# Notes on how bacterial sporulation triggered in Bacillus subtilis, before the σ factor cascade is activated

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February 5, 2018

*"Various conditions in the environment are monitored by a group of five sensor kinases. These function via a phosphotransfer relay system whose mechanism resembles that of a two-component regulatory system . . . but is considerably more complex (Figure 7.25). The net result of multiple adverse conditions is the successive phosphorylation of several proteins called sporulation factors, culminating with sporulation factor Spo0A. When Spo0A is highly phosphorylated, sporulation proceeds." (Anon, 2014)*

Many of the signals, both positive and negative, that affect this decision are interpreted through the phosphorelay signal transduction system (Burbulys et al. 1991).

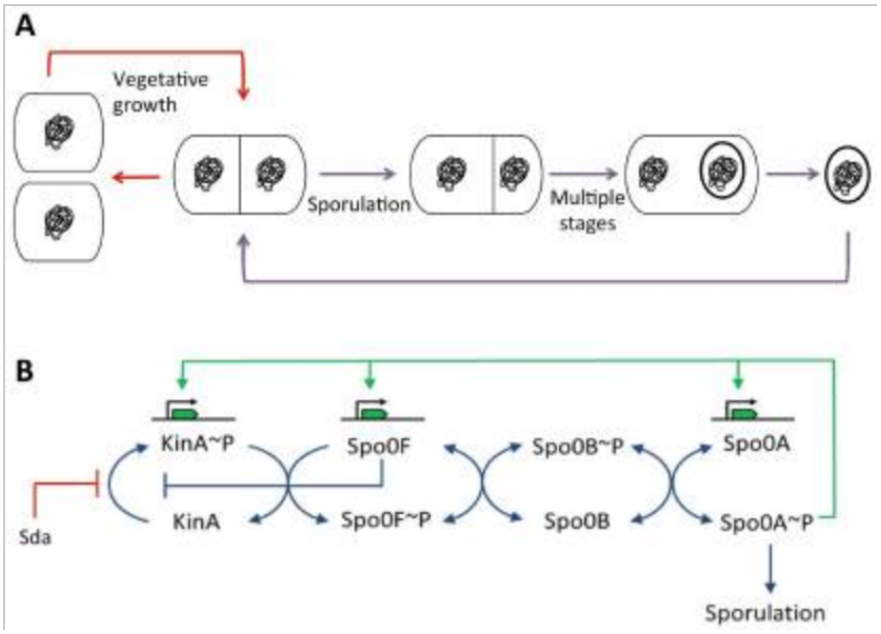
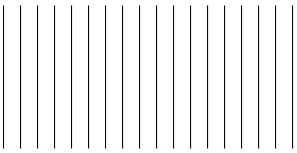



Figure 1: Phosphorelay leading to the induction of sporulation. A series of phosphoryl transfer reactions lead to the accumulation of threshold levels of Spo0A˜P needed for entry into the sporulation pathway. (Jameson & Wilkinson, 2017)

*"the capacity of* B. subtilis *for induced sporulation* ***reaches a peak about 15 min after chromosome***

***replication has begun**. This capacity **then declines rapidly,** but **can be restored by initiating a new round of deoxyribonucleic acid replication**." 1*

*"The main stimulus for sporulation is **starvation**. It is also important that the population density is **high**."* (Errington, 2003)



# ACTUAL STUFF

Recent reviews of how sporulation is triggered are in (Sonenshein, 2000) and this is the paper to end all other research. At least unless there's a newer review, which I haven't found. Until this reaches (Hoch, 1993), everything underneath this point will be from this paper.

There are 2 main triggers for sporulation.

Firstly, population density in the culture.

*"as the mass of a cell culture increases, certain secreted peptides accumulate in the extracellular medium[NOAH NOTE: THIS IS QUORUM SENSING]. When these peptides reach a critical concentration reflective of a particular population density, they are sensed by cell surface receptors"*

these then

*"initiate a sequential transfer of phosphate groups from ATP through histidine kinases (e.g. KinB, KinC) and two intermediate proteins, Spo0F and Spo0B, to a transcription factor, Spo0A"*

The phosphorylated form of Spo0A (Spo0A-P)'s main role during the exponential cell stage is to inhibit *abrB*, a gene which encodes an unstable repressor protein abrB, which represses many genes essential for both sporulation and the stationary cell phase. NOTE: Re-activating these genes, does not, in and of itself begin sporulation.

Secondly, external conditions (nutrient deficiency)

*"sources of carbon, nitrogen and phosphorus can be the relevant limiting growth substrates"*

The cells use guanine nucleotides, particularly GDP and GTP, as intracellular indicators of nutrient availability. Internal levels of GDP and GTP are measured with a CodY protein.

*"CodY, a global repressor of early stationary phase and sporulation genes . . . , binds GTP directly and that the GTP-bound state of CodY is the active state for repression"*

Such that " *These results lend themselves to a simple model: during rapid exponential growth, the intracellular concentration of GTP is high (1–3 mM) and stationary phase and early sporulation genes (including spo0A) are repressed. As cells experience nutrient limitation, the GTP level drops (because of reduced synthesis of*

*GTP or conversion of GTP to ppGpp via the stringent response or both) below a certain threshold level and CodY loses its ability to repress its target genes*"

It's important to note that:

*"most of the genes that are regulated by AbrB and CodY and are induced during the transition from rapid exponential growth to stationary phase are not involved in sporulation per se. They encode proteins whose activities help cells to adapt to poor nutritional conditions by swimming to a new site, by degrading macromolecules, by importing and catabolizing potential nutrients and by killing competitor organisms"*

The listed events above are then the cells last ditch attempt to STOP sporulation, by trying other avenues.

Stage 2 only occurs when

*'ενουγη Σπο0Α˜Π <αγγυμυλατεγ> το σερε αγ α ποσιτιε ρεγυλατορ οφ τρανσγριπτιον οφ γριτιγαλ, σπορυλατιον-εσσεντιαλ γενεγ (ε.γ. σποΙΙΑ, σποΙΙΓ, σποΙΙΕ) τηατ ινιτιατε τηε σ φαστορ γασγαδε . . . '*

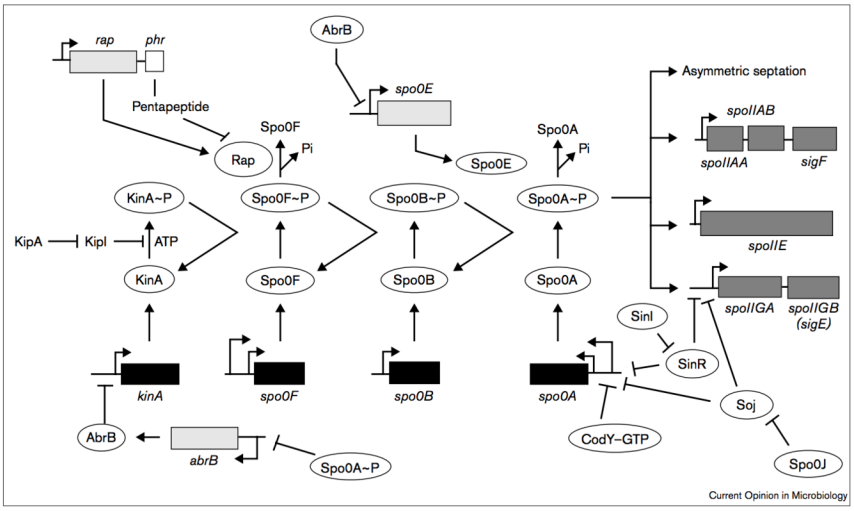



Figure 2: Regulatory pathways in early sporulating cells

**However for stage 2 to be activated, several more things have to be satisfied:** *"this transition depends on the synthesis of an efficient histidine kinase (KinA), on an increased rate of synthesis of Spo0A, and on the neutralization of various antagonists of the phosphorelay and repressors of Spo0A-dependent genes."*

*'Τηε γομβινεδ εφφεγτγ οφ ρελεασινγ ρεπρεσσιον βψ ΑβρΒ ανδ ὃδΨ, ανδ οφ αγτιατινγ σΗ (;), τηε προδυγτ οφ σπο0Η, λεαδ το ινδυγτιον οφ κινΑ, σπο0Φ ανδ σπο0Α (φρομ σΗ-δεπενδεντ προμοτερ) ανδ τηε ποτεντιαλ το προδυγε α ηιγηερ λεελ οφ Σπο0Α˜Π'*

**σH ις α σπεςιφις φορμ οφ PNA Πολψμερασε ρεχυιρεδ φορ συφφιςιεντ σψντηεσις λεελς οφ Σπο0A, Σπο0Φ ανδ κινA**

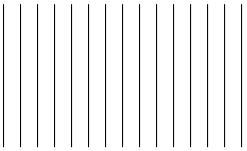
*'Τηε σπορυλατιον προμοτερς οφ σπο0A ανδ σπο0Φ, ας ωελλ ας τηε κινA προμοτερ, δεπενδ ον τηε σH φορμ οφ PNA πολψμερασε.'*

*¨σπο0H, τηε γενε τηατ ενςοδες σH, ις ρεπρεσσεδ βψ AβρB ανδ ις, τηερεφορε, εξπρεσσεδ ερψ ποορλψ ιν ςελλς ατ λοω ποπυλατιον δενσιτψ. Τηυς, σινςε Σπο0A ρεπρεσσες αβρB ανδ AβρB ρεπρεσσες σπο0H, Σπο0A˜Π ις αν ινδιρεςτ αυτοινδυςερ* <of the σH form of RNA polymerase>"

**"Turning on the kinA gene is not by itself sufficient to push a stationary phase cell toward the sporulation pathway"**

because

***Th*ere are many repressors that act as a checkpoint at this stage of sporulation (remember that sporulation is an 8 hour process that is incredibly energy intensive and so should ONLY be undertaken if absolutely necessary) *e.g "while the cell is accumulating KinA, it is also accumulating Spo0F˜P phosphatases (Rap proteins) and a Spo0A˜P phosphatase (Spo0E)"***



## INHIBITORS FOR STAGE 2

*"The **inhibitors include** at least **three Rap proteins*** [**Spo0F-P phosphatases**]*, **Spo0E [Spo0A-P phosphatase]**, KipI and SinR. Each has a specific antagonist."*

## *Rap proteins*

We currently (as of 2000) know of 3 Rap proteins.

*"For instance, RapA is inactivated by a pentapeptide encoded by phrA."*

ALL Rap proteins are inhibited by specific pentapeptides.

*"Recent work has identified the third known phosphatase for Spo0F˜P (RapE) and its antagonist (PhrE)"*

How the pentapeptides are regulated is currently unknown - it's also not known why 3 are needed.

*"How the rate of intracellular accumulation of phosphatase-inhibiting pentapeptides is regulated is also unclear; it may simply be a passive process, reflecting the time required for synthesis, secretion and processing of precursors of about 40 amino acids, and subsequent uptake of the pentapeptides. Alternatively, the cell may depend on further nutritional or physiological cues to set the time at which a sufficient level of Spo0F˜P accumulates to allow commitment to sporulation."*

## *KipI*

*"KipI, an inhibitor of KinA,*

> How is it repressed/inhibited?
>
> *"...is antagonized by KipA"*

## *SinR*

*"SinR, a tetrameric protein, is synthesized constitutively and binds to the promoter sites of the* spo0A *and* spoIIG *genes, repressing their transcription..."*

*"Relief of repression is mediated by SinI, a protein that is induced as cells enter stationary phase"*

> How is it repressed/inhibited?
>
> *"SinI forms a 1:1 complex with SinR monomers, driving SinR from its active, tetrameric state"*
>
> *'Τηε ιντεραστιον βετωεεν τηε τωο προτεινς ις μεδιατεδ βψ νεαρλψ ιδεντιςαλ α-ηελιςαλ δομαινσ'*

## *Soj*

<fill in>

## *ScoC*

*"ScoC ... has at least three important targets: the promoters of the opp and app oligopeptide transport operons and the sinI gene."*

*"The oligopeptide permeases are needed to import the signaling pentapeptides that inactivate Rap phosphatases and SinI is needed to antagonize SinR (see above). Thus, repression by ScoC must be relieved, by an unknown mechanism, for sporulation to ensue."*

## Structure of the Spo0A phosphorelay proteins

<fill in>

The phosphorelay system was first discussed in detail in (Hoch, 1993). The following quotes are from the linked article (emphasis mine).

*"The major signal-transduction pathway for the initiation of sporulation is the* ***phosphorelay****, which responds to* ***environmental, cell cycle, and metabolic signals****, and* ***phosphorylates the SpoOA transcription factor activating its function****."*

*"in a normal culture grown in the laboratory,* ***sporulation occurs during the stationary phase****, and many of the processes, alternate pathways, and enzymes formed during the early part of stationary phase are controlled along with sporulation because* ***the cell controls sporulation and many of the stationary-phase processes by a single transcription factor, SpoOA****."*

There are two main external factors that can trigger sporulation: nutrient starvation and bacterial population density.

Nutrient starvation can be detected inside the cell by utilising GTP/GDP concentrations. In high concentrations, GTP binds to the protein CodY. CodY-GTP acts as a repressor to many early sporulation genes.

Population density can be detected through quorum sensing.

If either one of these external conditions is detected, the cell initiates a phosphorylation cascade from the histidine kinase, KinA through two intermediate proteins, Spo0F & Spo0B, to the transcription factor Spo0A, forming Spo0A-P. Spo0A-P represses the transcription of AbrB, which is a repressor to many genes responsible survival strategies such as swimming to a new site, catabolizing macromolecules and killing competitors. AbrB is also a repressor to KinA transcription, hence repression of AbrB allows the potential increase of Spo0A-P concentrations if external conditions do not improve. In high concentrations Spo0A-P acts as a positive regulator for many sporulation genes such as spoIIA, spoIIGA and spoIIE, triggering the second stage of sporulation.

Thank you X. As X mentioned, I'm going to be talking a little bit about how sporulation is triggered, but more importantly I'm going to discuss some of the ways the cell tries to avoid sporulation.

Cells capable of sporulation are in a balancing act under poor conditions, because sporulation is a metabolically expensive process, that takes up to 8 hours to complete, which is a long time when you have a generation time of 20 minutes! It has to commit itself to the process but it can be too costly to come back from if its wrong. The cell does not want to form an endospore unless it is *certain* it is the right course of action, *however* if sporulation in this environment is inevitable, then the cell wants to make sure it sporulates *first*, before the bacteria surrounding it have, <for reasons we'll discuss later>. For now I'm going to focus on three main questions: How does the cell detect that the outside conditions have become so unfavourable? How does the cell make sure this isn't a false positive (because if the poor conditions are temporary the cell has made a *costly* mistake)? And providing it isn't, how does it begin the process of forming an endospore?

So let's look at the first question, how does the cell detect the unfavourable outside conditions? Well the first way is actually something we've covered, and that's quorum sensing. As you already know how this works, I'll skip over the details and tell you that detecting high concentrations outside the cell triggers a phosphorylation cascase, which looks like this. Our histidine kinase, KinA becomes phosphorylated, and passes this down the chain

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