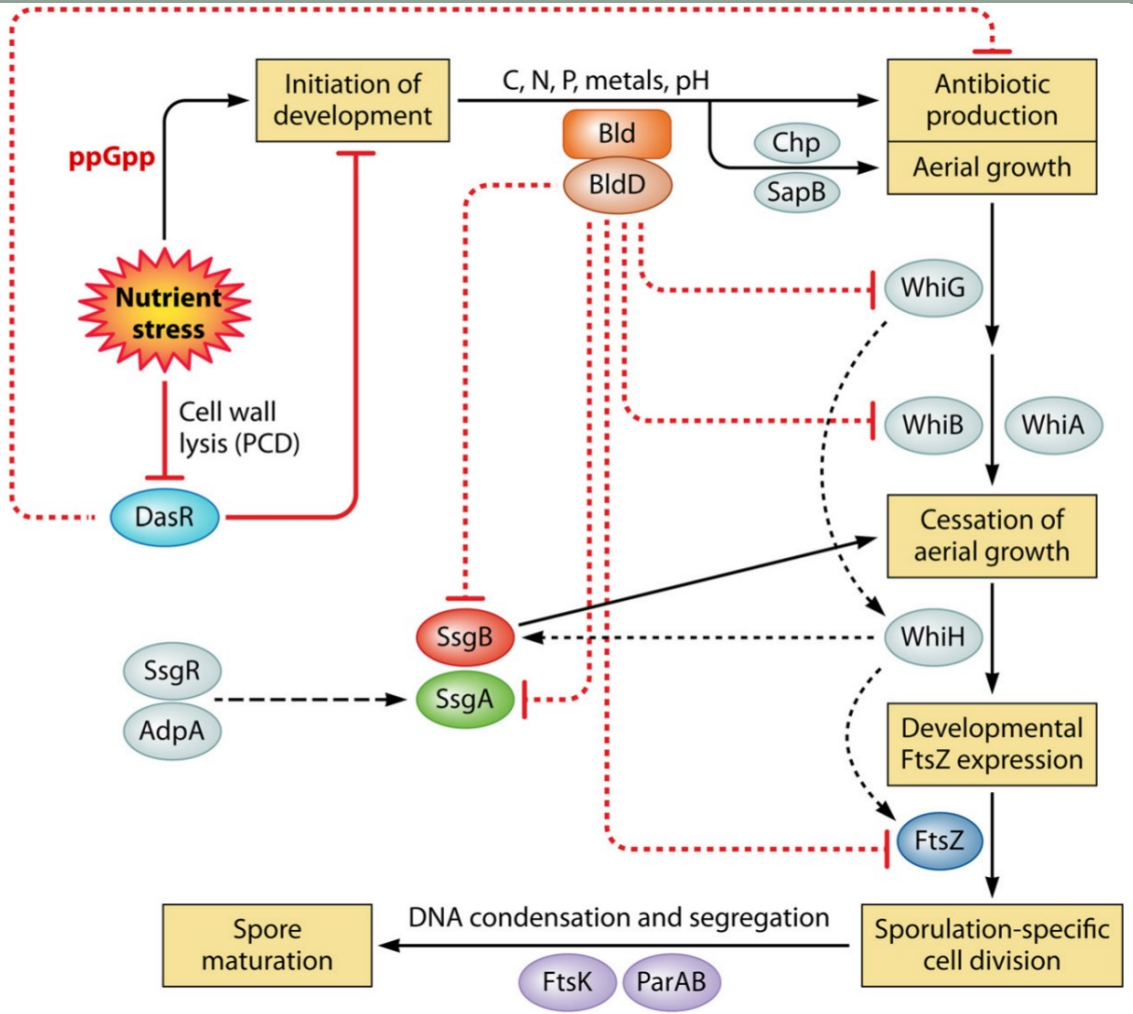
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FtsK
ParAB
Sporulation-specific cell division