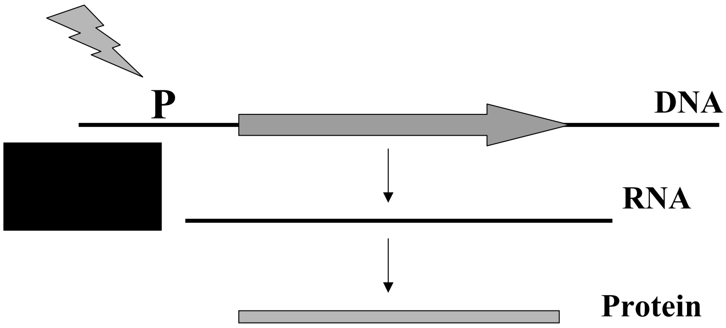
Transcriptional Regulation

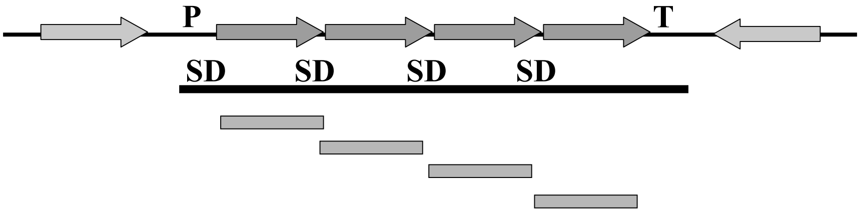


Transcriptional regulation

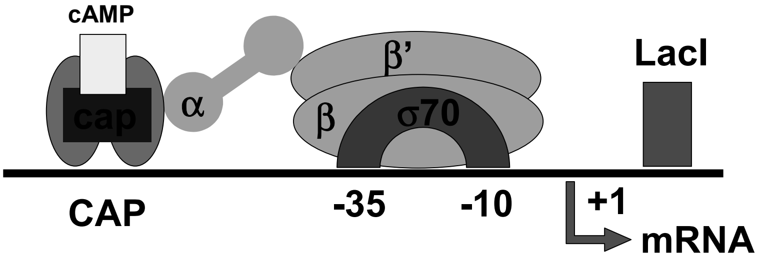
- Efficient
  - resources involved in mRNA and protein biosynthesis used when required
  - promoter interaction means groups of genes can be co-regulated
- Rapid
  - direct and rapid change in expression in response to changing conditions

Promoters: a reminder

- promoter characteristics
- lac promoter best characterised
- gene organisation in prokaryotes



The promoter



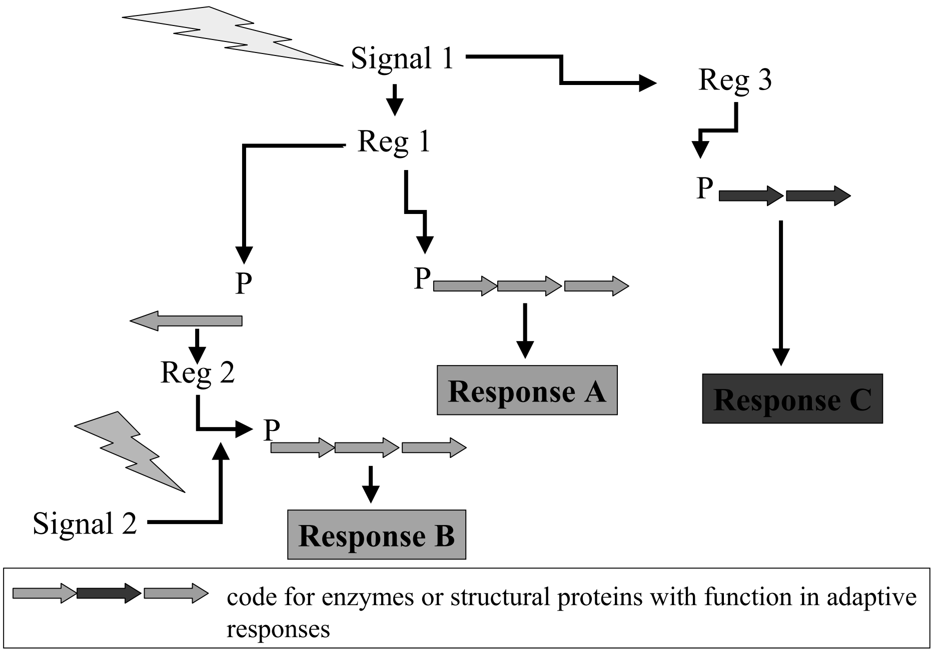
activator

RNA pol. binding

repressor binding

Promoters

- Promoter classes
  - Class I: alteration of DNA to make sigma-DNA interaction sites (-35&-10) aligned
  - Class II: binding of activator to promote RNA pol. binding.
- Points of control
  - need activator to enable RNA pol to bind to the promoter effectively
  - RNA pol. binding or polymerisation progression blocked by repressor
  - require specific sigma factor



code for enzymes or structural proteins with function in adaptive responses

Regulatory Pathways

- Global Regulators
  - affect several regulatory pathways with different cellular functions
  - eg Reg1 in illustration
  - Hns, Fur, OmpR, some sigmas
- Specific Regulators
  - not global (sic!)
  - eg Reg2 and Reg3
- Regulatory Cascade
  - regulator controls expression of another regulator….
  - eg Reg1 to Reg2 in example
  - sporulation cascade

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some definitions

- Regulon
  - All the genes that controlled by a particular regulator
  - eg Reg2, Response A and Response B genes are part of the Reg1 regulon.
- Stimulon
  - Genes whose expression changes in response to a particular stimulus
  - May involve several regulons
  - eg. Reg1, Reg3, Response A and Response C genes.

Happy Families

- Amino acid sequence comparisons between regulatory proteins reveals similarities between regions
- Paralogues (similar sequence & same job) and Orthologues (similar sequence & different job)
- Many regulators can be grouped into related families
- Regulators within family have related sequence, shared domains and presumably similar mechanisms of action
- Can further classify some into sub-families to reflect specific differences
  - Good example are the TCRs

Regulator families (cont.)

- Family often named after first found/best characterised
- Across bacterial genera
- LysR
- AraC
- MerR
- Lux
  - Quorum sensing
- Fur
  - good example of negative regulator

Examples of Regulatory Systems/Families.

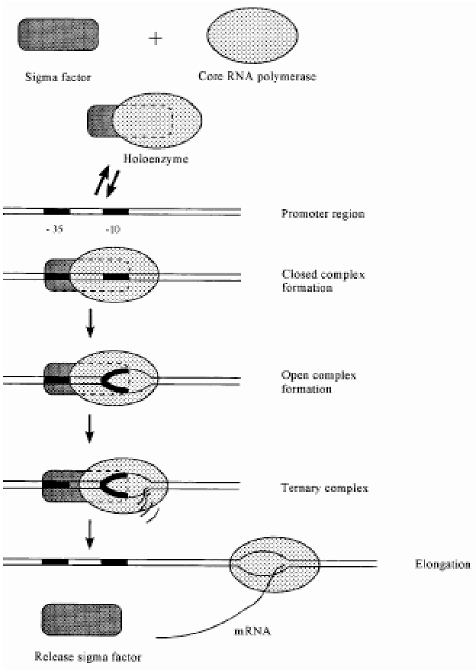
We will consider……

- Sigma Factors
  - RpoH and RpoE
  - Sporulation pathway (*B. subtilis*)
- Two Component Regulators
  - a large, important family
- Post transcriptional regulation

- *Escherichia coli* unless otherwise stated

Sigma Factors

- Most promoters (ie house-keeping genes during exponential phase) are transcribed by RNAP programmed by σ^{70}.
- Other promoters require RNAP programmed by different σ factors.
- Non-σ^{70} promoter contains different sigma-specific sequence motif
- Subset of promoters only bind RNAP when particular sigma present.
- Control specific sigma and control promoter activity.



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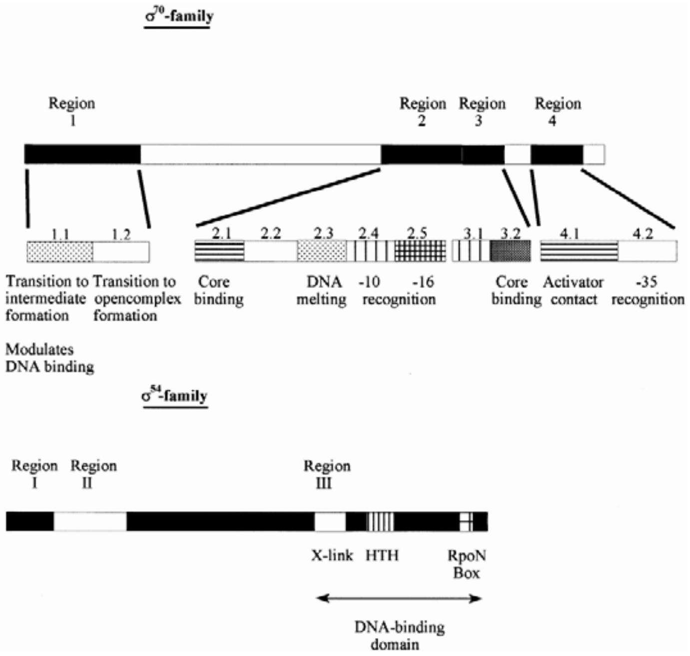
M. Wosten. FEMS Micro.
Rev. 1998 22:127-150

Sigma Factors

In ***Escherichia coli***:				
Group 1: Primary sigma Factor Family				
Major Sigma	RpoD	σ^{70}		Exponential phase
Sigma S	RpoS	σ^{38}	σ^{S}	Stationary phase
Sigma H	RpoH	σ^{32}		Heat Shock
Sigma E	RpoE	σ^{24}	σ^{e}	
Sigma F	FliA	σ^{28}		flagellum
Group 2: The sigma^{54} family				
Sigma54	RpoN	σ^{54}	σ^{N}	Nitrogen limitation



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concensus sequences

	-35	spacer	-10
σ^{70}	TTGACA	16-18	TATAAT
σ^{38}			CTATACT
σ^{32}	CTTGAAA	11-16	CCCATnT
σ^{24}	GAACTT	16-17	TCTRA
σ^{28}	TAAA	15	GCCGATAA
	-24	**spacer**	**-12**
σ^{54}	TGGCAC	5	TTGCW
N=any base; R=A or G; W=A or T.			

Detecting environmental change.

- Promoter will not recruit RNAP if specific sigma factor is not present or is not in active form.
- How link sigma to detection of environmental change?
- sigma does not have sensor domain or cognate sensor moiety.
- control sigma by:
  - additional regulator control of sigma gene expression
  - control sigma activity –anti-sigma factors

Anti-sigma factors

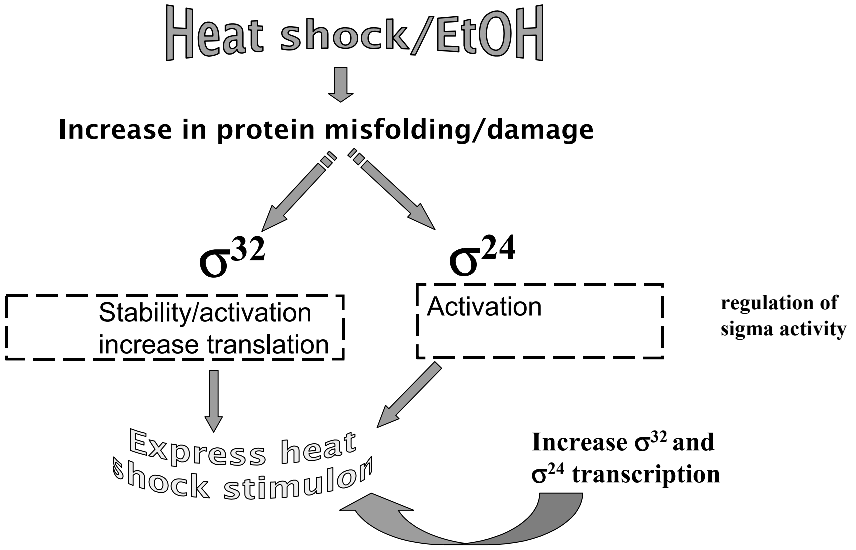
- anti-sigma factors negatively regulate transcription by binding to sigma thus stopping sigma programming RNAP
- Sigma can be pre-synthesised –quicker response
- examples:
- FliA and FlgM (α-σ28)
  - FlgM binds σ28 until first part of flagellum is constructed. FlgM then exported thru flagellar basal body and σ28 then allows expression of rest of genes. When complete FlgM stop being exported and σ28 activity shut down.
- RpoE and RpoH system
- Sporulation system:
  - α-σ factor SpoIIAB and α-α-σ factor SpoIIAA (binds α-σ factor).

Response to Stress

- Biological Context:
  - respond to high temp, EtOH, oxidative stress etc.
  - protein damage prevention and repair by stress regulon members
    - re-fold or degrade
  - in Ec* stress regulon controlled by $\sigma^{32(H)}$ and $\sigma^{24(E)}$
    - $\sigma^{32(H)}$ regulon inc. *dnaJ*, *dnaK*, *groEL*, *groES*, *rpoD*…+lots..
    - $\sigma^{24(E)}$regulon inc. *rpoH*, *rpoE*, *htrA*
  - example of up-regulation of σ synthesis & activity of anti- σ factors.

  - NB: *other systems (non σ) in other bacteria.

Sigma-mediated regulation of heat shock stimulon



Heat shock/EtOH

Increase in protein misfolding/damage

σ^{32}

Stability/activation
increase translation

σ^{24}

Activation

regulation of sigma activity

Express heat shock stimulon

Increase σ^{32} and σ^{24} transcription

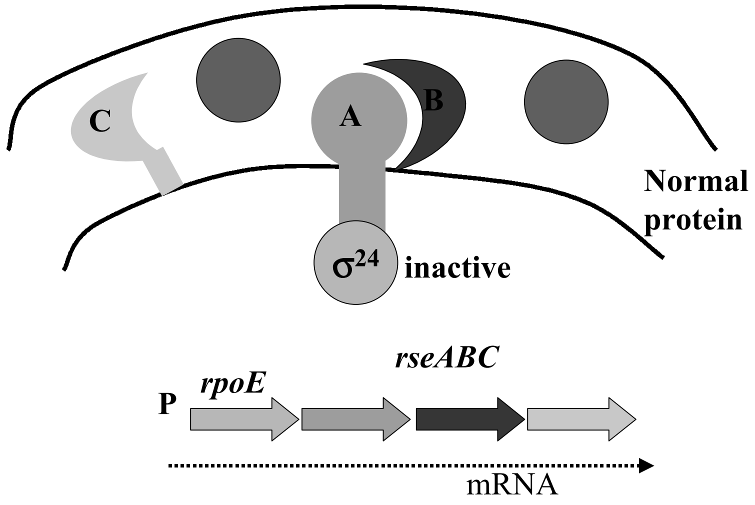
Anti-sigma factors: the RseABC system

- RpoE activated during heat shock –misfolded proteins
  - low level of transcription
- *rpoE* part of 4 gene operon –*rseA,B,C*
- RseA is anti-sigma factor
  - normal conditions bind & inactivate RpoE
  - *rseA* mutant –constitutive high expression of *rpoE*
  - over express RseA –inhibit RpoE
  - also shown with purified RseA *in vitro*
- RseA inner memb protein w/ periplasmic (C-term.) & cytoplasmic (N-term.) domains
  - N-term. domain inhibit RpoE

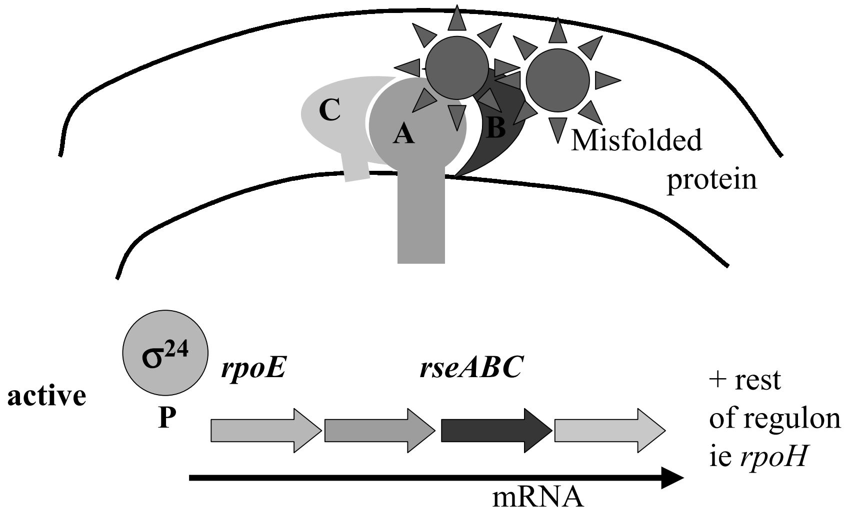
RseB&C

- RseB and C also affect system
- RseB: interact w/ RseA
  - periplasmic
  - interact with C-term domain RseA and mis-folded proteins
- RseC: inner membrane
  - promotes RpoE activity
  - anti-anti-sigma factor

- Yeast 2-hybrid system used to show interactions between RpoE, RseA, B, C.



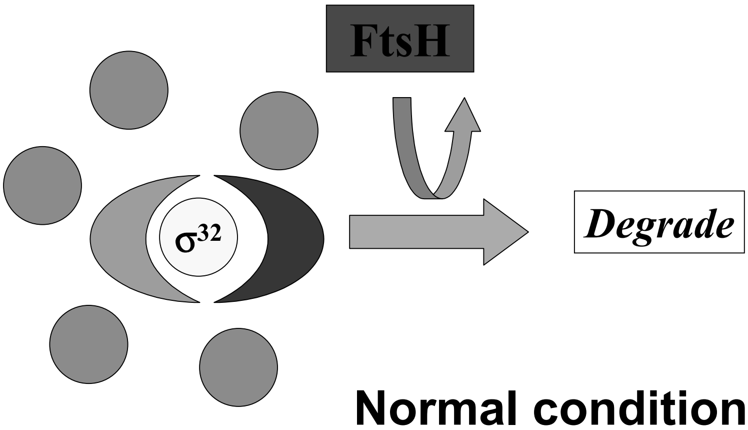
Normal conditions



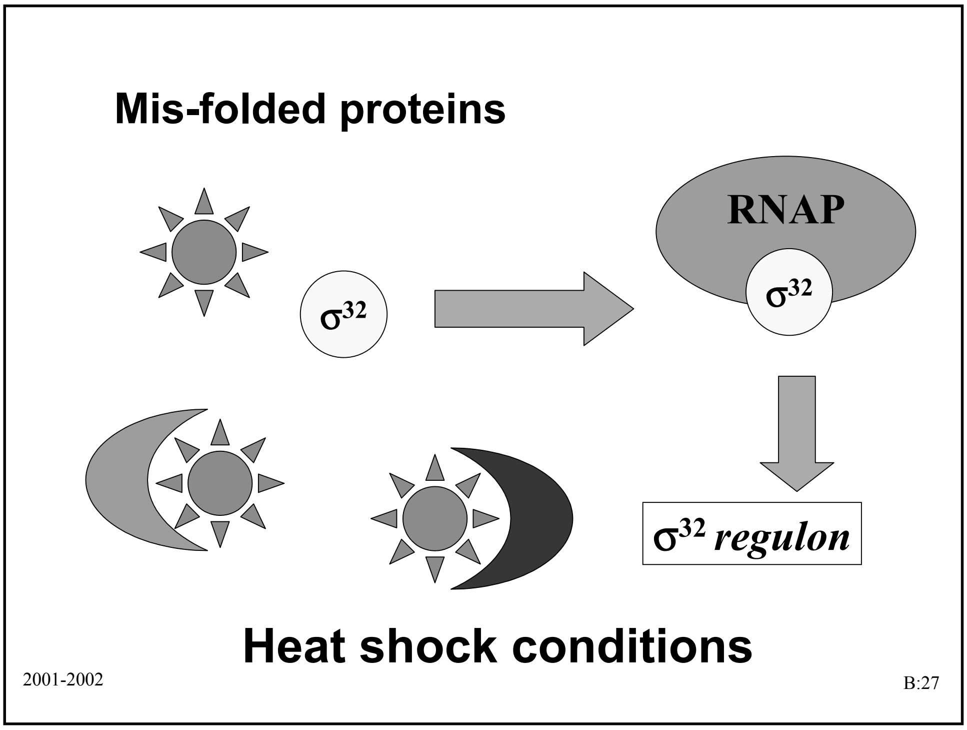
Heat shock conditions

Activation of RpoH

- Also activated in presence of mis-folded proteins
- RpoH in cytoplasm
- With normal proteins, DnaJ&K bind to RpoH
  - DnaJ-K-RpoH complex degraded by FtsH
- With mis-folded proteins, DnaK&J not bind to RpoH
  - DnaJ&K bind to mis-folded proteins
  - RpoH not degraded
  - RpoH bind to RNAP & regulon promoters

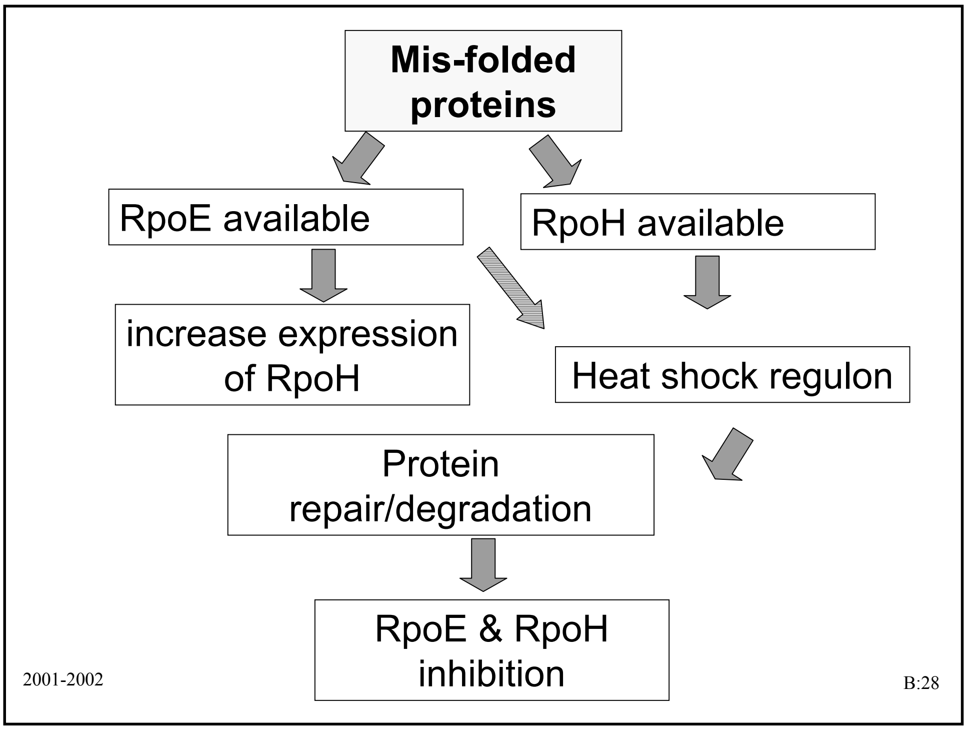


Normal conditions



Mis-folded proteins

Heat shock conditions



Mis-folded proteins

RpoE available

RpoH available

increase expression of RpoH

Heat shock regulon

Protein repair/degradation

RpoE & RpoH inhibition

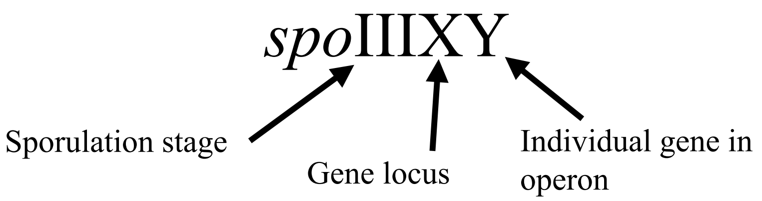
Sigma Factors in sporulation (*Bacillus subtilis*)

- A good example of:
  - specific sigma-specific regulons,
  - regulatory cascades
  - the interaction of regulators from different families.
- Expect understanding of the regulatory cascade (not developmental steps)

Sporulation by *Bacillus subtilis*

- Adaptation to starvation
  - Production of resistant spore and programmed mother cell death
- two cells with identical genomes initiate different programmes of gene expression
  - germ line and terminally differentiated cells
- important concept in developmental biology
- one of the most studied and best understood developmental systems
- two important aspects
  - *genetic control mechansims*
  - biochemical and morphological changes

- Nutrient replete conditions: x2 in size and divide into two identical cells- *vegetative cell growth*
- Nutrient limited conditions: developmental switch leading asymmetric division, death of one cell and production of tough dormant *endospore*
- sporulation process classified into stages
- gene nomenclature confusing
- early vs late id'ed spo genes
- ger, cot & others already named



*spo*IIIXY

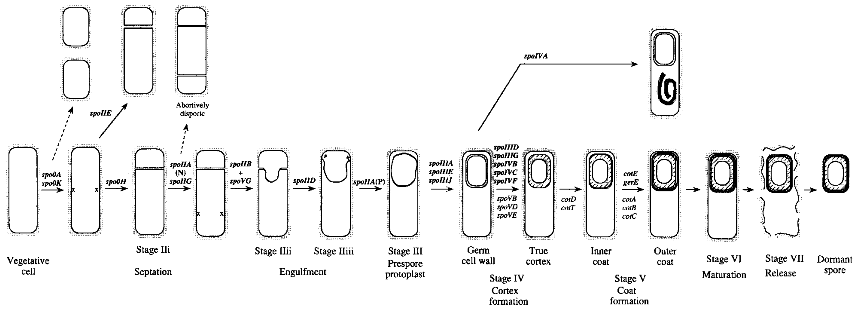
Sporulation stage

Gene locus

Individual gene in operon

2001-2002

Sporulation pathway



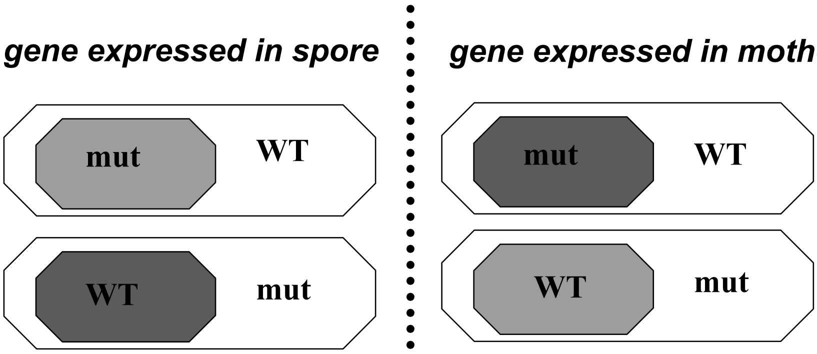
2001-2002

Techniques and approaches

- mutant phenotype itself
- lacZ gene fusions & mRNA levels
- when expressed?
- what regulate?   Regulon
- transcription vs translational reg
- product localisation
  - fractionation (difficult later satges)
  - ImmunoEM
    - Ab vs specific protein
    - Ab vs LacZ
  - compartmentalisation

Mosaic strains

- method to put wt & mutant chromo in sporulating cell
- Is spore heat resistant?



gene expressed in spore

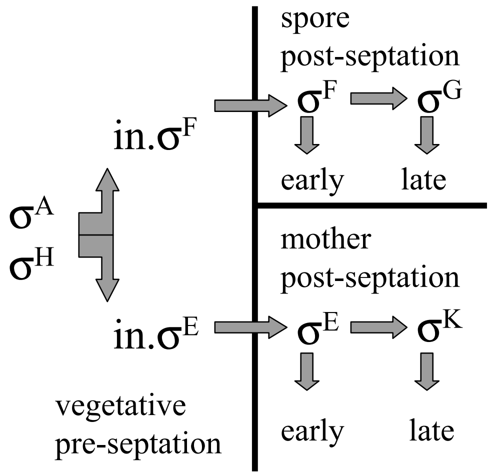
gene expressed in mother

red= heat resistant spore

Sigma cascade

- Most important regulatory system in spore development is changing sigma factors
- confer new promoter specificity
  - -35 and -10 sites
- wrt sporogenesis:
  - major veg: sigma A (=70)
  - minor veg: sigma H
  - major spore: sigma E
    sigma F
    sigma G
    sigma K
- How determine regulon of each sigma?
  - promoter sequence
  - *in vitro* transcription with pure RNApol + sigma
  - promoter fusions

σ controlled regulatory cascades

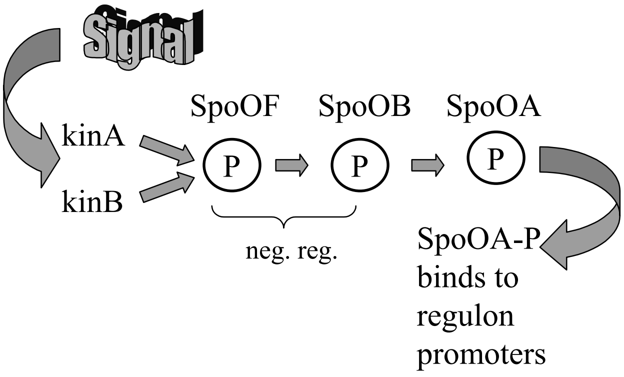


- •sigma factors form major regulatory control
- •other regulators control subsets of genes
  - •fine tune temporal regulation
- •Hierarchical regulatory cascade
- •compartmentalization
- •not describe morphogenesis and structure

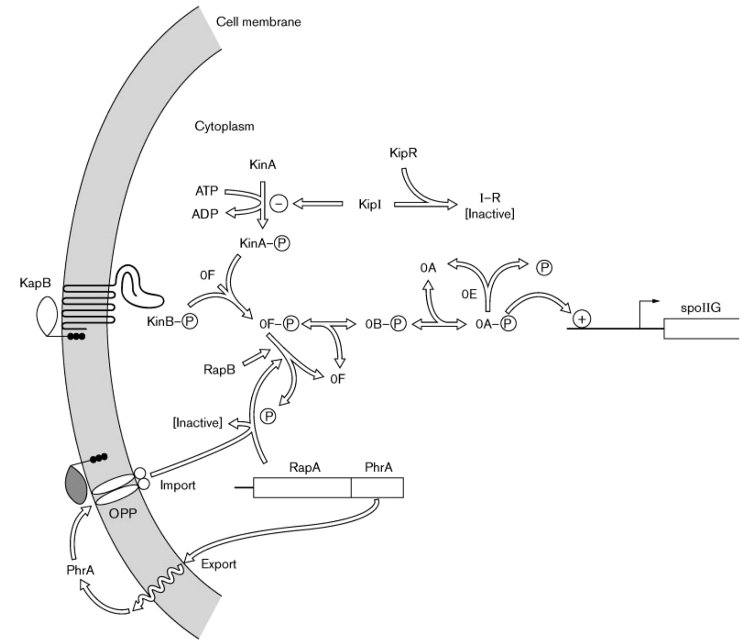
To sporulate or not to sporulate, that is the question

- What signals?
  - Nutrient availability
    - C, N & P levels
    - lead to change in GTP/GDP levels?
    - how connected to regulators?
  - Population density
    - pheromone oligopeptide concentration- quorum sensing
  - Stage of cell division
    - needs to be at certain stage
- sigmaH
  - transcription of gene (*spoOH*) in stationary phase. sigmaA promoter
  - also involved in other stationary phase responses (competence)
  - Most sigmaH activity after sporulation start (posttranscriptional control?)

- SpoOA phosphorelay system
- crucial role in initiation of sporulation
- first from sigmaA promoter then from a sigmaH promoter
- two component regulatory system
  - similar but more complex than paradigm of TCR like OmpR/EnvZ
- controls many genes, in particular,
  - *spoIIA* sigma F
  - *spoIIG* sigma E



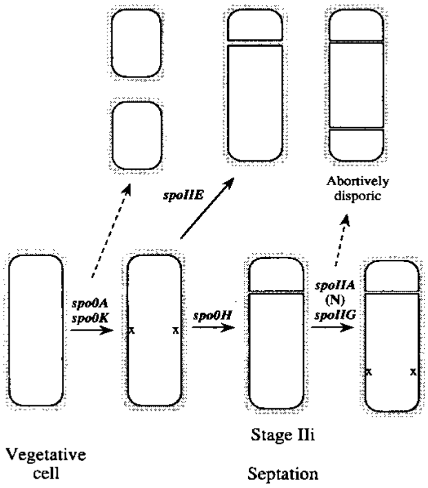
SpoOA-P binds to regulon promoters



Current Opinion in Microbiology

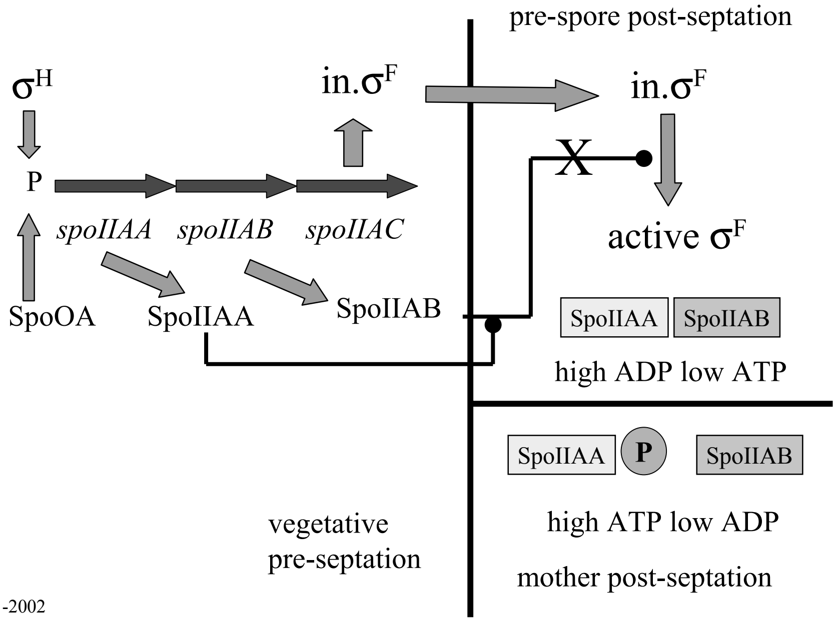
Getting things started

- Genes regulated by spoOA etc. responsible for formation of asymmetric septum
- both cells contain a chromosome
- can now compartmentalize gene expression
- SpoOA-P activate expression of
  - sigmaF (spoIIAC)
  - sigmaE (spoIIGB)
  - before septation but in inactive form
  - responsible for first level of spore specific and mother cell specific gene expression



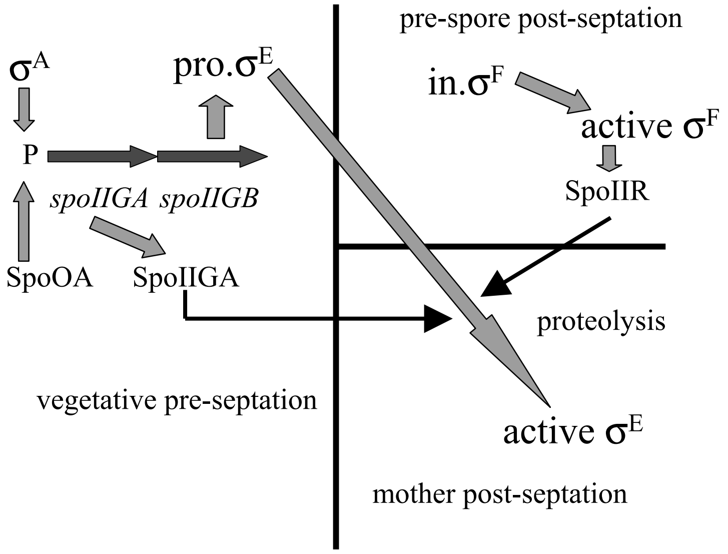
Compartments

- Activation of sigmaE and sigmaF dependant on septum
- differentiates between compartments. How?
  - ATP:ADP ratio
  - spore: ATP low, ADP high
  - mother: ATP high, ADP low
- ATP:ADP ratio affects binding of SpoIIAA to SpoIIAB
- In spore: low ATP
  - low SpoIIAA-P
  - high SpoIIAA-SpoIIAB
  - free in.sigmaF
- In mother: high ATP
  - high SpoIIAA-P,
  - low SpoIIAA-SpoIIAB
  - promotes in.sigmaF:SpoIIAB



Co-ordinating the sigma cascades

- conversion of pro-sigmaE to active sigma E depends on sigmaF controlled gene product
- Activation of sigma F leads to transcription of spoIIR gene
- SpoIIR protein activates SpoIIGA protease
- protease chops off part of pro-sigmaE to leave active sigmaE
- SpoIIR must enter mother cell. Therefore involves septum
- Important to co-ordinate spore and mother-specific events because:
  - functional spore requires mother cell input
  - if spore not finish programme but mother start then both cells die



pre-spore post-septation

σ^A

pro.σ^E

in.σ^F

active σ^F

P

spoIIGA *spoIIGB*

SpoIIR

SpoOA

SpoIIGA

proteolysis

vegetative pre-septation

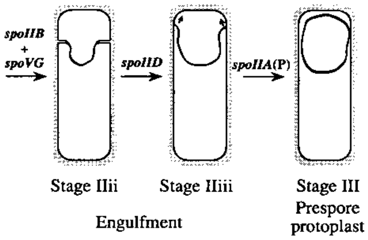
active σ^E

mother post-septation

Next stage: II to III

- When septum made Mother cell engulfs spore
- Cell wall between mother and pre-spore broken down
- predominantly controlled by sigmaE

- Not just sigma E
- temporal control in period when sigma E active involves action of other regulator
- SpoIIID
  - small protein with HTH
  - transcription sigmaE controlled
  - autoregulated by SpoIIID itself
  - repressor and activator
- Early sigmaE genes repressed by SpoIIID
- late sigmaE genes activated by SpoIIID

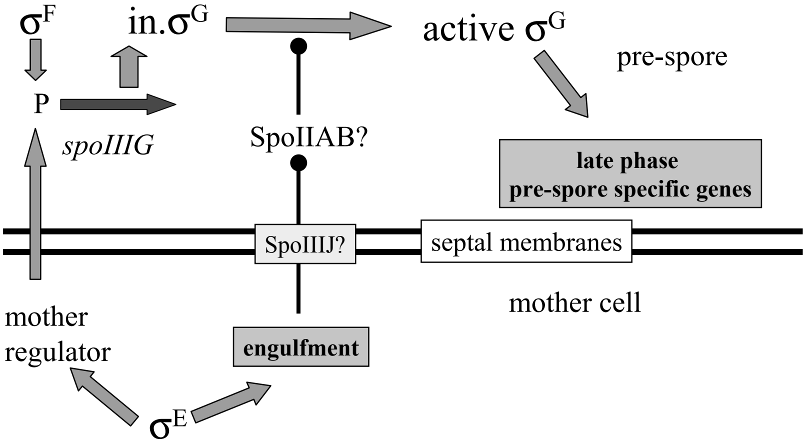


Spore cascade

- In pre-spore not many sigmaF controlled genes identified
- SigmaF does control transcription of gene encoding another sigma factor
- SigmaG (gene spoIIIG)

- SigmaG produced in inactive form
- signals derived from mother cell control conversion to active form
- via SpoIIAB system again?
- Perhaps inactive form so inhibit production of sigmaG until structural event occurred?
- engulfment?

- production of active sigmaG results in many spore specific genes being transcribed. For example:
  - resistance to UV
  - nutrient storage
  - germination proteins to sense when to germinate and initiate germination



σ^F

in.σ^G

active σ^G

pre-spore

P

spoIIIG

SpoIIAB?

late phase pre-spore specific genes

SpoIIIJ?

septal membranes

mother cell

mother regulator

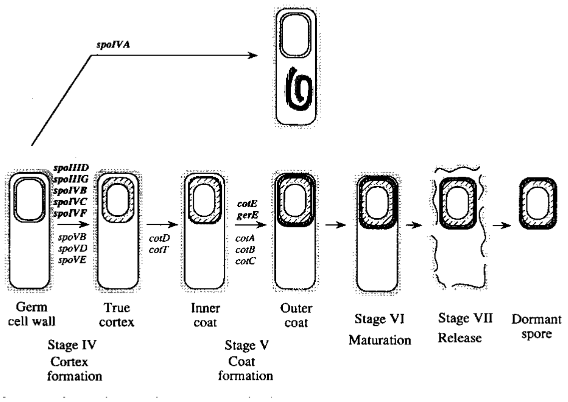
engulfment

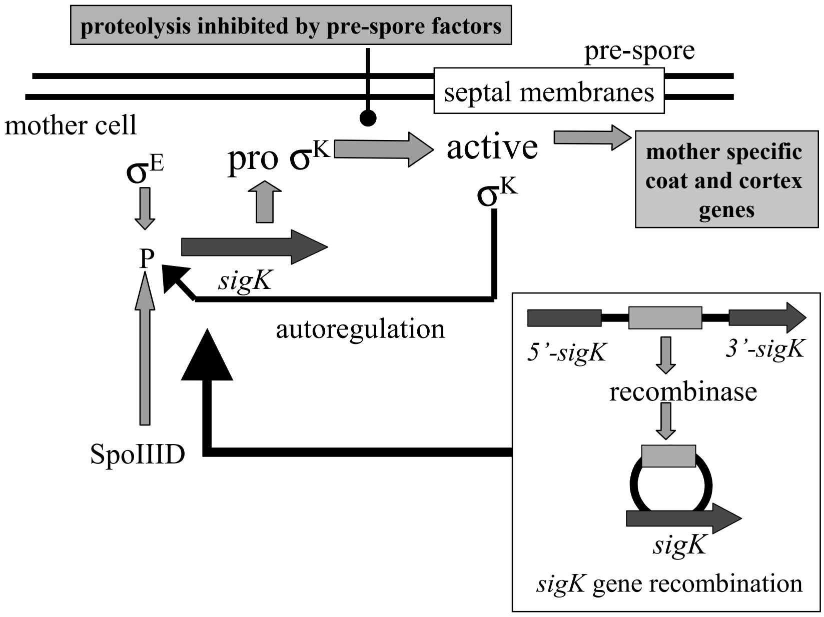
σ^E

Mum's job is nearly done….

- Another regulatory cascade initiated in mother cell
- sigmaE directs transcription of gene encoding sigmaK (*sigK*)
- unusual gene as requires recombination to be expressed properly

- sigmaK transcription factor for genes involved in sealing spore so important that not expressed too early
- spore coat and spore cortex genes





proteolysis inhibited by pre-spore factors

pre-spore

septal membranes

mother cell

σ^E

pro σ^K

active σ^K

mother specific coat and cortex genes

P

sigK

autoregulation

SpoIIID

5'-sigK

3'-sigK

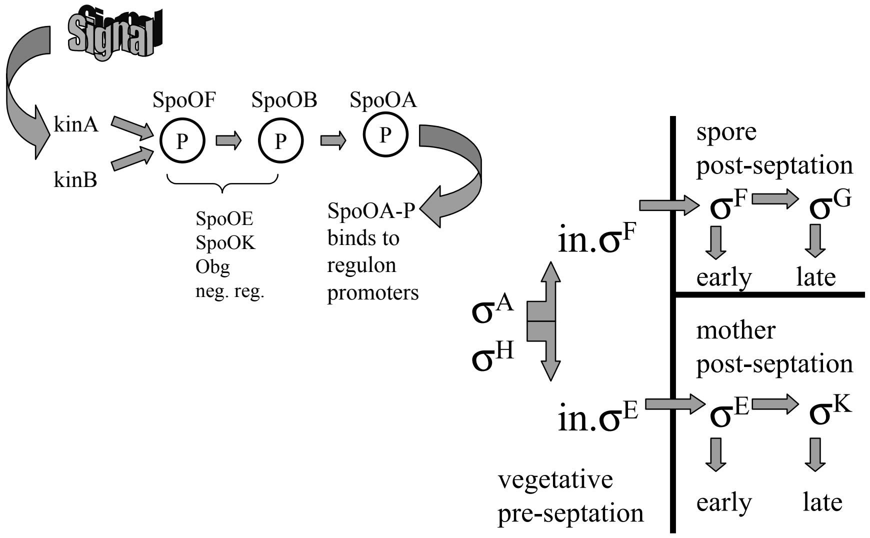
recombinase

sigK

sigK gene recombination

Complex development regulatory pathway

- Two cells with identical genomes initiate different programmes of gene expression
- results in different developmental pathways
- germ cell-like dormant spore
- terminally differentiated mother cell
- complex regulatory cascades to control gene expression with respect to time and position
- Network of different regulatory systems and strategies



Signal

kinA

kinB

SpoOF

SpoOB

SpoOA

P

P

P

SpoOE
SpoOK
Obg
neg. reg.

SpoOA-P
binds to
regulon
promoters

σ^A

σ^H

in.σ^F

in.σ^E

vegetative
pre-septation

spore
post-septation

σ^F → σ^G

early late

mother
post-septation

σ^E → σ^K

early late

Summary II

- Characteristics of Transcriptional regulation
- Aspects of promoter control –reminders
- Regulatory pathways –associated descriptive terms
- Families of bacterial transcriptional regulators

- Sigma Factors –groups and functional roles
- The σ^{32} and σ^{24} system
- The sporulation cascade