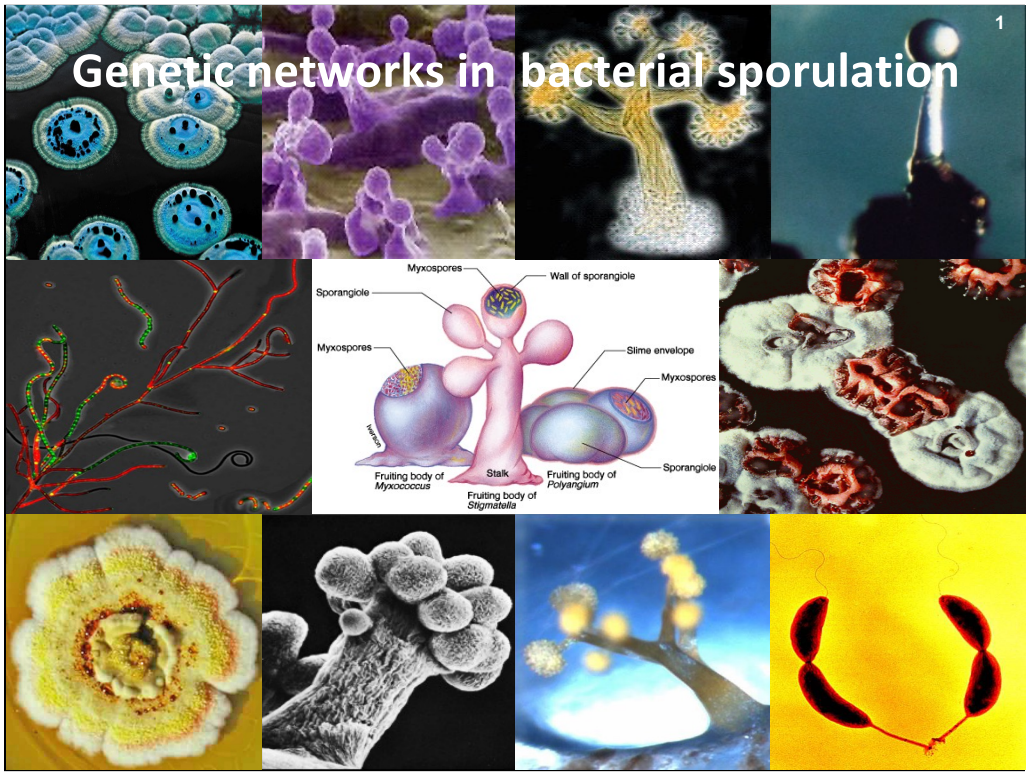
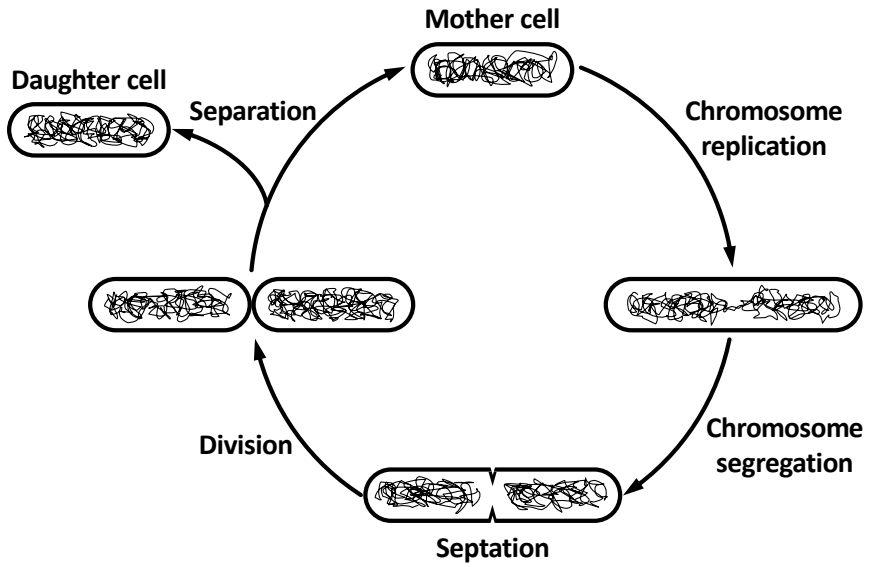
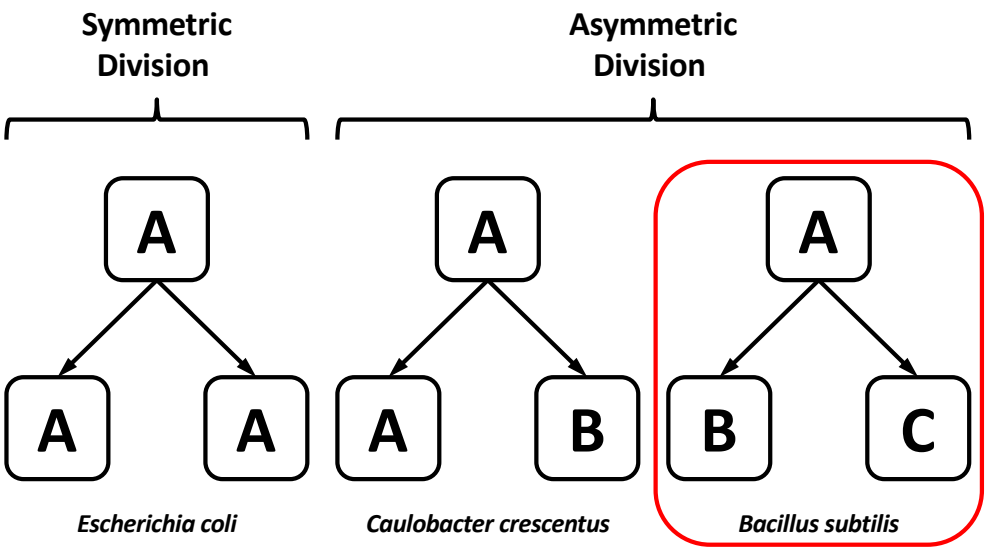

Genetic networks in bacterial sporulation
1
Myxospores
Wall of sporangiole
Sporangiole
Myxospores
Slime envelope
Myxospores
Sporangiole
Fruiting body of Myxococcus
Stalk
Fruiting body of Polyangium
Fruiting body of Stigmatella

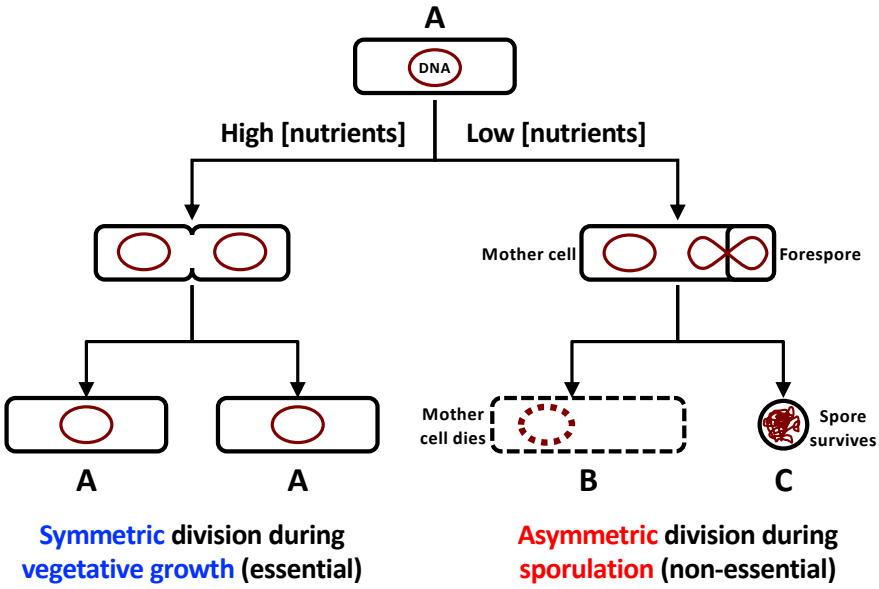
Bacteria divide *symmetrically* during vegetative growth



Bacteria divide *asymmetrically* during differentiation

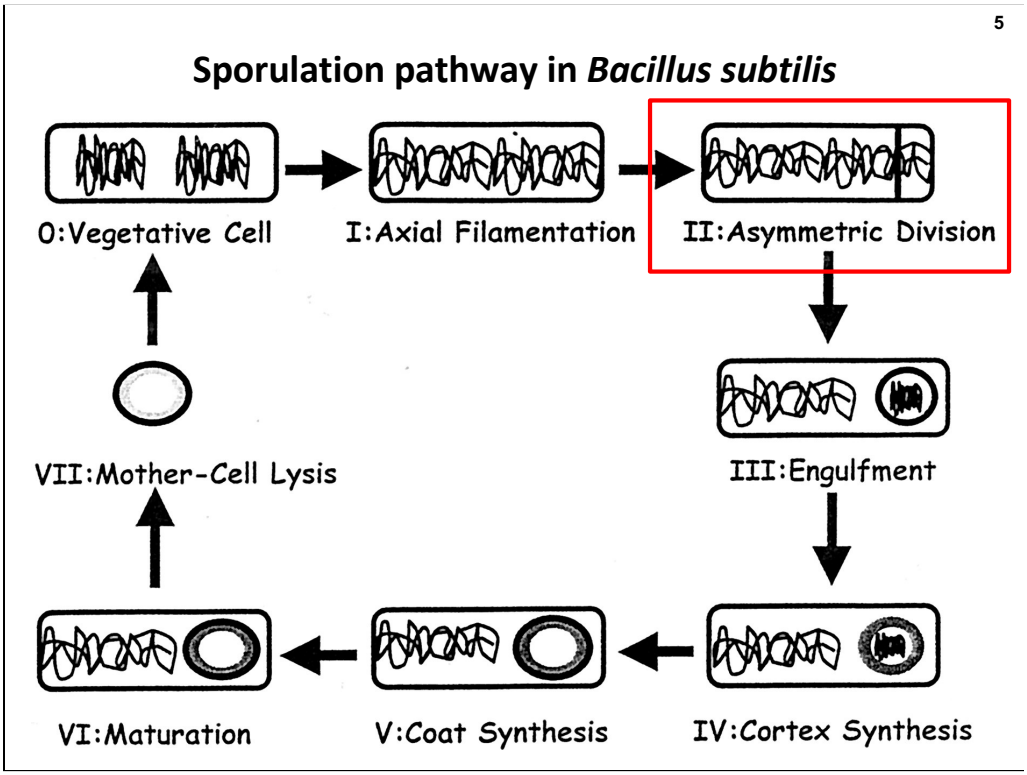


Two different cell division modes in *Bacillus subtilis*



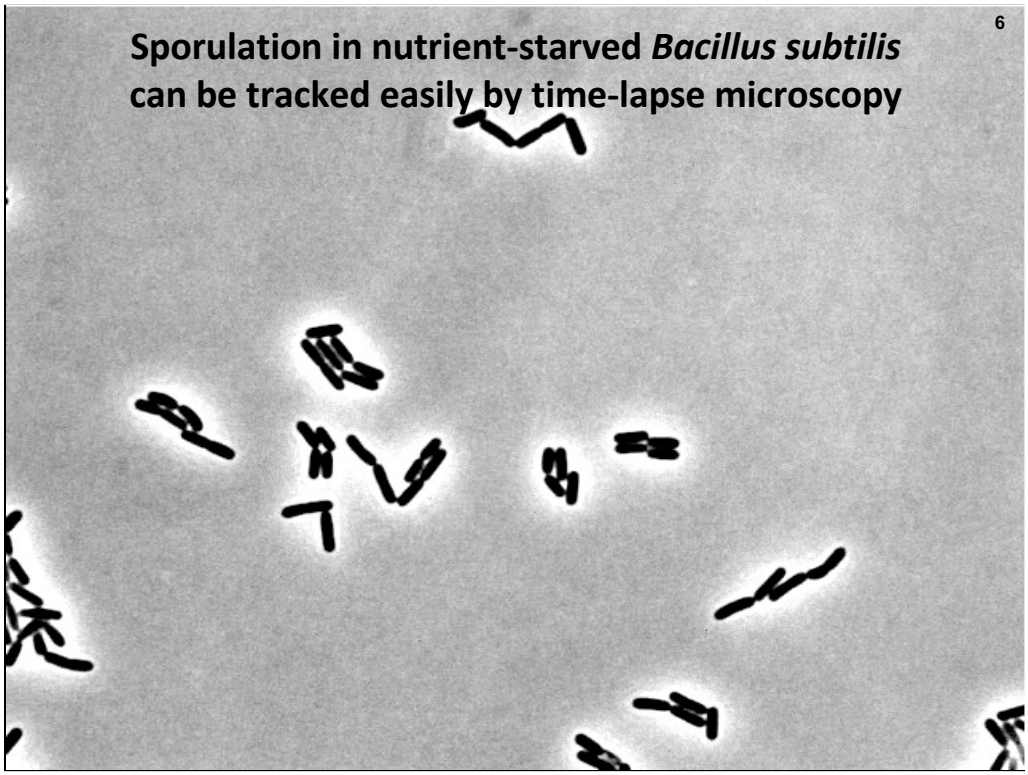
SOURCE: Errington J (2003) Regulation of endospore formation in *Bacillus subtilis*. *Nat Rev Microbiol* 1(2): 117-126 PMID:15035041.

SOURCE: Higgins D, Dworkin J (2012) Recent progress in *Bacillus subtilis* sporulation. *FEMS Microbiol Rev* 36(1): 131-148 PMID: 22091839.



SOURCE: Hilbert DW, Piggot PJ (2004) Compartmentalization of gene expression during *Bacillus subtilis* sporulation. *Microbiol Mol Biol Rev* 68(2): 234-262 PMID: 15187183. Figure 1.

Schematic representation of the stages of spore formation. A vegetatively growing cell is defined as **Stage 0**. It is shown as having completed DNA replication and containing two complete chromosomes (represented as disordered lines within the cells), although replication is not completed at the start of spore formation. Formation of an axial filament of chromatin, where both chromosomes (or a partially replicated chromosome) form a continuous structure that stretches across the long axis of the cell, is defined as **Stage I**. Asymmetric division occurs at **Stage II**, dividing the cell into the larger mother cell and smaller forespore (a.k.a."prespore" or "endospore"). At the time of division, only about 30% of a chromosome is trapped in the forespore, but over time the DNA translocase SpoIIIE will pump the remaining 70% into the forespore. **Stage III** is defined as completion of engulfment, and the forespore now exists as a free-floating protoplast within the mother cell enveloped by two membranes, represented by a single ellipse. Synthesis of the primordial germ cell wall and cortex (a distinctive form of peptidoglycan) between the membranes surrounding the forepore is defined as **Stage IV** and is represented as thickening and graying of the ellipse. Deposition of the spore coat, protective layers of proteins around the forespore, is defined as **Stage V**. The coat is represented as the black layer surrounding the engulfed forespore. Coincident with coat and cortex formation, the engulfed forespore is dehydrated, giving it a phase-bright appearance, represented here as a light grey shading. **Stage VI** is maturation, when the spore acquires its full stress-resistance properties, although no obvious morphological changes occur. **Stage VII** represents lysis of the mother cell, which releases the mature spore into the environment.



SOURCE: Movie provided by Prof. Jonathan Dworkin, Columbia University, New York, NY USA.

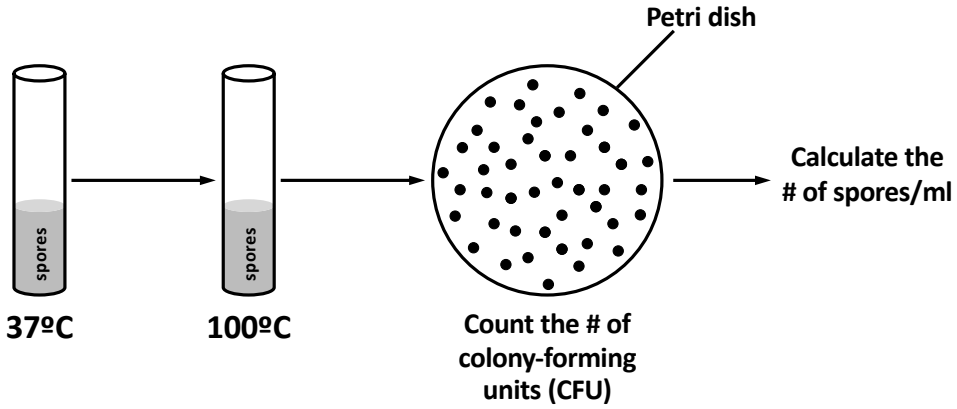
Mutants defective in sporulation (*spo* mutants) can be identified easily using indicator medium

Wild-type (*spo*$^+$)

Mutant (*spo*$^-$)

SOURCE: Photograph provided by Prof. Jonathan Dworkin, Columbia University, New York, NY USA.

Spore formation can be quantified easily using a "heat kill" assay and the "colony count" method

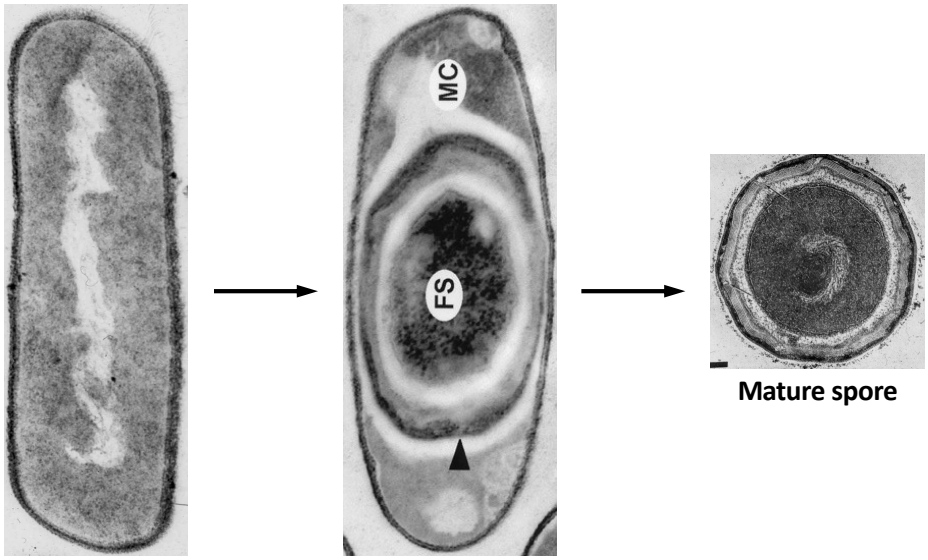


Spores can survive exposure to temperatures up to 150°C!

“It is a profound and necessary truth that the deep things in science are not found because they are useful: they are found because it was possible to find them.”

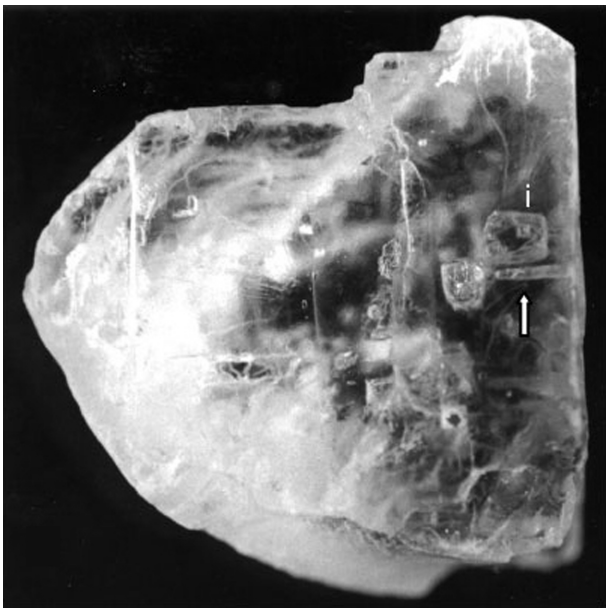
J. Robert Oppenheimer
a.k.a. “Father of the Atom Bomb”

Bacterial cell morphology changes during sporulation



SOURCE: Photographs provided by Prof. Jonathan Dworkin, Columbia University, New York, NY USA.

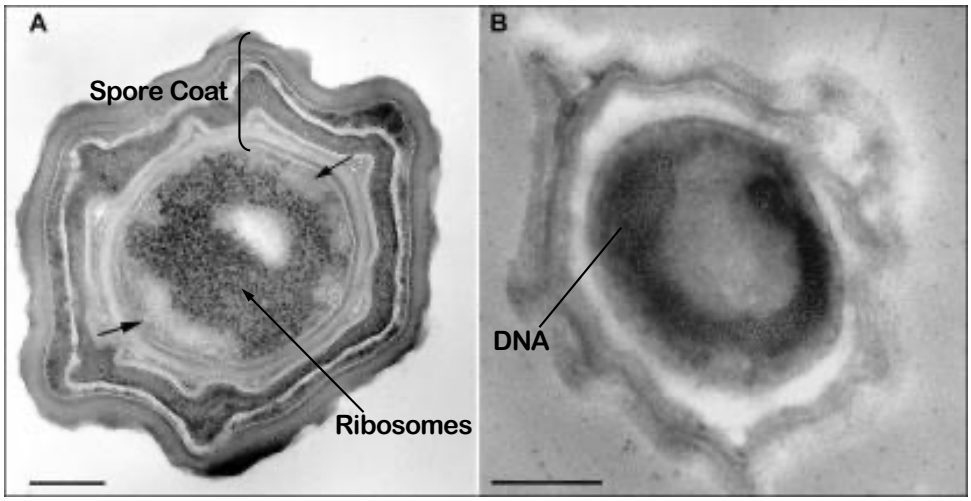
Germination of halophilic bacterial spores from an ancient (250-million-year-old) primary salt crystal!



SOURCE: Vreeland RH, Rosenzweig WD, Powers DW (2000) Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature* 407(6806): 897-900 PMID: 11057666. Figure 1.

Halite crystals taken from the dissolution pipe at the 569 m (1850 ft) level of the Salado Formation (air intake shaft18 for the Waste Isolation Pilot Plant, Carlsbad, New Mexico) in October 1998. The sample that yielded *Bacillus* strain 2-9-3 is shown. This crystal measured 3.5 x 3.5 x 2.5 cm. The inclusion that contained the bacterium (below i) measured approximately 3 x 3 x 1 mm (9 cubic mm). The drill hole made during sampling of inclusion B is visible above the arrow. The thickness of this crystal obscures some of the internal details (the drill hole for inclusion A) in the photograph.

Subcellular structure of a mature spore



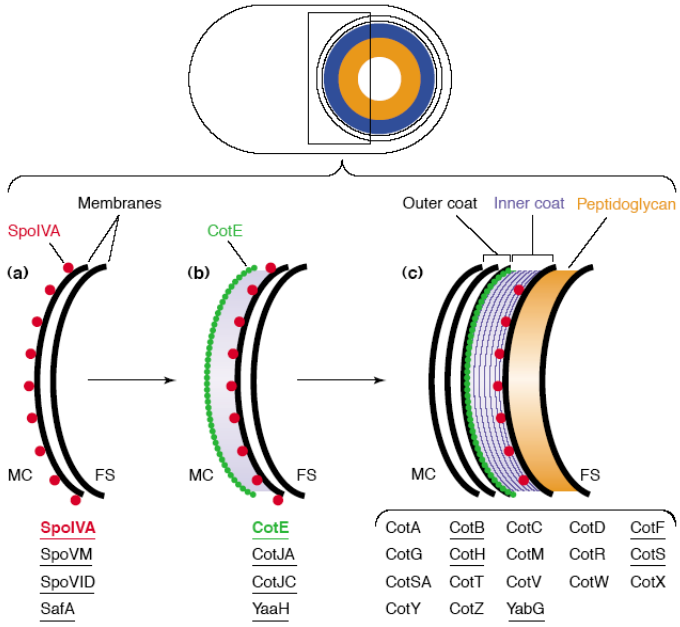
Ribosome stain **DNA stain**

SOURCE: Frenkiel-Krispin D, Sack R, Englander J, Shimoni E, Eisenstein M, Bullitt E, Horowitz-Scherer R, Hayes CS, Setlow P, Minsky A, Wolf SG (2004) Structure of the DNA-SspC Complex: Implications for DNA Packaging, Protection, and Repair in Bacterial Spores. *J Bacteriol* 186(11): 3525–3530 PMID: 15150240. Figure 4.

Electron microscopy of dormant *Bacillus subtilis* spores. **(A)** Spore stained with uranyl acetate. The densely stained particles are ribosomes. Ribosome-free spaces in the periphery of the spore core are indicated by arrows; these contain chromatin (DNA). **(B)** Specific DNA staining of a dormant spore, which results in darkly stained DNA, highlights the toroid morphology of the chromatin. Since the sections probed are ~70 nm thick, only a segment of the toroid can be detected. Scale bars, 200 nm.

Many genes are involved in assembly of the spore coat (which is synthesized by the mother cell, layer-by-layer)

13



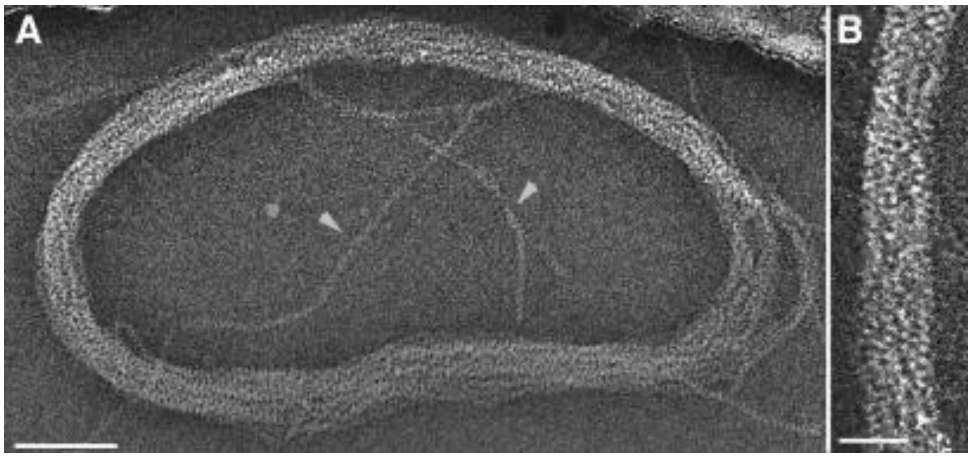
SOURCE: Driks A (2002) Maximum shields: the assembly and function of the bacterial spore coat. *Trends Microbiol* 10(6): 251-254 PMID: 12088650. Figure 2.

Model for spore coat assembly. The upper diagram depicts a cell containing a mature spore before mother cell lysis. The lower diagram shows spore coat assembly on an arc of the spore surface, boxed in the upper diagram. The mother cell (MC) and forespore (FS) sides of each arc are indicated. The blue region within the inner coat, called the matrix, connects the shells of SpoIVA and CotE. Its composition is unknown. Below the diagram are coat or coat-associated proteins in *Bacillus subtilis* that are synthesized and/or assembled at each stage of spore development. These proteins are synthesized in the mother cell compartment and "donated" to the forespore; this is an example of how biosynthetic activities that take place specifically in the mother cell (which will die during the process of sporulation) are essential for proper assembly and viability of the forespore. The underlined proteins have candidate homologs in *Bacillus anthracis* (the bacterium that causes anthrax, a deadly infectious disease).

You do not need to memorize any of the genes or gene products listed on this slide. The point is simply that construction of the spore coat is a complex process requiring a large number of genes that are essential for construction of a viable spore but non-essential for normal (vegetative) cell growth and division.

14

Spore DNA is tightly packed into filaments that are highly resistant to chemical and physical damage



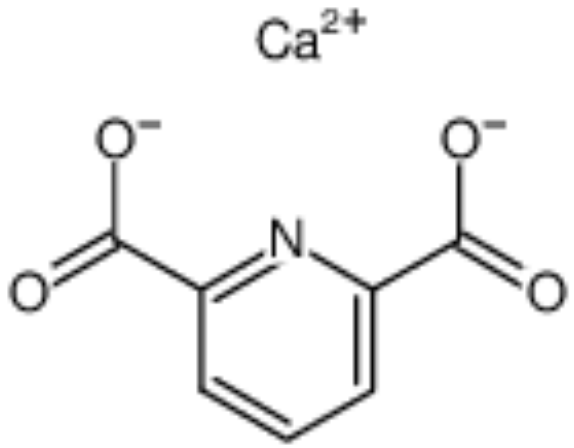
Spore DNA is packaged into rope-like filaments by "Small Acid-Soluble Proteins" (SASPs) that confer resistance to dessication, oxidation, UV irradiation, heat stress, mechanical stress…

SOURCE: Frenkiel-Krispin D, Sack R, Englander J, Shimoni E, Eisenstein M, Bullitt E, Horowitz-Scherer R, Hayes CS, Setlow P, Minsky A, Wolf SG (2004) Structure of the DNA-SspC complex: implications for DNA packaging, protection, and repair in bacterial spores. *J Bacteriol* 186(11): 3525-3530 PMID: 15150240. Figure 1.

Transmission electron microscopy of a DNA-SASP complex, where "SASP" stands for "Small Acid-Soluble Protein". **(A)** Image of a typical toroidal aggregate formed by DNA-SASP filaments. Arrowheads indicate DNA-SASP single filaments spreading out of the circle. **(B)** High magnification of a region from panel A, highlighting the tight packing of adjacent DNA-SASP filaments. Scale bars, 50 nm (A) and 10 nm (B).

15

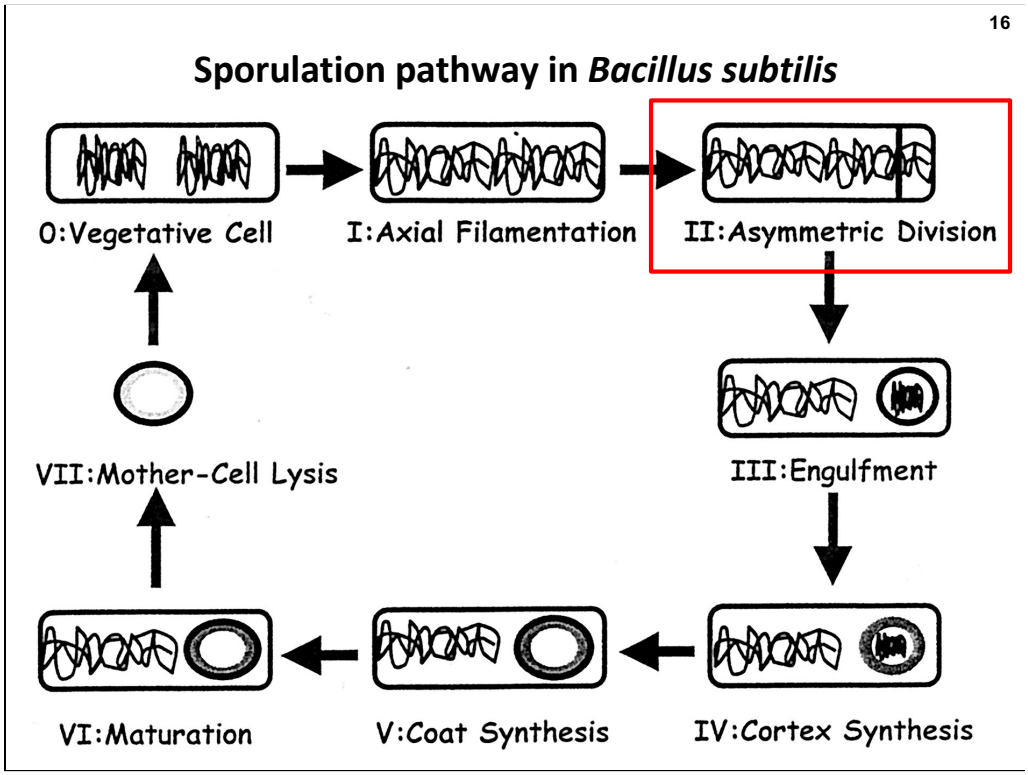
Dipicolinate-calcium crystals “dehydrate” the spore cytoplasm and protect DNA from heat denaturation



This compound accounts for about 20% of a spore’s dry weight!

Dipicolinate (negatively charged) forms a complex with calcium ions (positively charged) within the endospore core. The dipicolinate-calcium complex binds free water molecules, causing “dehydration” of the spore. As a result, the heat and other stress resistance of macromolecules (including DNA) within the spore increases.

This compound is highly specific for spores. To the best of my knowledge, it is not found anywhere else in nature.



SOURCE: Hilbert DW, Piggot PJ (2004) Compartmentalization of gene expression during *Bacillus subtilis* sporulation. *Microbiol Mol Biol Rev* 68(2): 234-262 PMID: 15187183. Figure 1.

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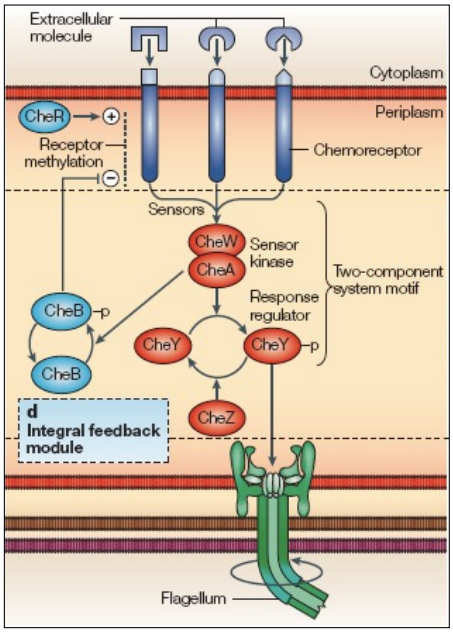
Refresher: bacterial regulatory circuits are modular



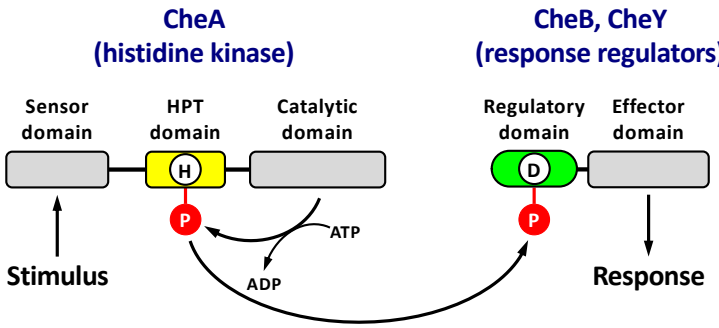
SOURCE: McAdams HH, Srinivasan B, Arkin AP (2004) The evolution of genetic regulatory systems in bacteria. *Nat Rev Genet* 5(3): 169-178 PMID: 14970819.

Figure 1. Example of a bacterial regulatory circuit. The figure illustrates the modular nature of the *Escherichia coli* chemotaxis network. **(A)** The **sensor module** includes several different chemoreceptors that are sensitive to different extracellular molecules. Diverse collections of hundreds or thousands of these chemoreceptors are assembled into large membrane-associated arrays that are localized predominantly at the cell poles (Ref. 62). External molecules bind to receptors on the cell surface and activate the CheW/CheA sensor kinase. **(B)** The **transduction module** comprises biochemical reactions between different chemotaxis molecules that create a pathway that communicates a signal to the distant flagella. This signal changes the frequency of reversal of the flagella motor, the **actuator module (C)** in a manner that causes the bacteria to swim generally towards attractive chemical sources and away from hazardous sources. **(D)** The **feedback loop** within the sensor module, which involves methylation of the receptor, allows the network to operate over wide concentration ranges of the external molecule that is being sensed. The histidine sensor kinase-response regulator reaction, labeled as a **two-component system** motif, is part of the communication link that signals the status of the chemoreceptors to the motor. Because of its strong **modular organization**, the chemotaxis system is evolutionarily flexible, as shown by the diversity of chemical signals and response regulator functions that are found among motile bacteria.

Earlier in this class (week 7), we studied the bacterial chemotaxis network as an example of a system comprising four functional modules designed to sense and respond to time-dependent changes in the concentration of specific chemicals in the extracellular milieu. This general modular design (sensor, signal transduction, actuator, feedback) is typical of many bacterial networks performing a variety of functions.

18

Chemotaxis two-component phosphorelay system

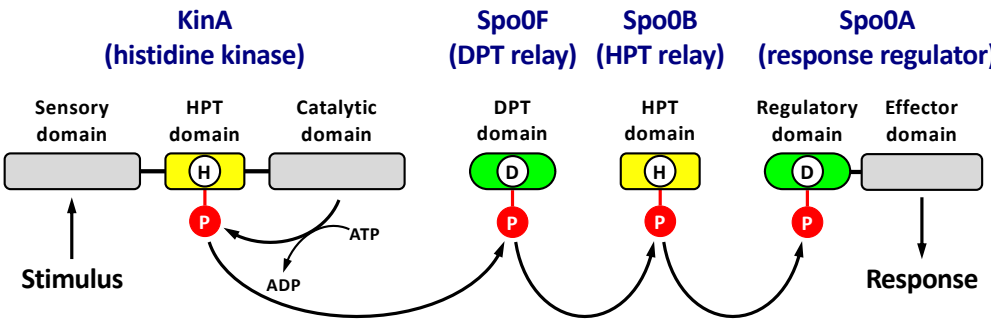


SOURCE: West AH, Stock AM (2001) Histidine kinases and response regulator proteins in two-component signaling systems. *Trends Biochem Sci* 26(6) 369-376 PMID: 11406410. Figure 1.

The schematic shows a typical two-component phospho-relay system; in the example shown, the system controls chemotaxis in *Escherichia coli*. Two-component systems typically consist of a dimeric transmembrane sensor **histidine kinase** (**HK**) and a cytoplasmic **response regulator** (**RR**), although there are many various on this theme (e.g., some histidine kinases are cytoplasmic proteins and some response regulators are transmembrane proteins). Histidine kinases typically comprise three domains: an N-terminal **sensor domain** that senses the cognate environmental stimulus; a central **HPT domain** that mediates histidine phospho-transfer; a C-terminal **catalytic domain** that mediates ATP hydrolysis and concomitant phosphorylation of the **regulatory domain** on the response regulator protein.

Histidine kinases catalyze ATP-dependent autophosphorylation of a specific conserved histidine residue (H) in their HPT domain. The activities of histidine kinases are modulated by environmental signals/stimuli. The phosphoryl group (P) is then transferred to a specific aspartate residue (D) located within the conserved **regulatory domain** of a linked response regulator protein. Phosphorylation of the response regulator typically activates an associated (or downstream) **effector domain**, which ultimately elicits a specific cellular response.

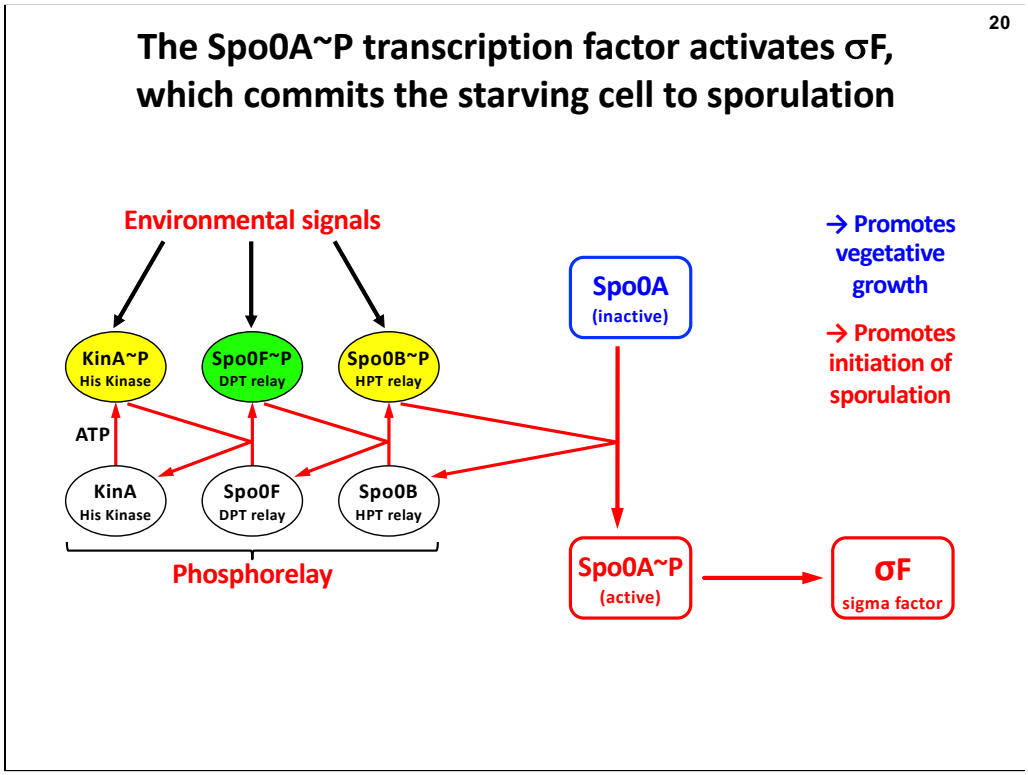
Sporulation two-component phosphorelay system



SOURCE: West AH, Stock AM (2001) Histidine kinases and response regulator proteins in two-component signaling systems. *Trends Biochem Sci* 26(6) 369-376 PMID: 11406410. Figure 1.

The schematic shows a typical multi-component phospho-relay system; in the example shown, the system controls sporulation in *Bacillus subtilis*. Two-component systems typically consist of a dimeric transmembrane sensor histidine kinase (HK), a cytoplasmic response regulator (RR), and two or more intermediate phospho-transfer relay proteins. More than one histidine (H) to aspartate (D) phosphoryl transfer reaction takes place, and the scheme usually involves an aspartate phospho-transfer (DPT) protein as well as a histidine phospho-transfer (HPT) protein that serve as aspartate-phosphorylated or histidine-phosphorylated intermediates in the phospho-relay.

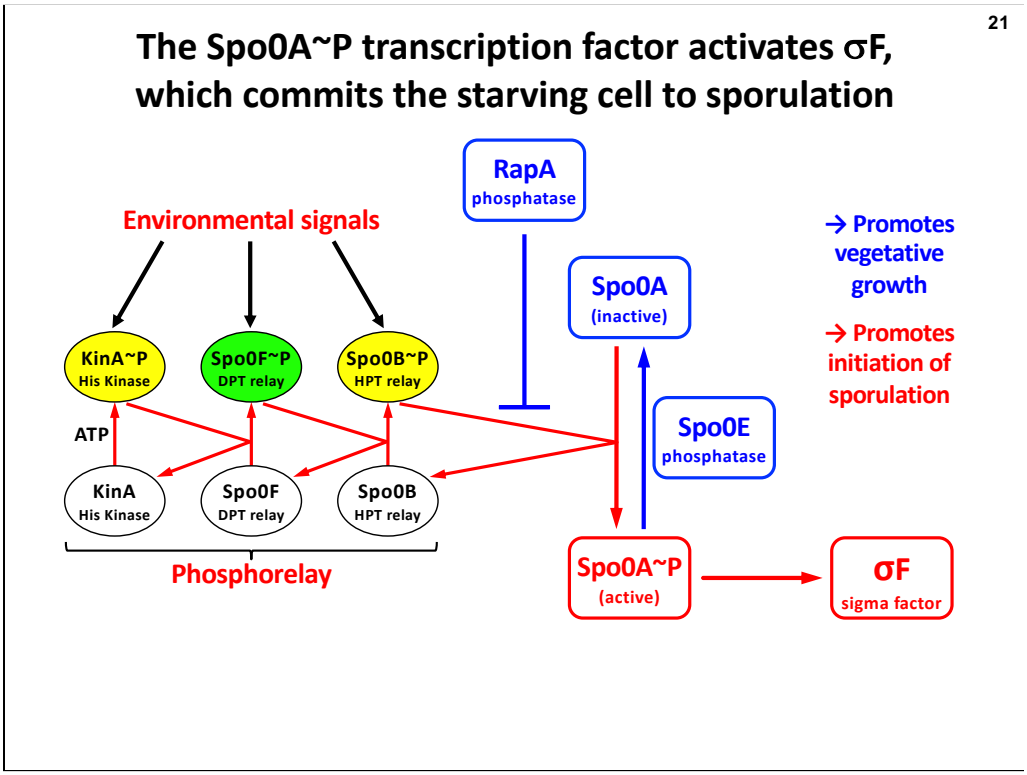
Abbreviations: HK, histidine kinase; RR, response regulator; HPT, histidine phospho-transfer; DPT, aspartate phospho-transfer.



SOURCE: Veening JW, Hamoen LW, Kuipers OP (2005) Phosphatases modulate the bistable sporulation gene expression pattern in *Bacillus subtilis*. *Mol Microbiol* 56(6): 1481-1494 PMID: 15916600.

Figure 1. Simplified schematic representation of the regulatory network that governs initiation of sporulation. Perpendiculars and arrows represent the negative and positive actions respectively.

Spore formation in *Bacillus subtilis* is a complex adaptive response to starvation. The process of sporulation is governed by a **multicomponent phosphorelay** which consists of five **histidine kinases** (KinA, KinB, KinC, KinD, and KinE) and two phosphorelay proteins (Spo0F and Spo0B). Multiple environmental and physiological signals are fed into this system, and under appropriate conditions this leads to **phosphorylation** of **Spo0A**, the key sporulation transcription factor. Within an isogenic culture of sporulating *B. subtilis*, some cells initiate the developmental program of sporulation, whereas others do not. Therefore, initiation of sporulation is a regulatory process with a **bistable** outcome. Phenotypic variation could benefit the fitness of the species, because the heterogeneous population is able to quickly react to changing environments. As sporulation is an energy-intensive process, and irreversible after its earliest stage, cells that are delayed to commit to sporulate could have an advantage over sporulating cells if food resources were to become plentiful again. Theoretical modelling and experiments in both prokaryotic and eukaryotic model systems have demonstrated that **positive feedback** of a transcriptional regulator, together with a **non-linear response** to an activator, can lead to a **bimodal probability distribution** in expression. It is believed that stochastic fluctuations causes some cells to reach the threshold level to activate the **feed-forward loop** and these cells will end up in the 'high expressing' population, whereas others do not reach this threshold and remain in the 'low expressing' state. In the study cited above, the authors examined whether the positive feedback architecture of spo0A regulation is responsible for bistability in sporulation gene expression and studied the influence of the RapA and Spo0E phosphatases on the maintenance of bistability.



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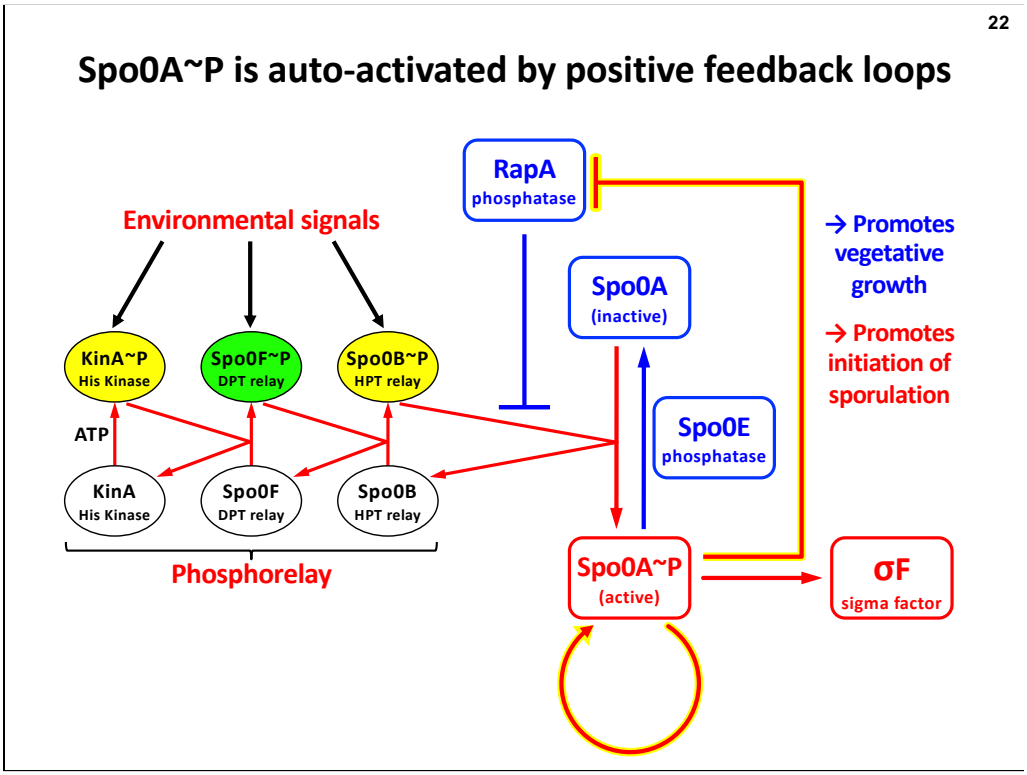

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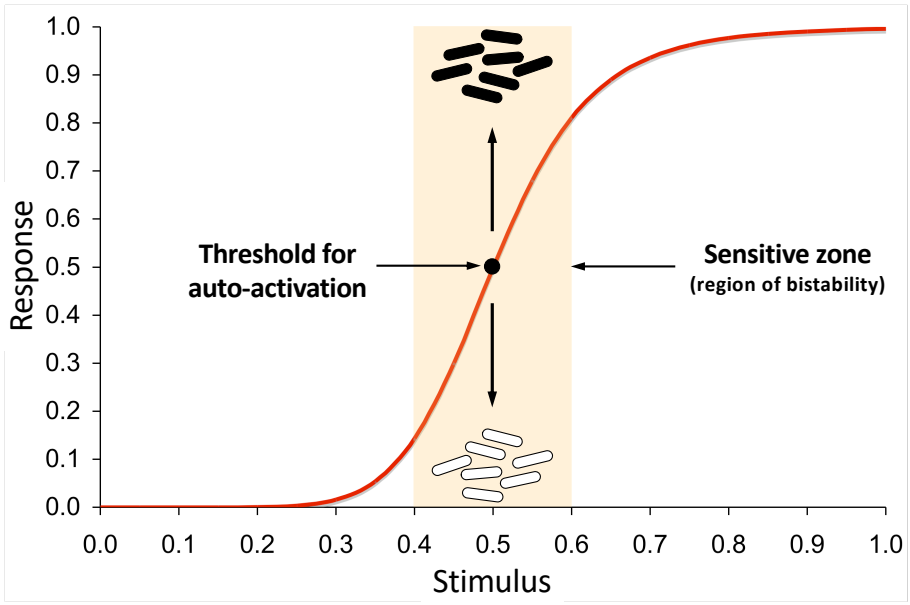
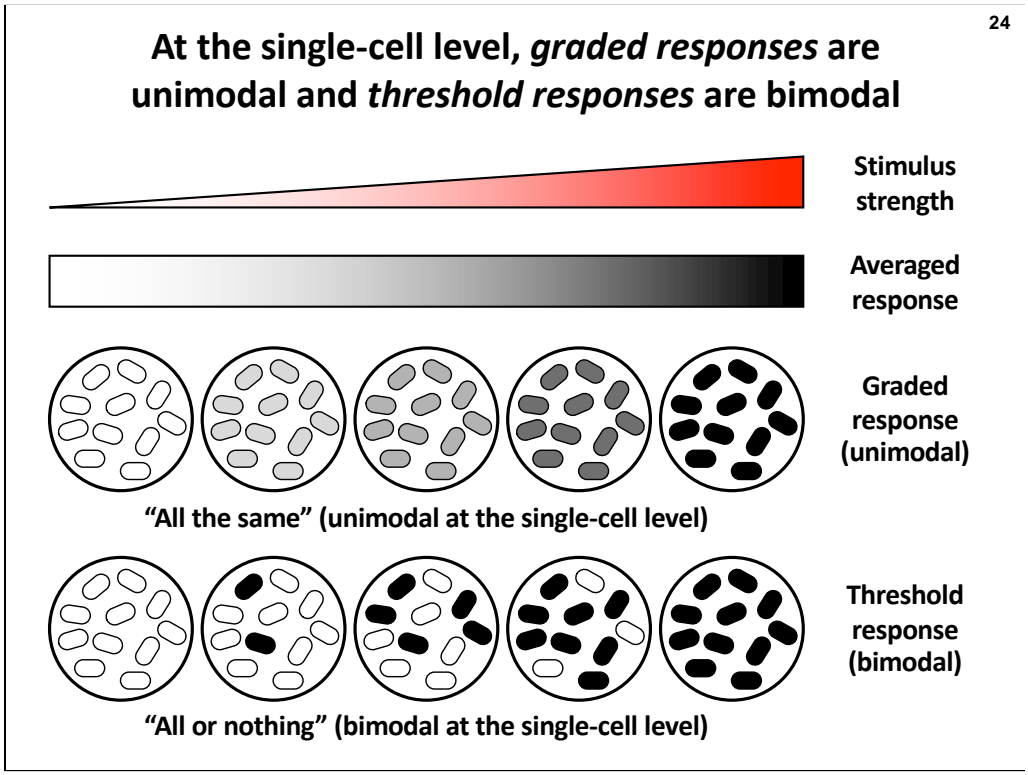
Positive feedback loops generate sigmoidal stimulus-response curves that are bimodal



SOURCE: Ozbudak EM, Thattai M, Lim HN, Shraiman BI, van Oudenaarden A (2004) Multistability in the lactose utilization network of *Escherichia coli*. *Nature* 427(6976): 737-740 PMID: 14973486. Figure 4.



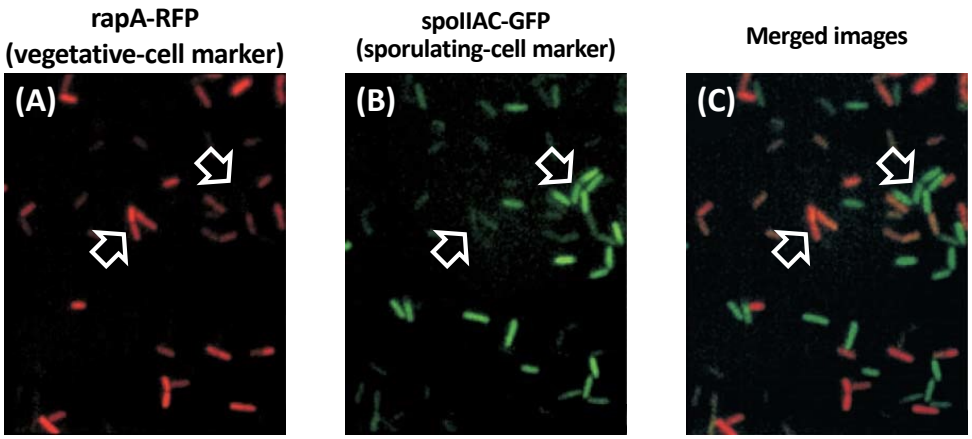
SOURCE: Kringstein AM, Rossi FM, Hofmann A, Blau HM (1998) Graded transcriptional responses to different concentrations of a single transactivator. *Proc Natl Acad Sci USA* 95(23): 13670-13675 PMID: 9811858.

SOURCE: Becskei A, Séraphin B, Serrano L (2001) Positive feedback in eukaryotic gene networks: cell differentiation by graded to binary response conversion. *EMBO J.* 20(10): 2528-2535 PMID: 11350942.

SOURCE: Biggar SR, Crabtree GR (2001) Cell signaling can direct either binary or graded transcriptional responses. *EMBO J* 20(12): 3167-3176 PMID: 11406593.

SOURCE: Kurakin A (2005) Self-organization vs. watchmaker: stochastic gene expression and cell differentiation. *Dev Genes Evol* 215(1): 46-52 PMID: 15645318.

Bimodal and mutually exclusive expression of rapA and spoIIAC in sporulating bacteria



SOURCE: Veening JW, Hamoen LW, Kuipers OP (2005) Phosphatases modulate the bistable sporulation gene expression pattern in *Bacillus subtilis*. *Mol Microbiol* 56(6): 1481-1494 PMID: 15916600. Figure 6.

Reciprocal and mutually exclusive expression of **rapA** (which is highly expressed during **vegetative growth**) and **spoIIAC** (which is highly expressed during **sporulation**). A dual reporter strain of *Bacillus subtilis* expressing a red fluorescent marker of vegetative growth (**rapA-RFP**) and a green fluorescent marker of sporulation (**spoIIAC-GFP**) was grown in sporulation medium. Cells were collected before and after the transition point between the exponential and stationary growth phases for analysis by fluorescence microscopy and flow cytometry. **(A)** Red cells: the *rapA* promoter driving expression of red fluorescent protein (**rapA-RFP**). **(B)** Green cells: the σF-dependent *spoIIA* promoter driving expression of green fluorescent protein (**spoIIA-GFP**). **(C)** Merged images from panels (A) and (B). Note that individual cells are red or green but not both (except transiently, as cells transition from vegetative growth to sporulation).

The **rapA** gene encodes a phosphatase that dephosphorylates proteins in the phosphorelay (KinA-Spo0F-Spo0B) that phosphorylates Spo0A (see slide 21). The **spoIIAC** gene encodes the sigmaF transcription factor (see slide 21).

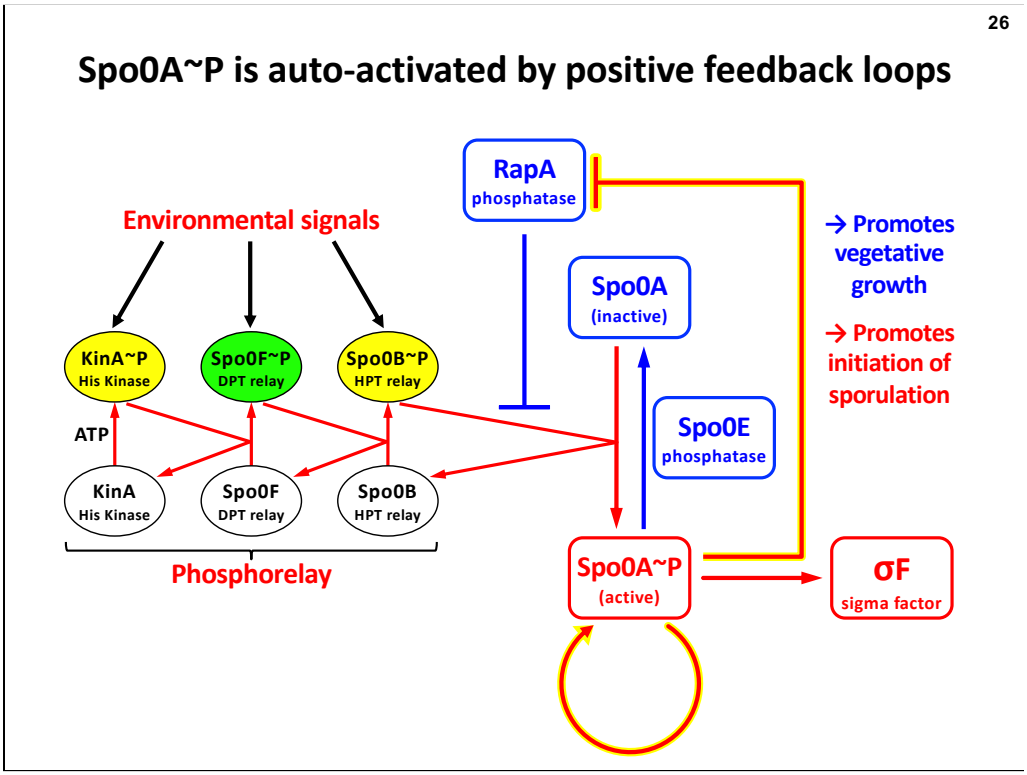


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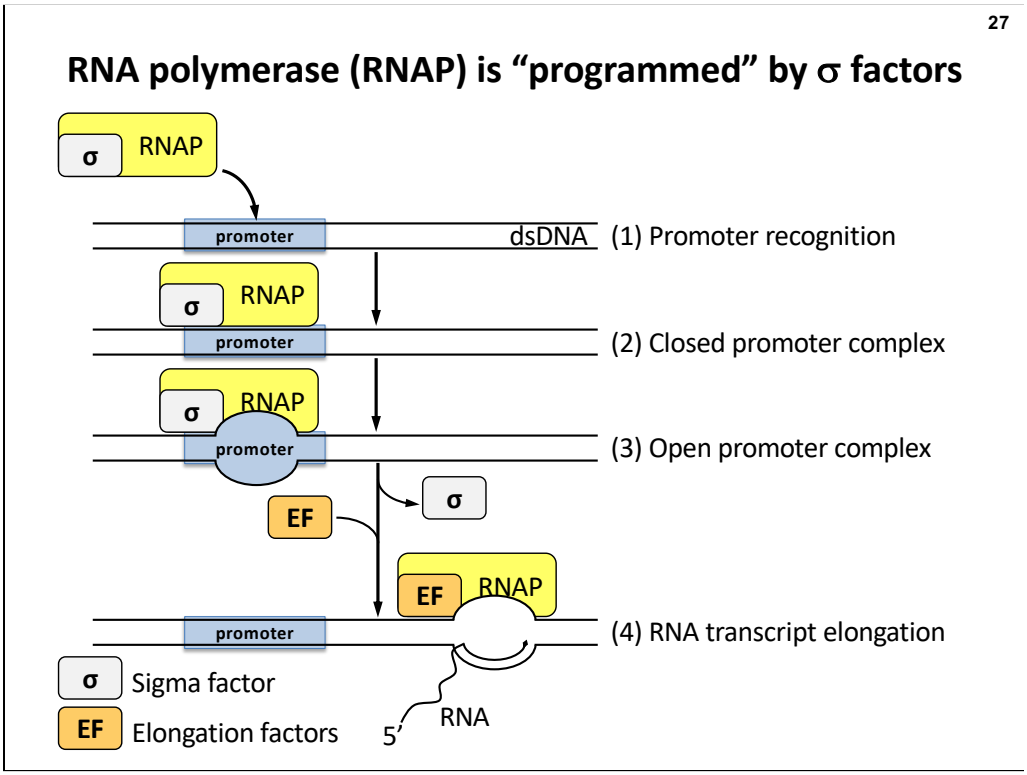
Figure 1. Simplified schematic representation of the regulatory network that governs initiation of sporulation. Perpendiculars and arrows represent the negative and positive actions respectively.

Spore formation in *Bacillus subtilis* is a complex adaptive response to starvation. The process of sporulation is governed by a **multicomponent phosphorelay** which consists of five **histidine kinases** (KinA, KinB, KinC, KinD, and KinE) and two phosphorelay proteins (Spo0F and Spo0B). Multiple environmental and physiological signals are fed into this system, and under appropriate conditions this leads to **phosphorylation** of **Spo0A**, the key sporulation transcription factor. Within an isogenic culture of sporulating *B. subtilis*, some cells initiate the developmental program of sporulation, whereas others do not. Therefore, initiation of sporulation is a regulatory process with a **bistable** outcome. Phenotypic variation could benefit the fitness of the species, because the heterogeneous population is able to quickly react to changing environments. As sporulation is an energy-intensive process, and irreversible after its earliest stage, cells that are delayed to commit to sporulate could have an advantage over sporulating cells if food resources were to become plentiful again. Theoretical modelling and experiments in both prokaryotic and eukaryotic model systems have demonstrated that **positive feedback** of a transcriptional regulator, together with a **non-linear response** to an activator, can lead to a **bimodal probability distribution** in expression. It is believed that stochastic fluctuations causes some cells to reach the threshold level to activate the **feed-forward loop** and these cells will end up in the 'high expressing' population, whereas others do not reach this threshold and remain in the 'low expressing' state. In the study cited above, the authors examined whether the positive feedback architecture of spo0A regulation is responsible for bistability in sporulation gene expression and studied the influence of the RapA and Spo0E phosphatases on the maintenance of bistability.



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 4: Molecular Information Flow and Protein Processing (pp. 138-172), published by Pearson Education Inc., San Francisco © 2019.

SOURCE: Feklístov A, Sharon BD, Darst SA, Gross CA (2014) Bacterial sigma factors: a historical, structural, and genomic perspective. *Annu Rev Microbiol* 68: 357-376 PMID: 25002089.

Figure 4.19. Transcription: steps in RNA synthesis. The transcription initiation site ("promoter") and termination site are specific nucleotide sequences on the DNA. RNA Polymerase (RNA Pol) moves along the DNA chain, temporarily opening the double helix and transcribing one of the DNA strands.

Sigma Factors Program Transcription by RNA Polymerase. On its own, the "core" RNA polymerase binds only weakly and non-specifically to DNA. Binding of a "sigma factor" to the core RNA polymerase allows the "holoenzyme" (comprising the core RNA polymerase plus a sigma factor) to recognize and bind to transcription promoter sequences. The sigma factor is required only for the early steps of transcription and it is replaced by "elongation factors" when RNA polymerase moves beyond the promoter.

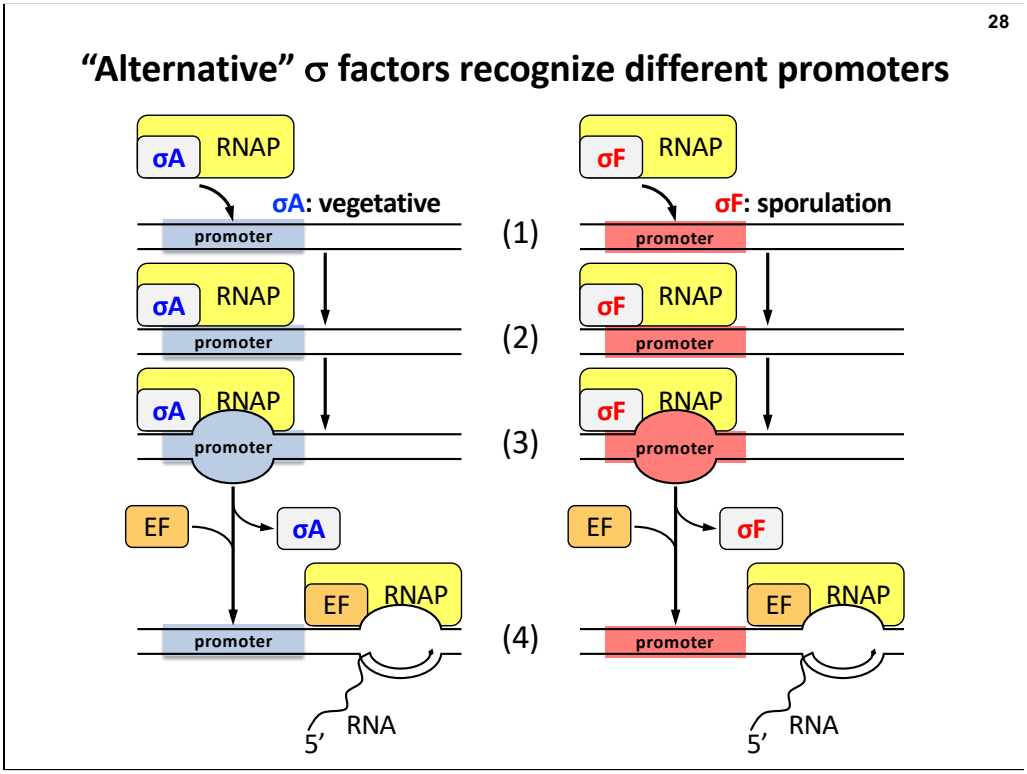
Steps in RNA synthesis:

(1) Promoter recognition by RNA polymerase. The RNA polymerase holoenzyme (core RNA polymerase plus sigma factor) recognizes a transcriptional promoter sequence on the DNA upstream of a transcribed region, which is typically an open reading frame encoding a protein or a structural RNA.

(2) Closed promoter complex. The RNA polymerase holoenzyme binds to the promoter sequence with high affinity and DNA sequence specificity.

(3) Open promoter complex. The DNA-bound RNA polymerase holoenzyme unzips a small region of the bound DNA to form a bubble-like stretch of single-stranded DNA.

(4) RNA transcript elongation. RNA polymerase transcribes the DNA into a single-stranded RNA molecule, which is synthesized from the 5' end to the 3' end. Movement of RNA polymerase beyond the promoter is accompanied by unbinding of the sigma factor and binding of elongation factors.



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Alternative Sigma Factors. Bacteria typically encode multiple sigma factors. *Bacillus subtilis* encodes 10 different sigma factors, but some bacterial species encode much larger numbers of different sigma factors (for example, *Streptomyces coelicolor* encodes 59 different sigma factors!). Each type of sigma factor programs the core RNA polymerase to recognize a different type of promoter sequence. In *Bacillus subtilis*, **sigmaA** is the "housekeeping" sigma factor that is responsible for transcription during vegetative growth, while **sigmaF** is an "alternative" sigma factor that is required for the early stages of sporulation under conditions of nutrient starvation. Promoter sequences recognized by RNA polymerase bound to sigmaA are not recognized by RNA polymerase bound to sigmaF. Conversely, promoter sequences recognized by RNA polymerase bound to sigmaF are not recognized by RNA polymerase bound to sigmaA. Thus, RNA polymerase programmed with sigmaA and RNA polymerase programmed with sigmaF transcribe completely different sets of genes: genes required for vegetative growth (sigmaA) and genes required for the early stages of sporulation (sigmaF).

Steps in RNA synthesis:

(1) Promoter recognition by RNA polymerase. The RNA polymerase holoenzyme (core RNA polymerase plus sigma factor) recognizes a transcriptional promoter sequence on the DNA upstream of a transcribed region, which is typically an open reading frame encoding a protein or a structural RNA.

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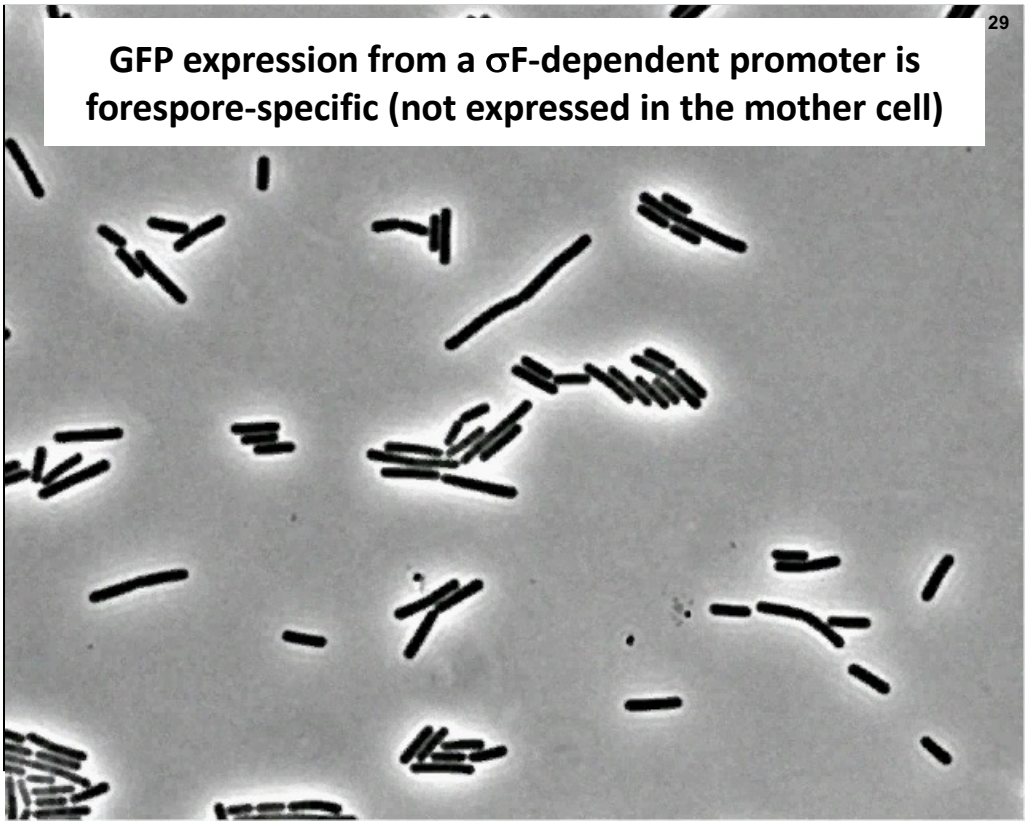
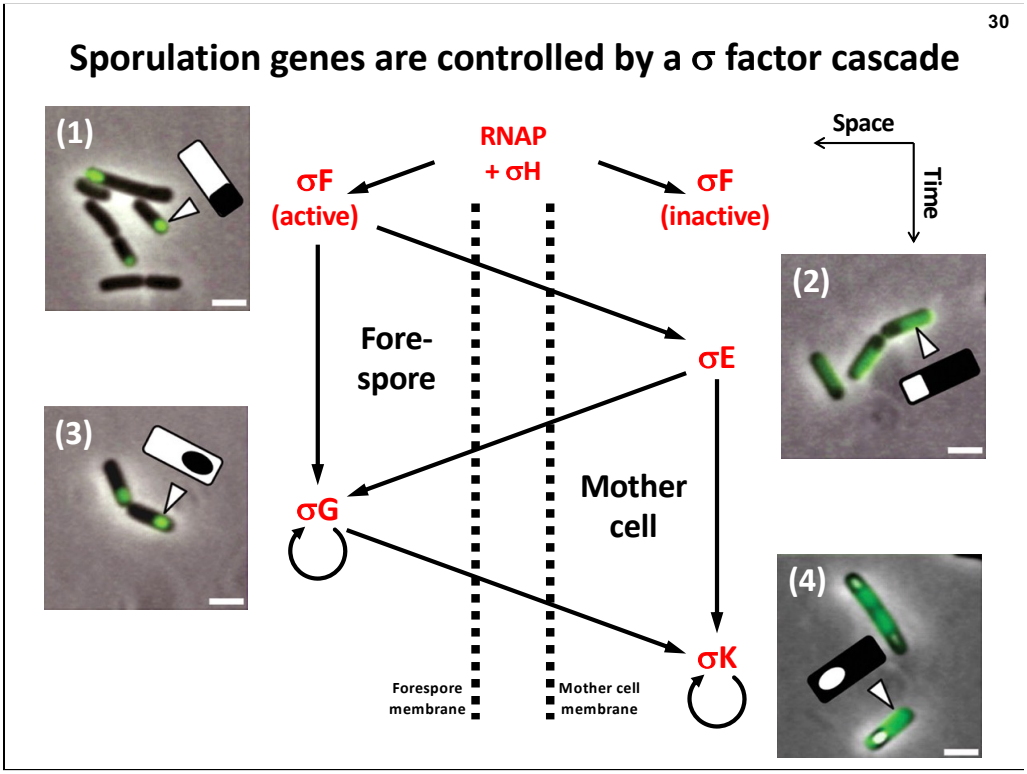


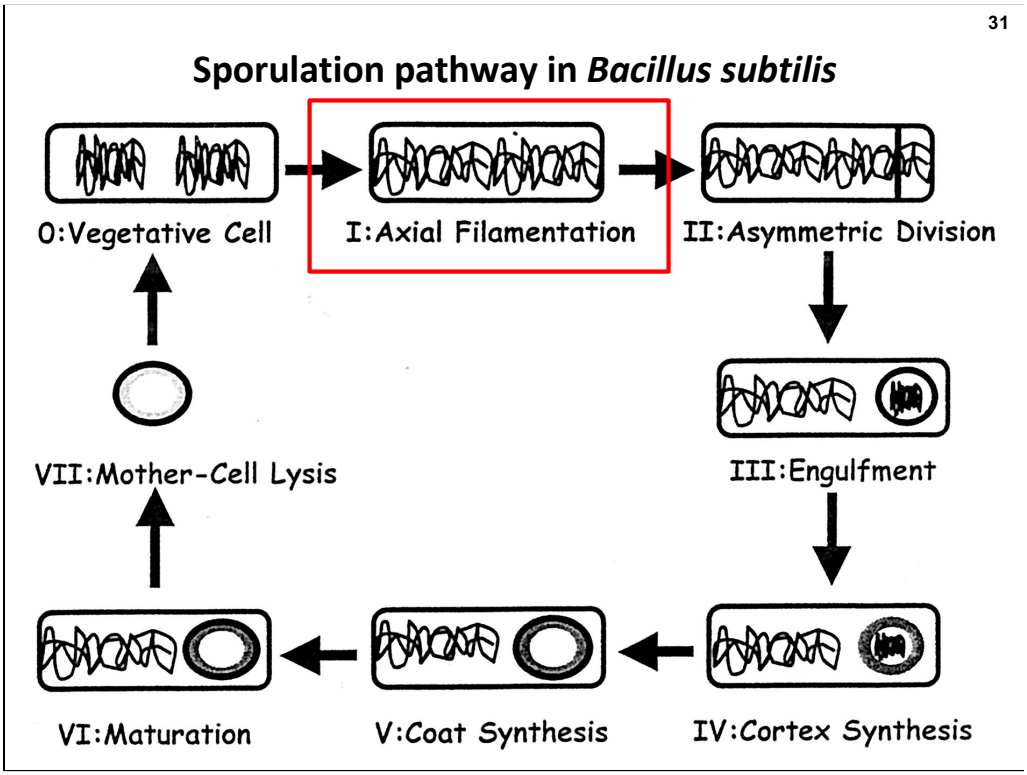
SOURCE: Movie provided by Prof. Jonathan Dworkin, Columbia University, New York, NY USA.



SOURCE: Steil L, Serrano M, Henriques AQ, Völker U (2005) Genome-wide analysis of temporally regulated and compartment-specific gene expression in sporulating cells of *Bacillus subtilis*. *Microbiology* 151(2): 399-420 PMID:15699190. Figure 5.

SOURCE: Hilbert DW, Piggot PJ (2004) Compartmentalization of gene expression during *Bacillus subtilis* spore formation. *Microbiol Mol Biol Rev* 68(2): 234-262 PMID: 15187183. Figure 2.

Compartment-specific gene expression (in the mother cell or forespore compartments) of representatives of each sporulation-specific sigma-factor regulon using appropriate green fluorescent protein (GFP) fusions. Scale bars, 2 µm.



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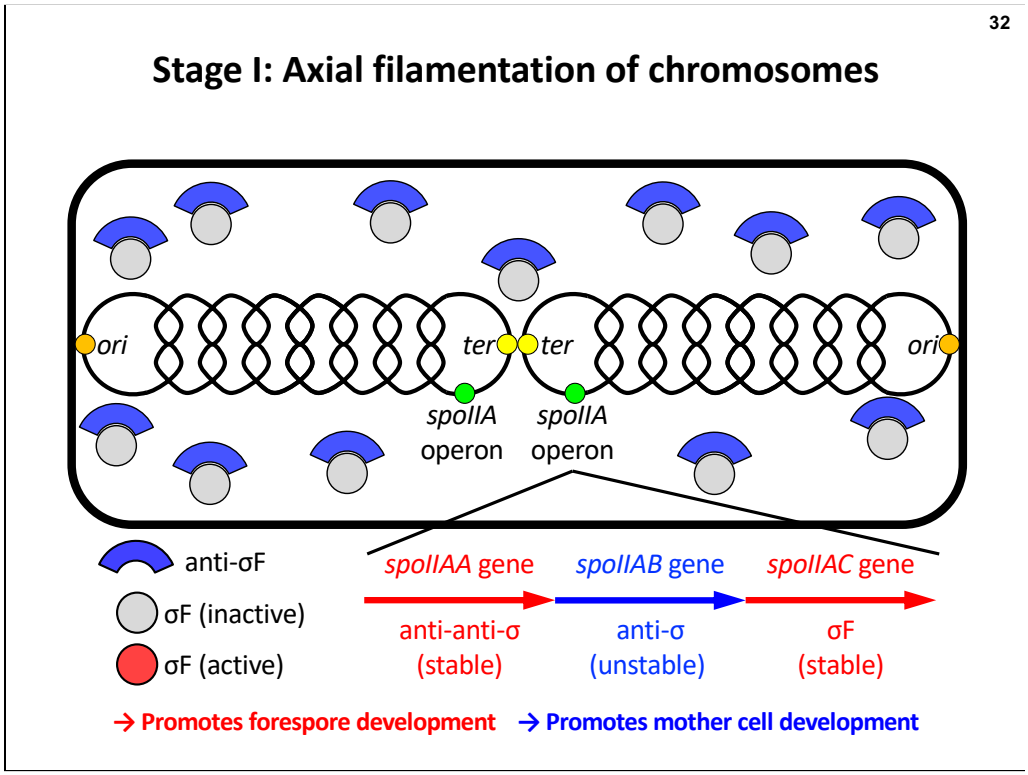
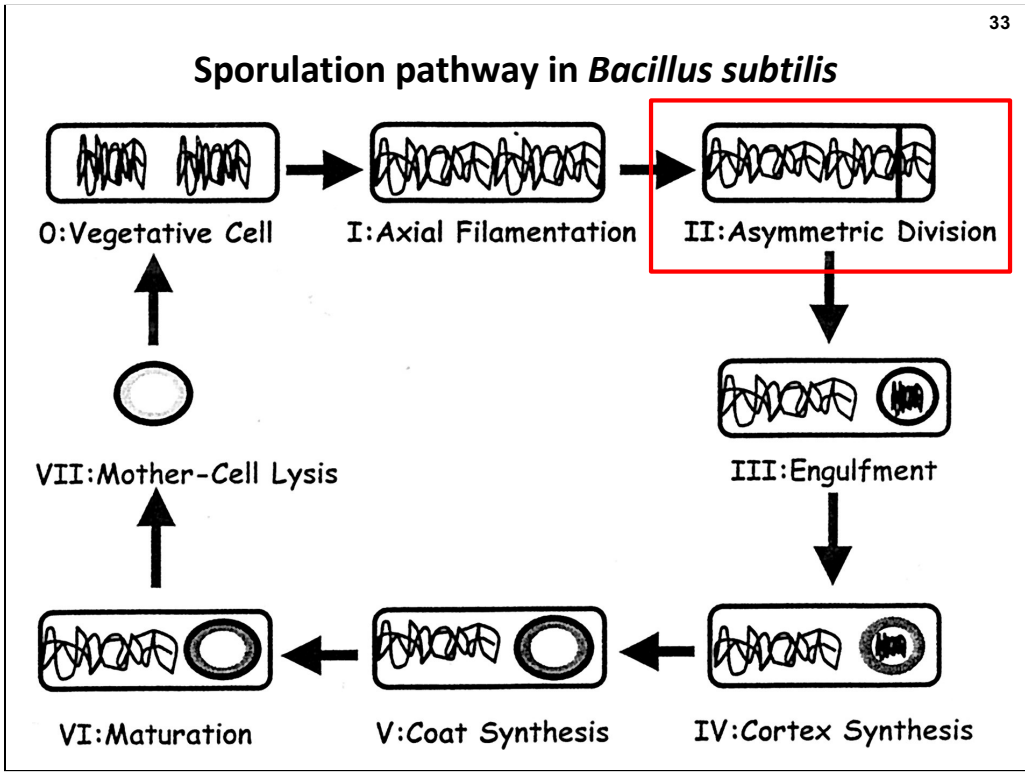
Schematic representation of the stages of spore formation. A vegetatively growing cell is defined as **Stage 0**. It is shown as having completed DNA replication and containing two complete chromosomes (represented as disordered lines within the cells), although replication is not completed at the start of spore formation. Formation of an axial filament of chromatin, where both chromosomes (or a partially replicated chromosome) form a continuous structure that stretches across the long axis of the cell, is defined as **Stage I**. Asymmetric division occurs at **Stage II**, dividing the cell into the larger mother cell and smaller forespore (a.k.a."prespore" or "endospore"). At the time of division, only about 30% of a chromosome is trapped in the forespore, but over time the DNA translocase SpoIIIE will pump the remaining 70% into the forespore. **Stage III** is defined as completion of engulfment, and the forespore now exists as a free-floating protoplast within the mother cell enveloped by two membranes, represented by a single ellipse. Synthesis of the primordial germ cell wall and cortex (a distinctive form of peptidoglycan) between the membranes surrounding the forepore is defined as **Stage IV** and is represented as thickening and graying of the ellipse. Deposition of the spore coat, protective layers of proteins around the forespore, is defined as **Stage V**. The coat is represented as the black layer surrounding the engulfed forespore. Coincident with coat and cortex formation, the engulfed forespore is dehydrated, giving it a phase-bright appearance, represented here as a light grey shading. **Stage VI** is maturation, when the spore acquires its full stress-resistance properties, although no obvious morphological changes occur. **Stage VII** represents lysis of the mother cell, which releases the mature spore into the environment.



SOURCE: Hilbert DW, Piggot PJ (2004) Compartmentalization of gene expression during *Bacillus subtilis* spore formation. *Microbiol Mol Biol Rev* 68(2): 234-262 PMID: 15187183. Figure 3.

Chromosome partitioning and transient genetic asymmetry during sporulation in *Bacillus subtilis*. Initially, when asymmetric division occurs at stage II of sporulation, the newly formed forespore compartment contains only one-third of a chromosome (always the one-third containing the origin (*ori*) of replication), while the mother cell contains the other two-thirds of the same chromosome (always the two-thirds containing the terminus (*ter*) of replication). This temporarily "spatially partitioned" chromosome will eventually become the forespore chromosome. The mother cell also contains one entire chromosome, which will eventually become the mother cell chromosome. This peculiar partitioning of chromosomes results in a transient genetic asymmetry between the prespore and mother cell compartments. Within about 20 minutes after asymmetric division, the SpoIIIE DNA pump transfers the remaining two-thirds of the forespore chromosome into the forespore compartment, thus restoring genetic symmetry between the forespore and mother cell compartments. However, this transient genetic asymmetry is sufficient to result in activation of sigmaF in the forespore compartment but not in the mother cell compartment.



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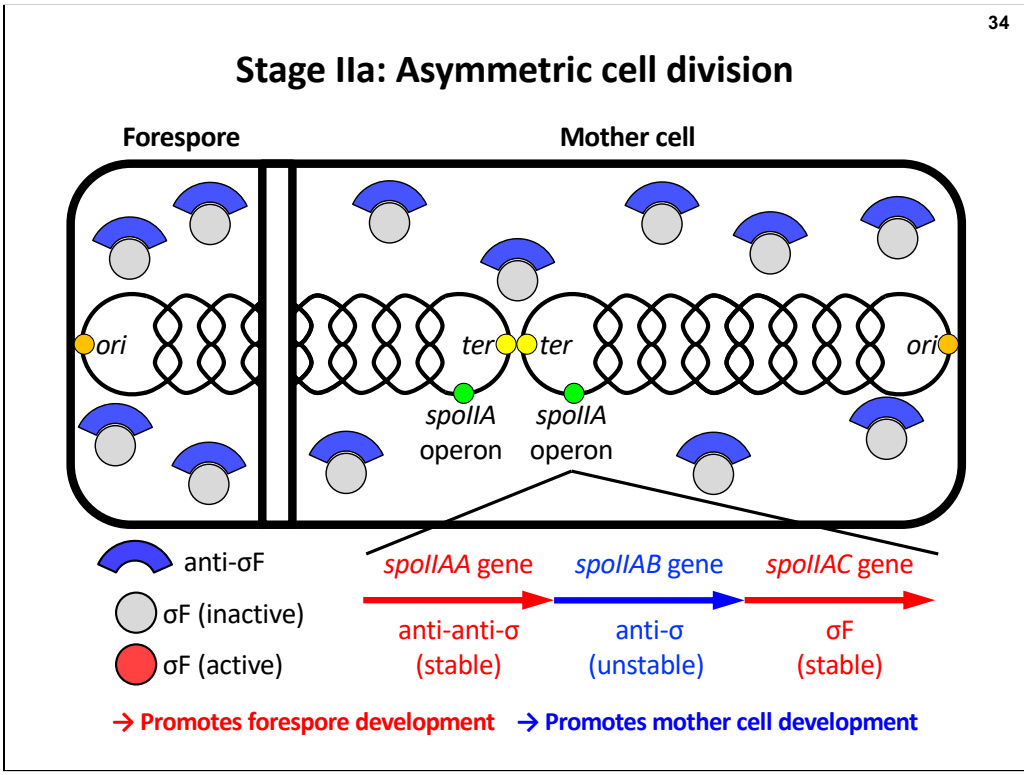

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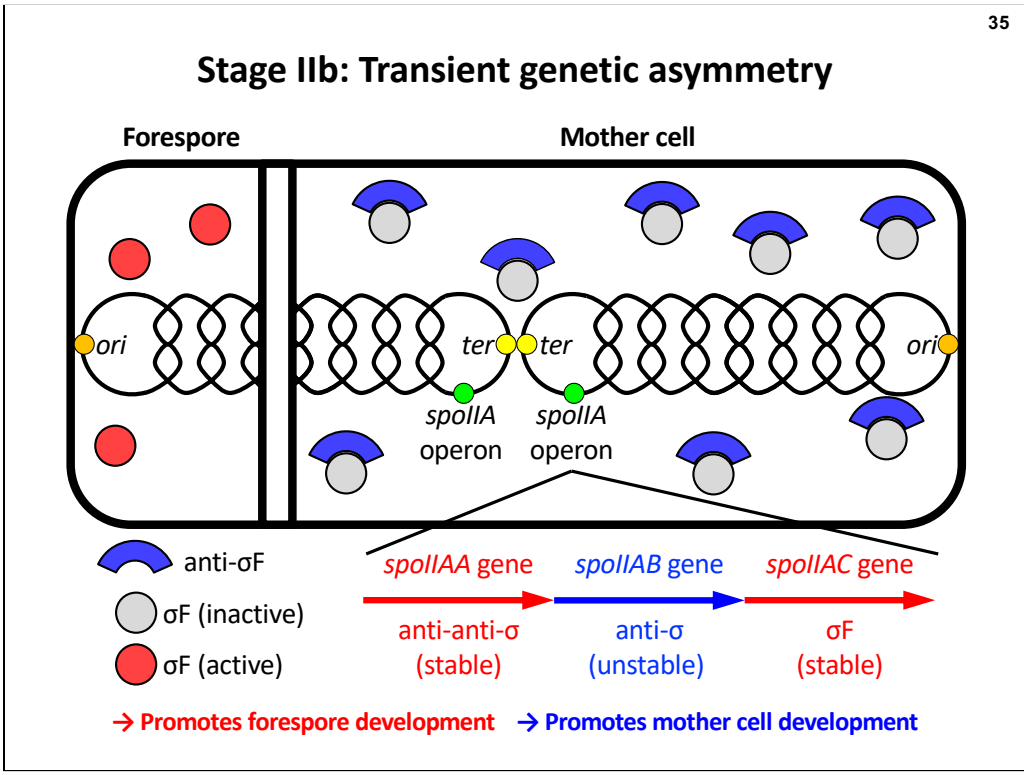

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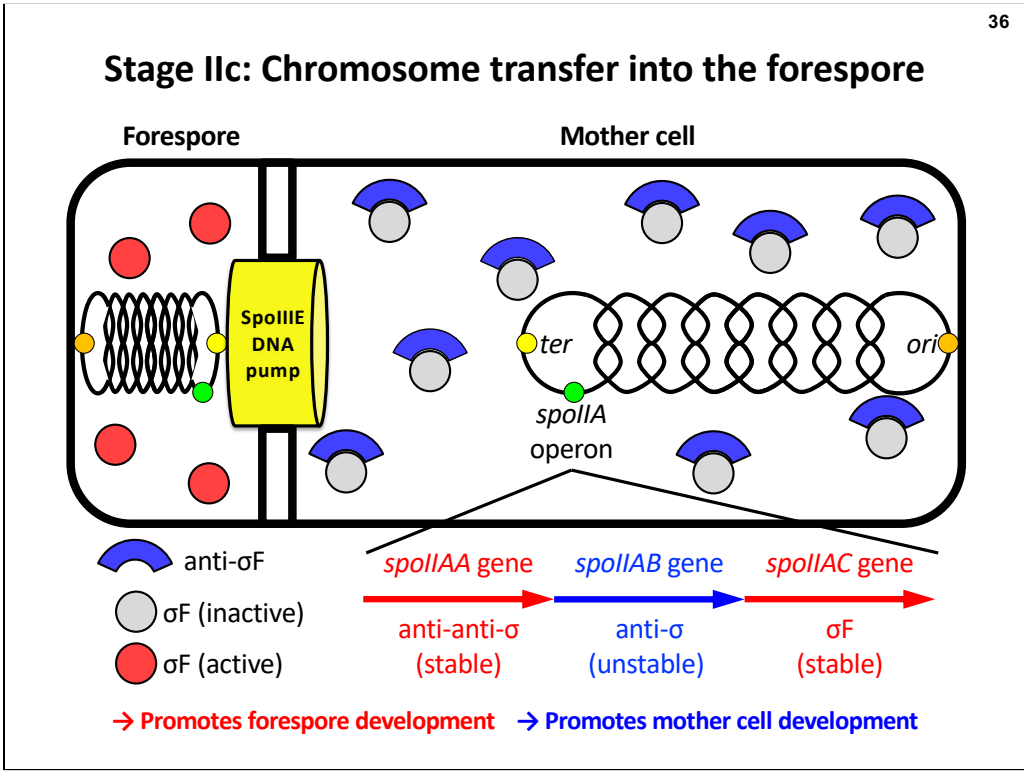

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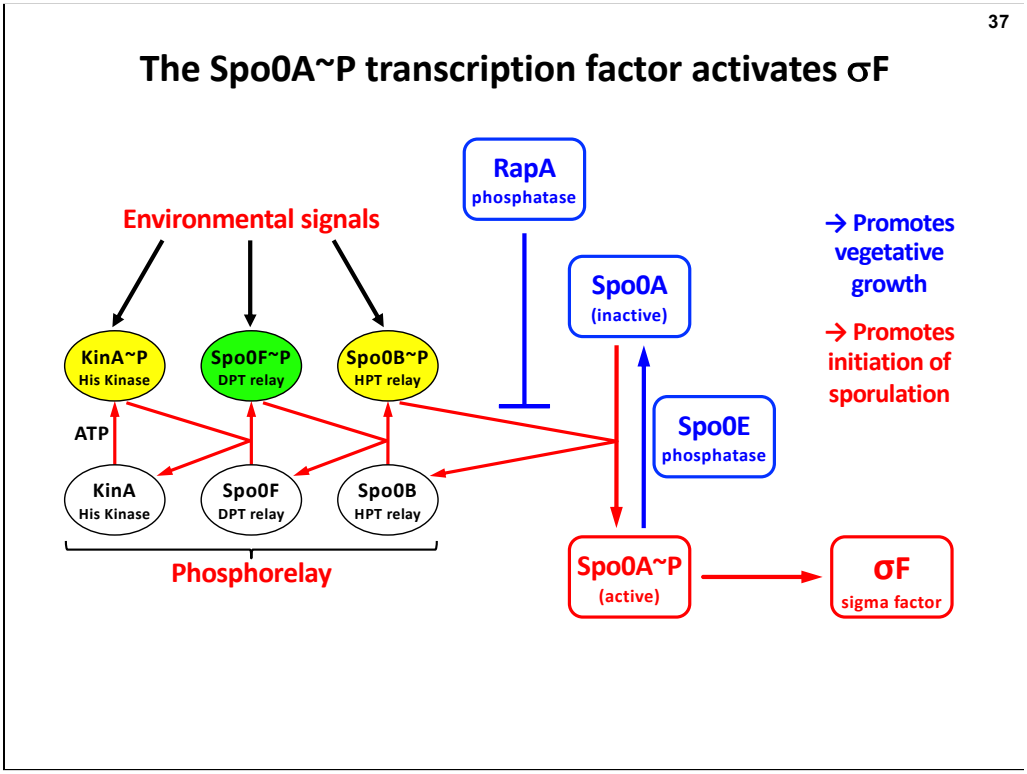
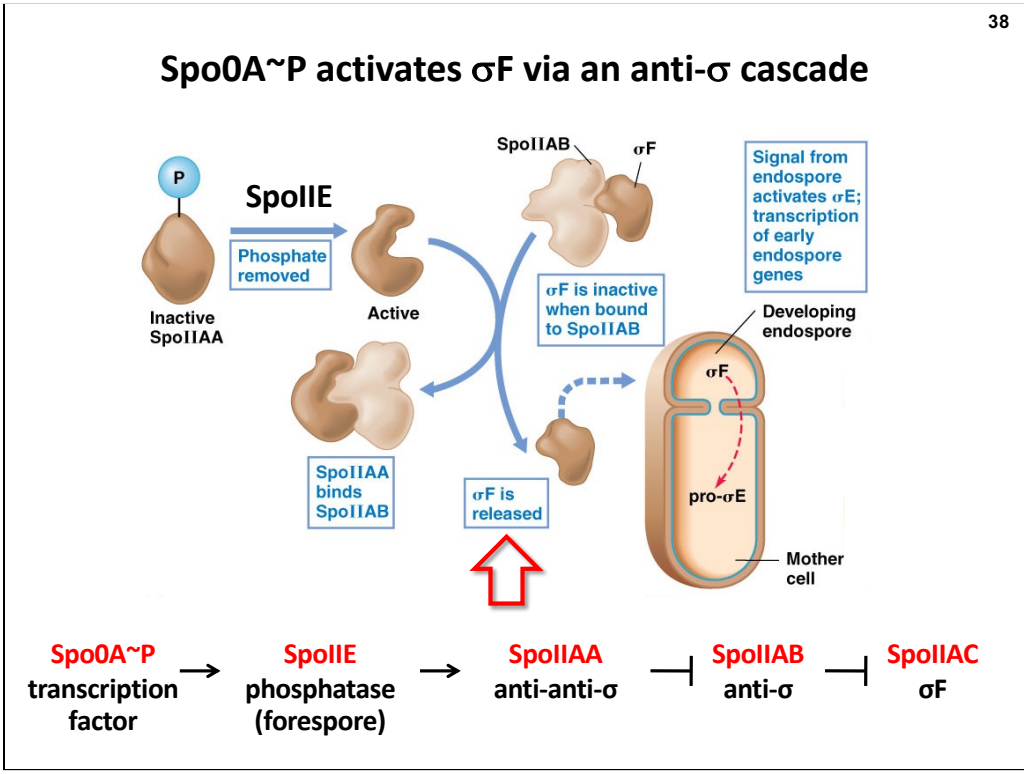


SOURCE: Veening JW, Hamoen LW, Kuipers OP (2005) Phosphatases modulate the bistable sporulation gene expression pattern in *Bacillus subtilis*. *Mol Microbiol* 56(6): 1481-1494 PMID: 15916600.

Figure 1. Simplified schematic representation of the regulatory network that governs initiation of sporulation. Perpendiculars and arrows represent the negative and positive actions respectively.

Spore formation in *Bacillus subtilis* is a complex adaptive response to starvation. The process of sporulation is governed by a **multicomponent phosphorelay** which consists of five **histidine kinases** (KinA, KinB, KinC, KinD, and KinE) and two phosphorelay proteins (Spo0F and Spo0B). Multiple environmental and physiological signals are fed into this system, and under appropriate conditions this leads to **phosphorylation** of **Spo0A**, the key sporulation transcription factor. Within an isogenic culture of sporulating *B. subtilis*, some cells initiate the developmental program of sporulation, whereas others do not. Therefore, initiation of sporulation appears to be a regulatory process with a **bistable** outcome. Phenotypic variation could benefit the fitness of the species, because the heterogeneous population is able to quickly react to changing environments. As sporulation is an energy intensive process, and irreversible after its earliest stage, cells that are delayed to commit to sporulate could have an advantage over sporulating cells if food resources were to become plentiful again. Theoretical modelling and experiments in both prokaryotic and eukaryotic model systems have demonstrated that **positive feedback** of a transcriptional regulator, together with a **non-linear response** to an activator, can lead to a **bimodal probability distribution** in expression. It is believed that stochastic fluctuations causes some cells to reach the threshold level to activate the **feed-forward loop** and these cells will end up in the 'high expressing' population, whereas others do not reach this threshold and remain in the 'low expressing' state. In the present study, we examined whether the positive feedback architecture of spo0A regulation is responsible for bistability in sporulation gene expression and studied the influence of various phosphatases on the maintenance of bistability.



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 7: Molecular Biology of Microbial Growth (pp. 238-258), published by Pearson Education Inc., San Francisco © 2019. Figure 7.15.

Figure 7.15. Control of endospore formation in *Bacillus subtilis*. After an external signal (such as nutrient starvation) is received, a cascade of sigma factors controls differentiation. **(a)** Active SpoIIAA binds the anti-sigma factor SpoIIAB, thus liberating the first sigma factor, sigmaF. **(b, not shown here; see slide 28)** sigmaF then initiates a cascade of sigma factors, some of which already exist and need to be activated, others of which are not yet present and whose genes must be expressed. These sigma factors then promote transcription of genes needed for endospore development.

Spo0A~P, the phosphorylated and activated form of the Spo0A transcription factor, activates expression of the SpoIIE phosphatase. SpoIIE activates the anti-anti-sigma factor SpoIIAA~P by converting it to the unphosphorylated form. Active (unphosphorylated) SpoIIAA binds the anti-sigma factor SpoIIB, thus liberating the first sporulation-specific sigma factor, sigmaF. Liberated sigmaF can then bind to the core RNA polymerase to activate transcription of early sporulation genes in the forespore.

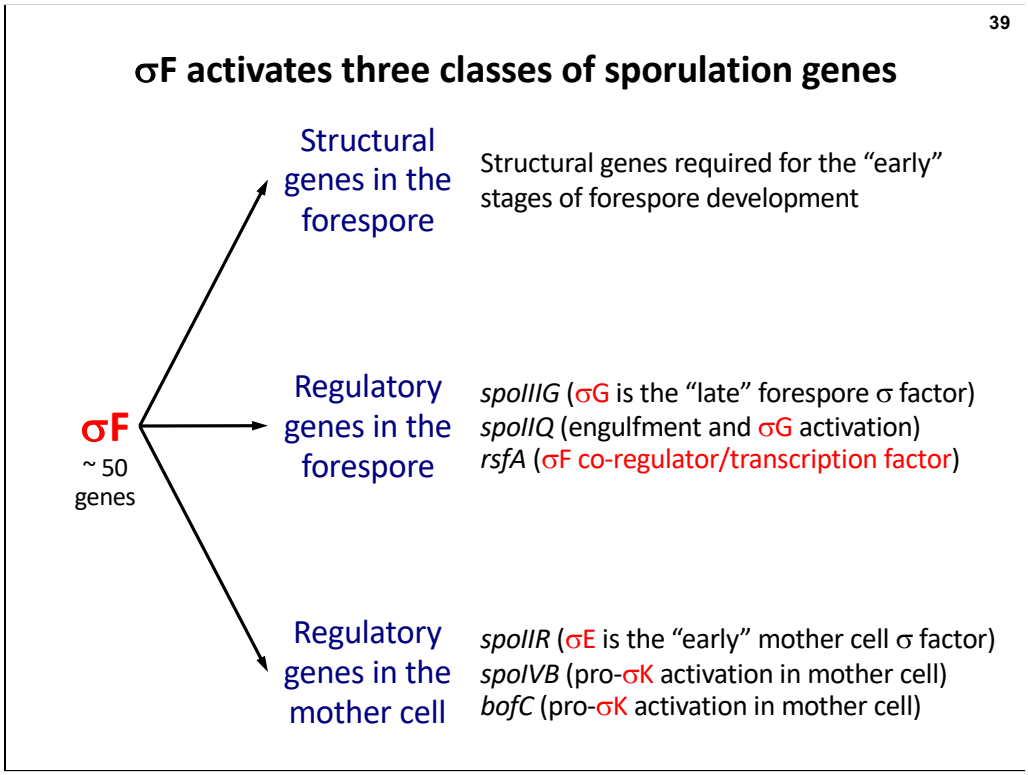
Activated sigmaF also initiates a cascade of sigma factors in the forespore and mother cell (**see slide 28**), some of which already exist and need to be activated (the "early" sigma factors sigmaF and sigmaE), others of which are not yet present and whose genes must be expressed (the "late" sigma factors sigmaG and sigmaK). The sigma factors then promote transcription of genes that are required for endospore formation in the correct compartment and at the correct time:

sigmaF activates early genes in the prespore

sigmaG activates late genes in the prespore

sigmaE activates early genes in the mother cell

sigmaK activates late genes in the mother cell

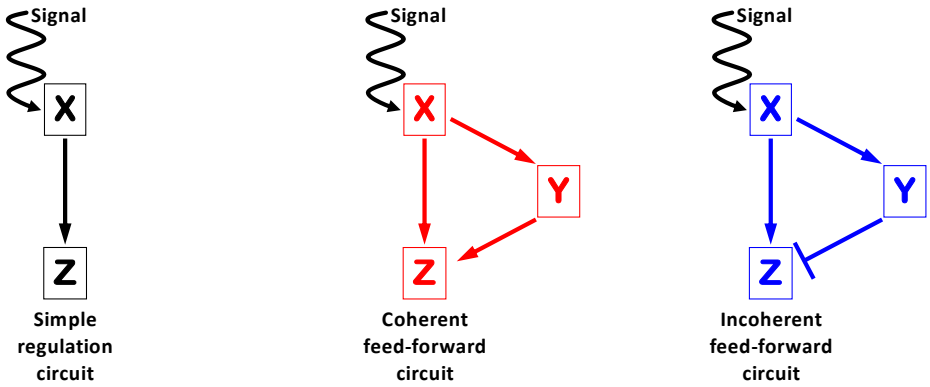


SOURCE: Hilbert DW, Piggo, PJ (2004) Compartmentalization of gene expression during *Bacillus subtilis* spore formation. *Microbiol Mol Biol Rev* 68(2): 234-262 PMID: 15187183.

SigmaF controls the transcription of three classes of genes:

1. In the prespore: genes encoding factors that affect the activity of sigmaF and sigmaG.
2. In the mother cell: genes encoding factors that affect the activity of sigmaE and sigmaK.
3. In the prespore: genes encoding "early stage" spore-formation genes.

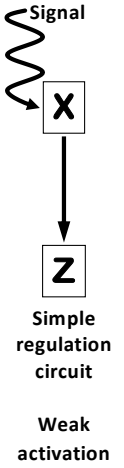
Gene expression network motifs: feed-forward circuits

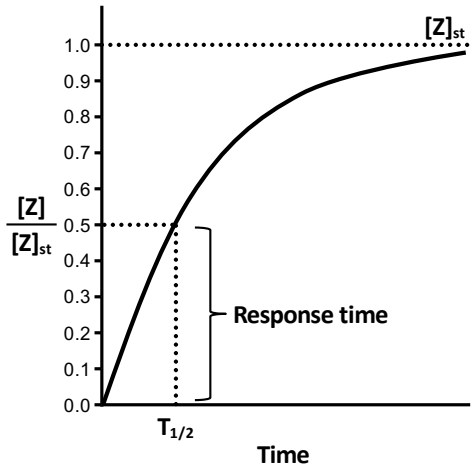


X = Master regulator (σF)
Y = Co-regulator (RsfA)
Z = Target genes

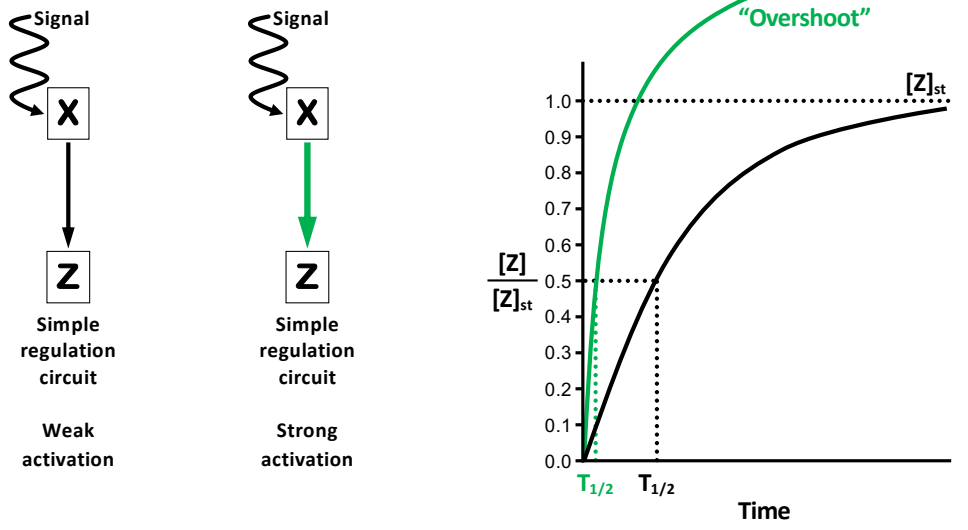
SOURCE: Alon U (2007) Network motifs: theory and experimental approaches. *Nature Rev Genet* 8(6): 450-461 PMID:17510665.

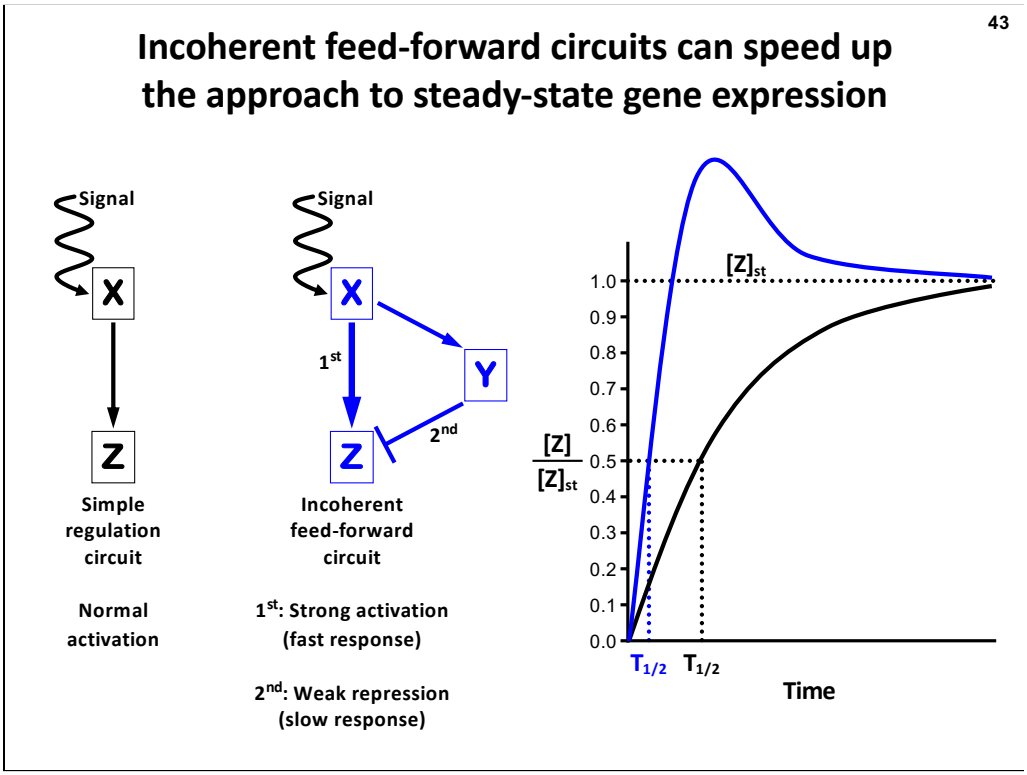
Simple gene regulation circuits generate a slow and asymptotic approach to steady-state gene expression





Simple gene regulation circuits generate a slow and asymptotic approach to steady-state gene expression





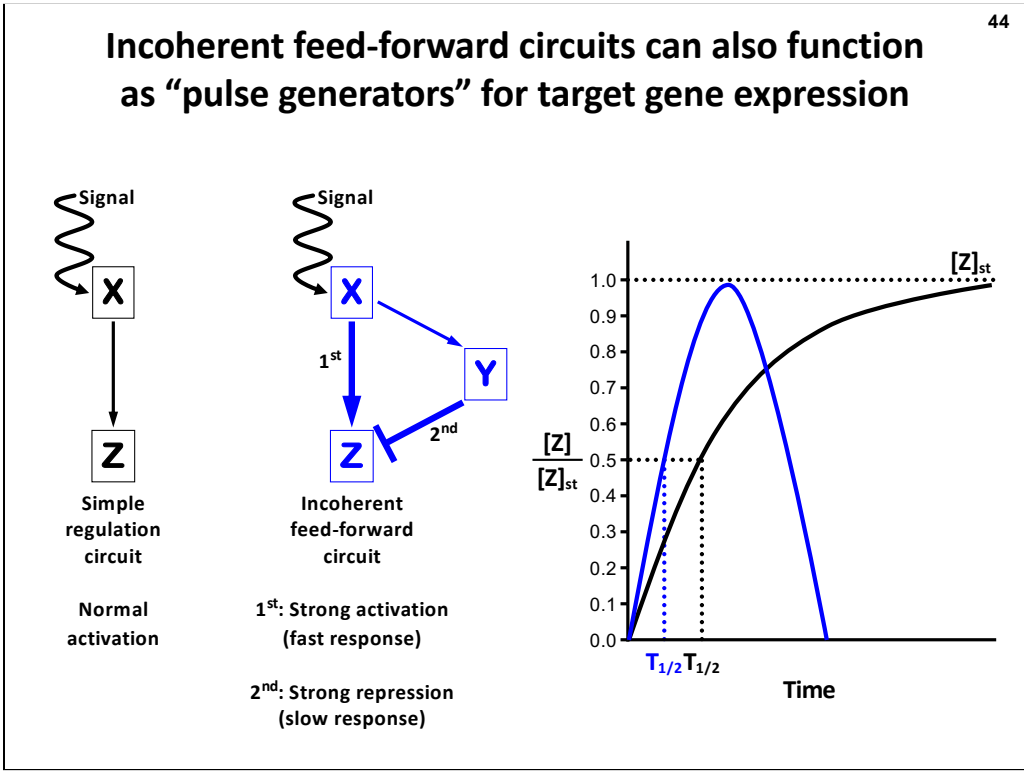
SOURCE: Rosenfeld N, Elowitz MB, Alon U (2002) Negative autoregulation speeds the response times of transcription networks. *J Mol Biol* 323(5): 785-793 PMID: 12417193.

SOURCE: Mangan S, Alon U (2002) Structure and function of the feed-forward loop network motif. *Proc Natl Acad Sci USA* 100(21): 11980-11985 PMID: 14530388.

SOURCE: Mangan S, Itzkovitz S, Zaslaver A, Alon U (2006) The incoherent feed-forward loop accelerates the response-time of the *gal* system of *Escherichia coli*. *J Mol Biol* 356(5): 1073-1081 PMID: 16406067.

SOURCE: Alon U (2007) Network motifs: theory and experimental approaches. *Nature Rev Genet* 8(6): 450-461 PMID: 17510665.

Figure 4. The incoherent feed-forward loop. The incoherent feed-forward loop (blue) can generate a pulse of Z expression in response to a stimulus. This occurs because once the concentration of Y has passed its DNA-binding threshold it starts to repress Z. The incoherent feed-forward loop shows faster response time for the concentration of protein Z than a simple-regulation circuit *with the same steady-state expression level*.



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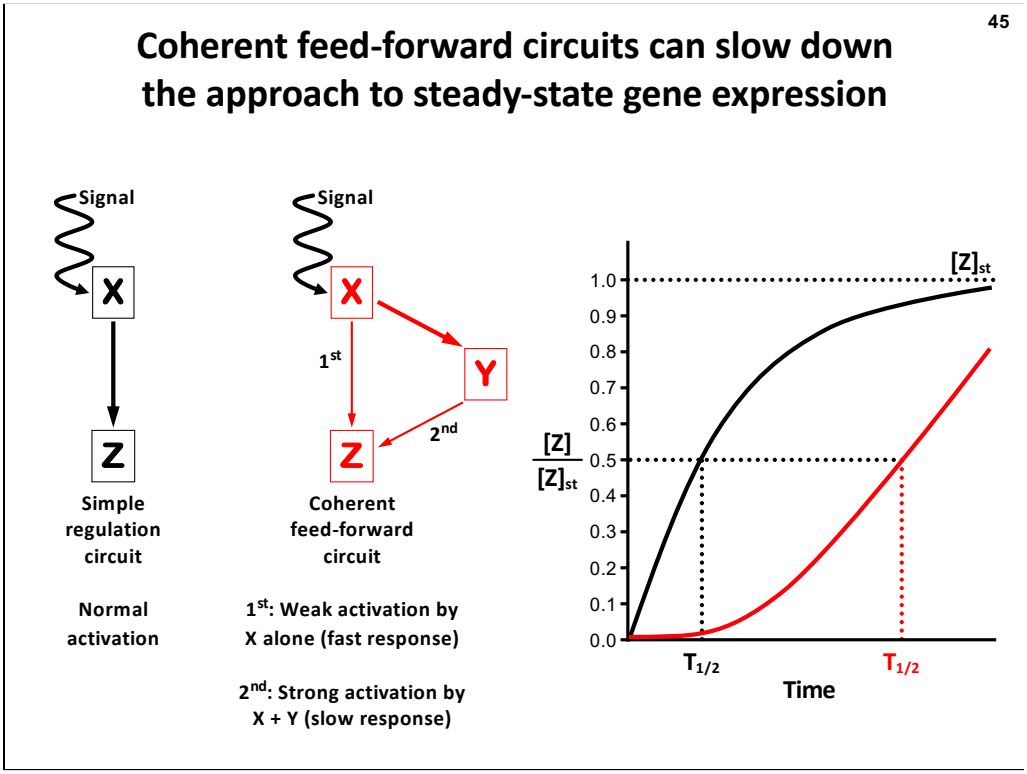
SOURCE: Alon U (2007) Network motifs: theory and experimental approaches. *Nature Rev Genet* 8(6): 450-461 PMID: 17510665.

Figure 4. The incoherent feed-forward loop. The incoherent feed-forward loop (blue) can generate a transient pulse of Z expression in response to a stimulus. This occurs because once the concentration of Y has passed its DNA-binding threshold it starts to repress Z. If the action of Y on Z (repressing) is completely dominant over the action of X on Z (activating) then a transient pulse of Z gene expression will result.



SOURCE: Mangan S, Alon U (2002) Structure and function of the feed-forward loop network motif. *Proc Natl Acad Sci USA* 100(21): 11980-11985 PMID: 14530388.

SOURCE: Alon U (2007) Network motifs: theory and experimental approaches. *Nature Rev Genet* 8(6): 450-461 PMID: 17510665.

The coherent feed-forward loop. The coherent feed-forward loop delays the transcriptional response to a stimulus because two inputs (X and Y) are required to activate Z expression and there is a delay in accumulation of Y due to the time required for transcription and translation of Y after activation of X by the signal. Only once Y has achieved a sufficient "activation threshold" will expression of the Z gene be activated. The coherent feed-forward loop can also serve as a "noise filter" because transient (non-sustained) upward "blips" in the signal strength will not result in sufficient accumulation of Y to permit activation of Z gene expression.

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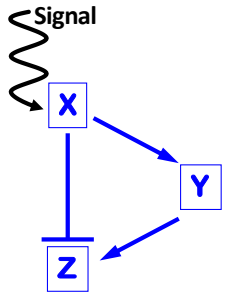
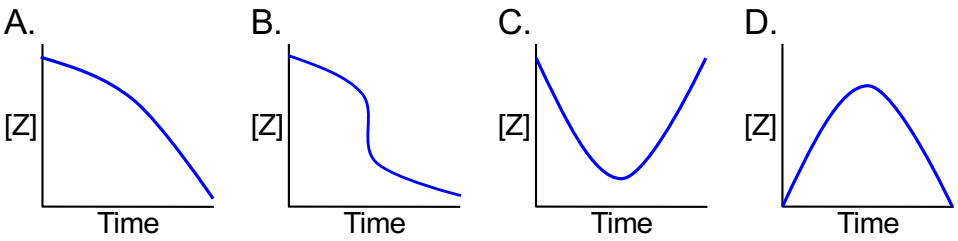
Imagine a genetic network motif with this structure:



Where X is a master regulator, Y is a co-regulator, Z is a target gene. After the signal activates X, what would be the shape of the gene expression response curve for [Z]?



Answer: (C)

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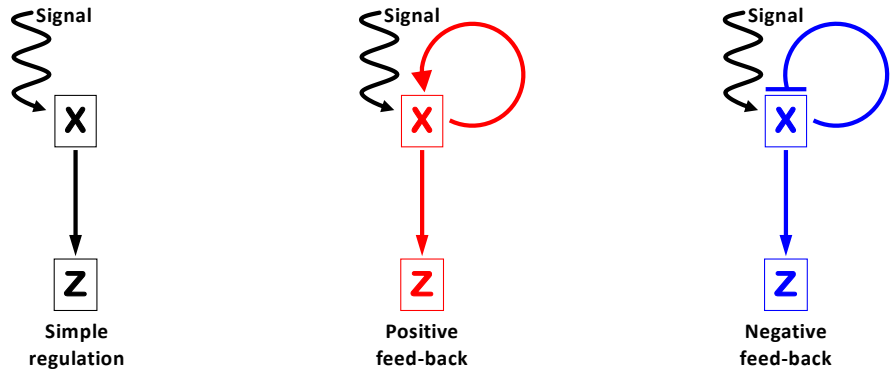
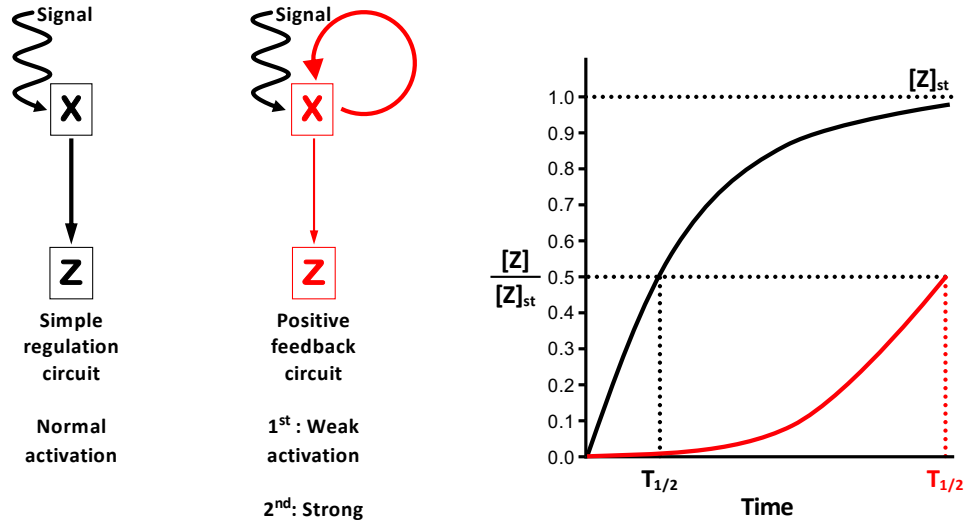
Gene expression network motifs: feed-back circuits



X = Master regulator (σG, σK)
Z = Target genes

SOURCE: Alon U (2007) Network motifs: theory and experimental approaches. *Nature Rev Genet* 8(6): 450-461 PMID:17510665.

Positive feed-back circuits slow down the approach to steady-state gene expression



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SOURCE: Alon U (2007) Network motifs: theory and experimental approaches. *Nature Rev Genet* 8(6): 450-461 PMID: 17510665.

Simple regulation and positive autoregulation. Left (green): In simple regulation, transcription factor X is activated by a signal. When active, X binds the promoter of gene Z to enhance its transcription rate. Middle (red): In positive autoregulation, X is a transcription factor that activates the Z promoter *and* activates its own promoter. Right (graph): Positive autoregulation **slows down (increases)** the response time (i.e., the time τ_r required for expression of Z to reach 50% of the steady-state level) relative to a simple-regulation system that reaches the same steady-state expression level. This seems counter-intuitive, right? The explanation is that with positive autoregulation of X the intrinsic activity of the promoter driving Z must be weak in order not to overshoot the final target (steady-state) concentration of Z after the level of X increases due to positive autoregulation.

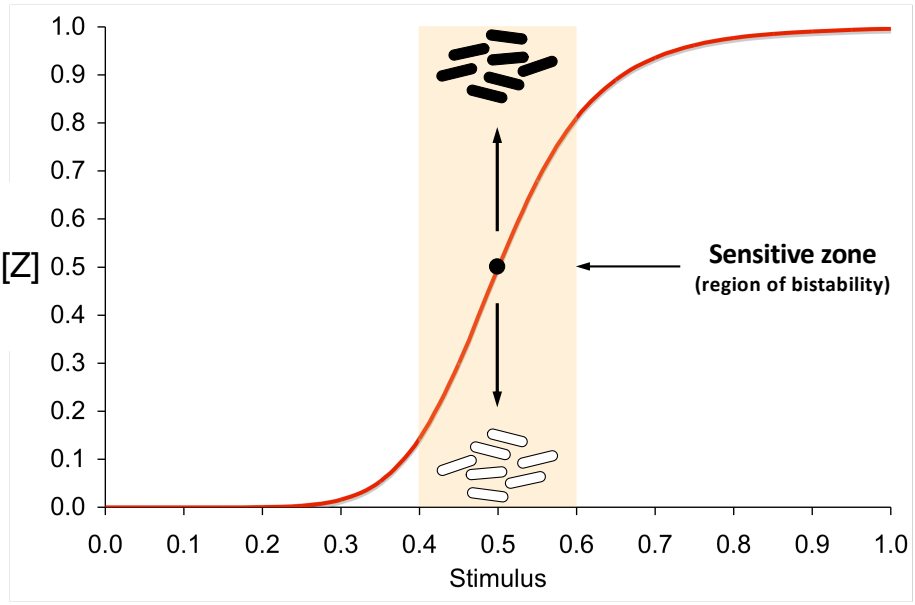
[Z] = concentration of Z.

$[Z]_{1/2}$ = concentration of Z at 50% of the final steady-state level.

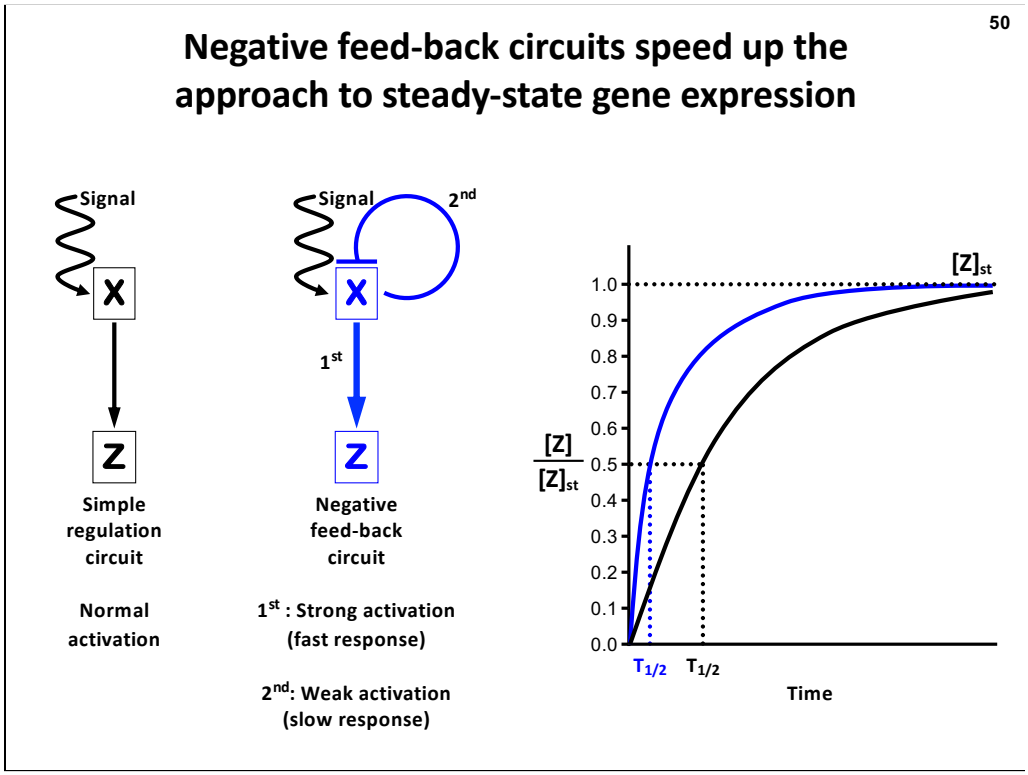
$[Z]_{st}$ = concentration of Z at 100% of the final steady-state level.

t_r = time required for [Z] to reach 50% of the final steady-state level; also known as the “rise time”.

Positive feedback loops generate sigmoidal stimulus-response curves that are bimodal



SOURCE: Ozbudak EM, Thattai M, Lim HN, Shraiman BI, van Oudenaarden A (2004) Multistability in the lactose utilization network of *Escherichia coli*. *Nature* 427(6976): 737-740 PMID: 14973486.



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SOURCE: Alon U (2007) Network motifs: theory and experimental approaches. *Nature Rev Genet* 8(6): 450-461 PMID: 17510665.

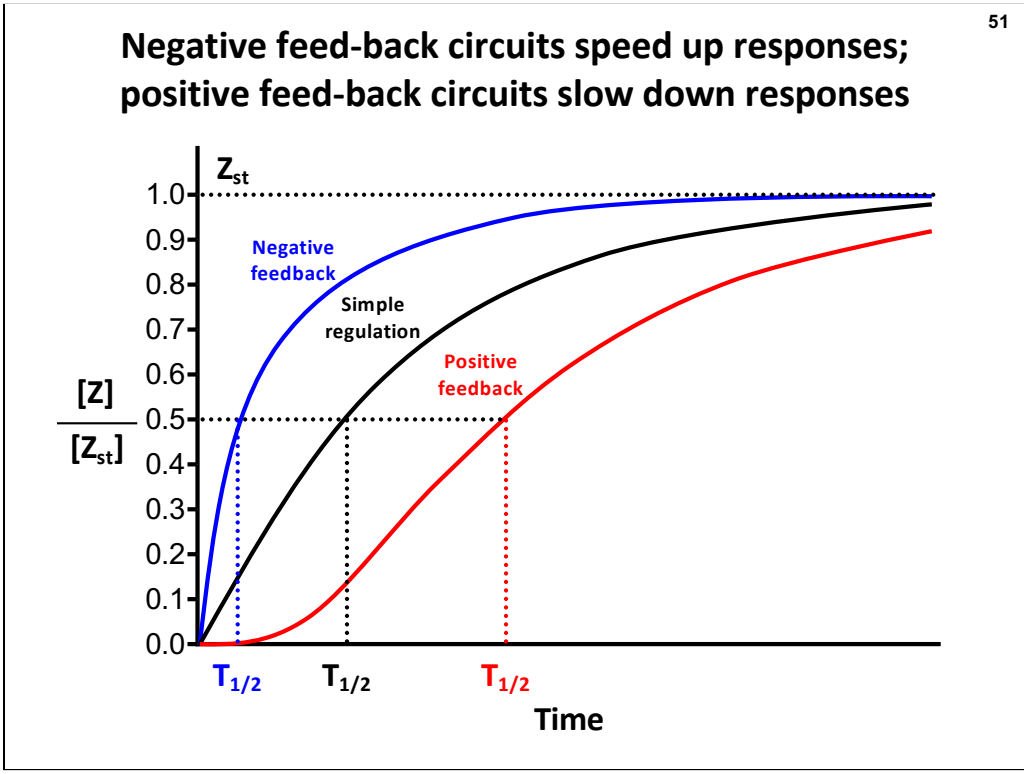
Figure 1. Simple regulation and negative autoregulation. Left (blue): In simple regulation, transcription factor X is activated by a signal. When active, X binds the promoter of gene Z to enhance its transcription rate. Middle (green): In negative autoregulation, X is a transcription factor that activates the Z promoter but represses its own promoter. Right (graph): Negative autoregulation **speeds up (decreases)** the response time (i.e., the time τ_r required for expression of Z to reach halfway to the steady-state level) relative to a simple-regulation system that reaches the same steady-state expression level. This seems counter-intuitive, right? The explanation is that with negative autoregulation of X the intrinsic activity of the promoter driving Z must be strong in order to maintain the final target (steady-state) concentration of Z after the levels of X decrease due to negative autoregulation.

[Z] = concentration of Z.

$[Z]_{1/2}$ = concentration of Z at 50% of the final steady-state level.

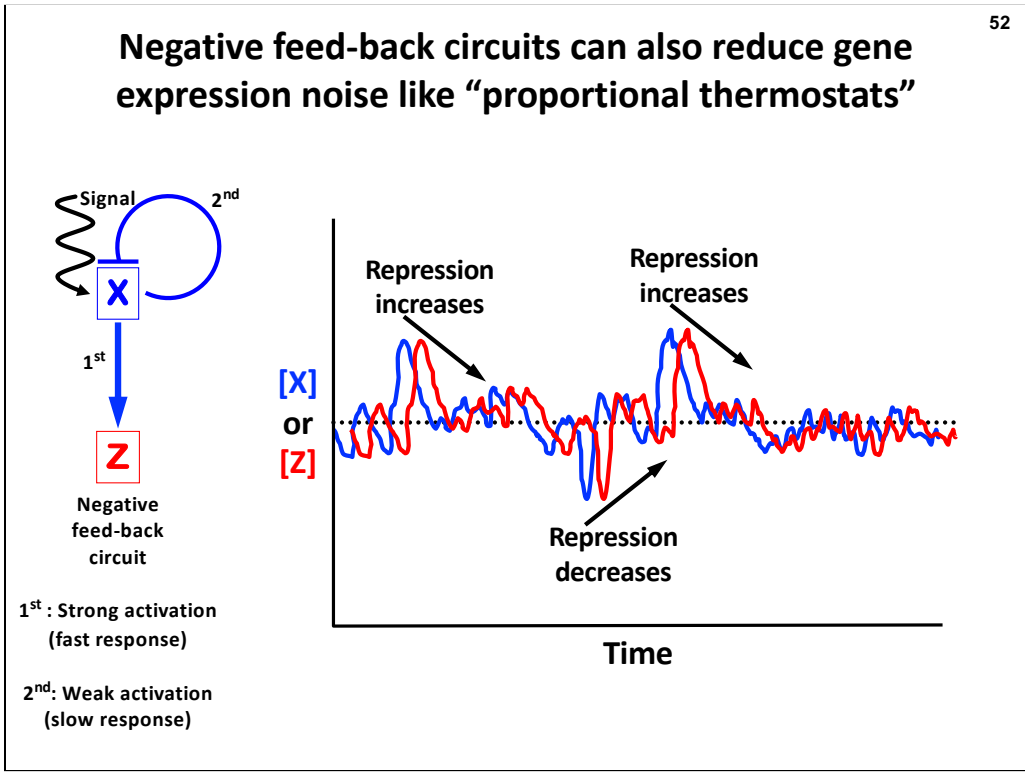
$[Z]_{st}$ = concentration of Z at 100% of the final steady-state level.

t_r = time required for [Z] to reach 50% of the final steady-state level; also known as the “rise time”.



SOURCE: Alon U (2007) *An Introduction to Systems Biology: Design Principles of Biological Circuits*, published by Chapman & Hall, CRC Press, New York © 2007.

Figure 3.5. Dynamics of negatively autoregulated gene, a simply regulated gene, and a positively autoregulated gene. The negatively autoregulated genes and positively autoregulated genes have a Hill-input function with Hill coefficient n = 1. Shown is protein concentration normalized by its steady-state value, X/Xst, following an increase in production rate. Time is in number of cell generations, or for actively degraded proteins, log(2)/α, where alpha is the protein degradation/dilution rate. Note that the response-time (i.e., the time to reach 50% of the steady-state value of X) is $T_{1/2}$ = log(2) / α = 1 for simple regulation, $T_{1/2}$ = 0.21 for negative autoregulation, and $T_{1/2}$ ~ 2 for positive autoregulation with the given parameters. The response time is constructed by the intersect of the dynamics with horizontal line at X/Xs = 0.5.



SOURCE: Kaern M, Eston TC, Blake WJ, Collins JJ (2005) Stochasticity in gene expression: from theories to phenotypes. *Nature Rev Genet* 6(6): 451-464 PMID: 15883588.

Gene networks with negative feedback. By analogy to electrical circuits, negative feedback can provide a noise-reduction mechanism in gene expression circuits. To test this in the context of gene regulation, Becskei and Serrano (PMID:10850721) engineered a single-gene negative-feedback system in *Escherichia coli*. They compared the variability generated by this regulatory network with that generated in the absence of feedback control. This comparison revealed a decrease in gene-expression variability in the negative-feedback network, therefore confirming that negative autoregulation provides a noise-reduction mechanism.

Feed-back control with a “proportional thermostat”