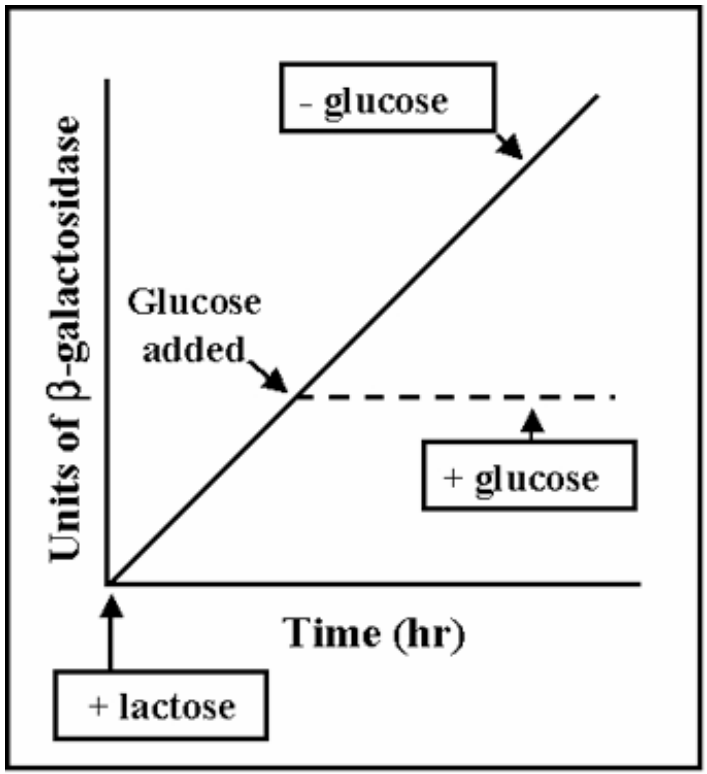
When times are good and when times are bad:

Stringent response
Stationary phase

Reading
Chapter 13 p,571-572, 573-579, 580-581, 582-584, 554-556,, 598-602

Example of catabolite control



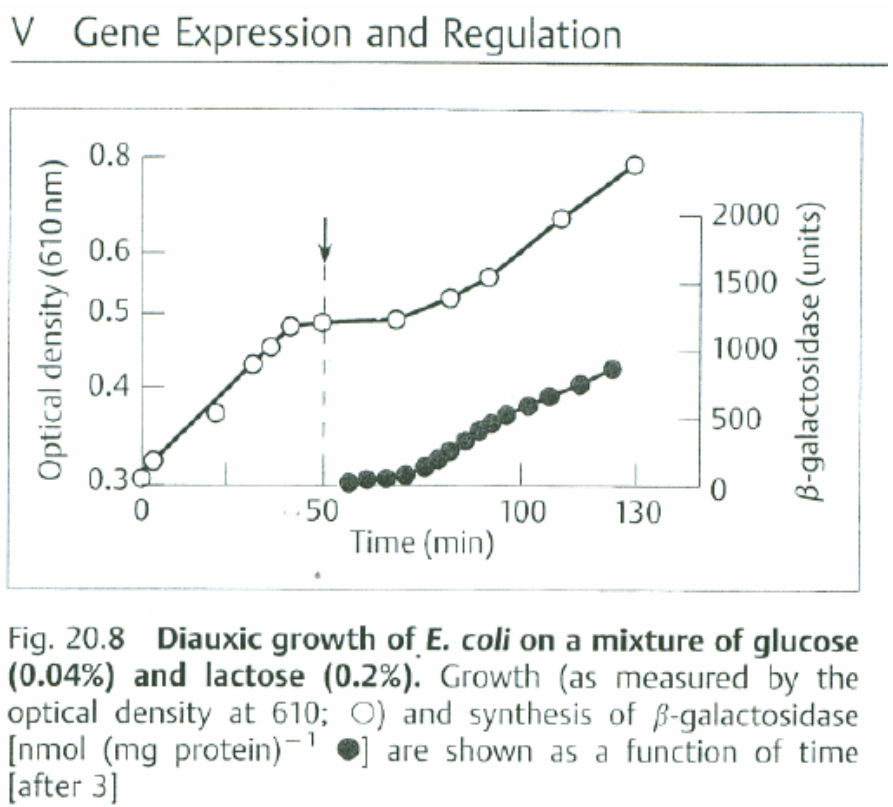


Fig. 20.8 **Diauxic growth of *E. coli* on a mixture of glucose (0.04%) and lactose (0.2%).** Growth (as measured by the optical density at 610; ○) and synthesis of β-galactosidase [nmol (mg protein)$^{-1}$ ●] are shown as a function of time [after 3]

Cells shifted from one medium to another have profound changes in overall metabolism.
These changes are brought about by global changes in the availability to synthesize the machinery for protein synthesis.

Table 13.1

TABLE 13.1 A sampling of *E. coli* global regulatory systems

System	Response	Regulatory gene(s) (protein[s])	Category of mechanism	Some genes, operons, regulons, and stimulons
Nutrient limitation				
Carbon	Catabolite repression	*crp* (CAP, also called CRP)	DNA-binding activator or repressor	*lac, ara, gal, mal,* and numerous other C source operons
	Control of fermentative vs. oxidative metabolism	*cra* (*fruR*) (CRA)	DNA-binding activator or repressor	Enzymes of glycolysis, Krebs cycle
Nitrogen	Response to ammonia limitation	*rpoN* (NtrA)	Sigma factor (σ^{54})	*glnA* (GS) and operons for amino acid degradation
		ntrBC (NtrBC)	Two-component system	
Phosphorus	Starvation for inorganic orthophosphate (P_i)	*phoBR* (PhoBR)	Two-component system	>38 genes, including *phoA* (bacterial alkaline phosphatase) and *pst* operon (P_i uptake)
Growth limitation				
Stringent response	Response to lack of sufficient aminoacylated-tRNAs for protein synthesis	*relA* (RelA), *spoT* (SpoT)	(p)ppGpp metabolism	rRNA, tRNA, ribosomal proteins
Stationary phase	Switch to maintenance metabolism and stress protection	*rpoS* (RpoS)	Sigma factor (σ^S)	Many genes with σ^S promoters; complex effects on many operons
Oxygen	Response to anaerobic environment	*fnr* (Fnr)	CAP family of DNA-binding proteins	>31 transcripts, including *narGHJI* (nitrate reductase)
	Response to presence of oxygen	*arcAB* (ArcAB)	Two-component system	>20 genes, including *cob* (cobalamin synthesis)
Stress				
Osmoregulation	Response to abrupt osmotic upshift	*kdpDE* (KdpD, KdpE)	Two-component system	*kdpFABC* (K^+ uptake system)
	Adjustment to osmotic environment	*envZ*/*ompR* (EnvZ/OmpR)	Two-component system	OmpC and OmpF outer membrane proteins
		micF	Antisense RNA	*ompF* (porin)
Oxygen stress	Protection against reactive oxygen species	*soxS* (SoxS)	AraC family of DNA-binding proteins	Regulon, including *sodA* (superoxide dismutase) and *micF* (antisense RNA regulator of *ompF*)
		oxyR (OxyR)	LysR family of DNA-binding proteins	Regulon, including *katG* (catalase)
Heat shock	Tolerance of abrupt temperature increase	*rpoH* (RpoH)	Sigma factor (σ^{32})	Stimulon, Hsps (heat shock proteins), including *dnaK, dnaJ,* and *grpE* (chaperones), and *lon, clpP, clpX,* and *hflB* (proteases)
Envelope stress	Misfolded Omp proteins	*rpoE* (RpoE)	Sigma factor (σ^E)	>10 genes, including *rpoH* (σ^{32}) and *degP* (encoding a periplasmic protease)
	Misfolded pilus	*cpxAR* (CpxAR)	Two-component system	Overlap with RpoE regulon
pH shock	Tolerance of acidic environment	Many	Many	Complex stimulon

Stringent Control Definition:
the coupling of rRNA and tRNA synthesis and levels during amino acid and nutrient starvation.

1. Ribosome and tRNA synthesis controlled by amino acid levels.
2. rRNA and tRNA levels also controlled by transcription rate which is also constrolled by translation rate-coupled.
3. Ribosome level is also controlled by growth rate and growth phase: log vs stationary phase.

Why bother?

1. Provides the cell with an efficient method to regulate the most abundant molecules in the cell
2. Upregulates genes encoding metabolic enzymes, especially those needed for amino acid biosynthesis
3. Shuts off synthesis of pathways utilized during growth phase

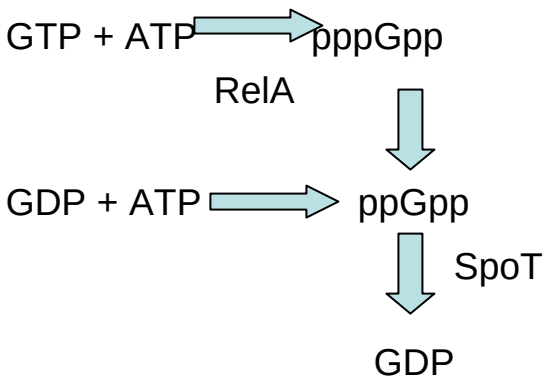
Stringent Response: Mechanisms

1. When cells are in a condition where there is an insufficient supply of amino acids to sustain protein, the stringent response is activated.
2. Stringent response causes a 10-20x reduction in the synthesis of fRNA and tRNA. This causes a reduction of about 10% of the mRNA in the cell.
3. Protein degradation is increased: Why? (hint amino acids can be food)
4. Reduction in membrane lipid, carbohydrate, and nucleotides: ie the cells are not going to be dividing.

The stringent response is accompanied by the increase of the alarmones ppGpp and pppGpp: guanosine tetraphosphate with diphosphates attached to the 5' and 3' ends of guanosin. Also guanosine pentaphosphate with a 5' triphosphate group and 3' diphosphate.

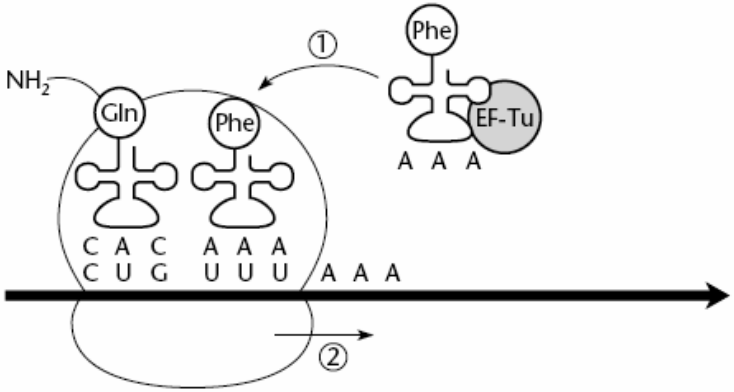
Collectively, these are known as (p)ppGpp.

Synthesis of pppGpp



RelA: ppGpp synthetase
SpoT: degrades ppGpp

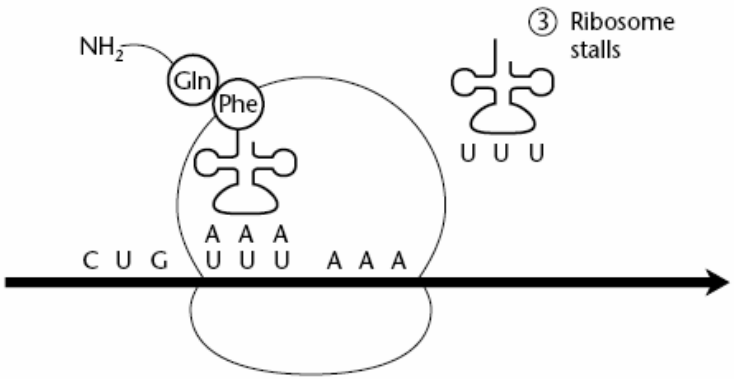
-About 5% of the ribosomes in the cell has RelA attached.
-RelA is activated when an uncharged tRNA enters the A site
-Ribosome idles on the mRNA waiting for a charged tRNA.
-RelA and ppGpp function to dislodge the uncharged tRNA.
-Ribosomal protein L11 in the 50s subunit is near the A-site.
-Change in conformation of the uncharged A site is transferred to the L11 protein and activates RelA



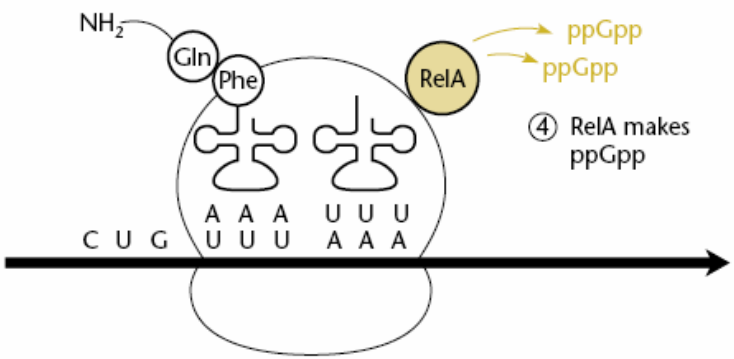
Charged tRNA binds to the A-site in ribosome

Amino acid starvation results in an uncharged tRNA

-deacylated tRNA will bind to the A-site empty



An uncharged tRNA is a signal for RelA binding to the ribosome and synthesizes ppGpp guanosine 3’5’ bisphophate, also pppGpp



ppGpp now interacs with RNA polymerase at key metabolic pathways

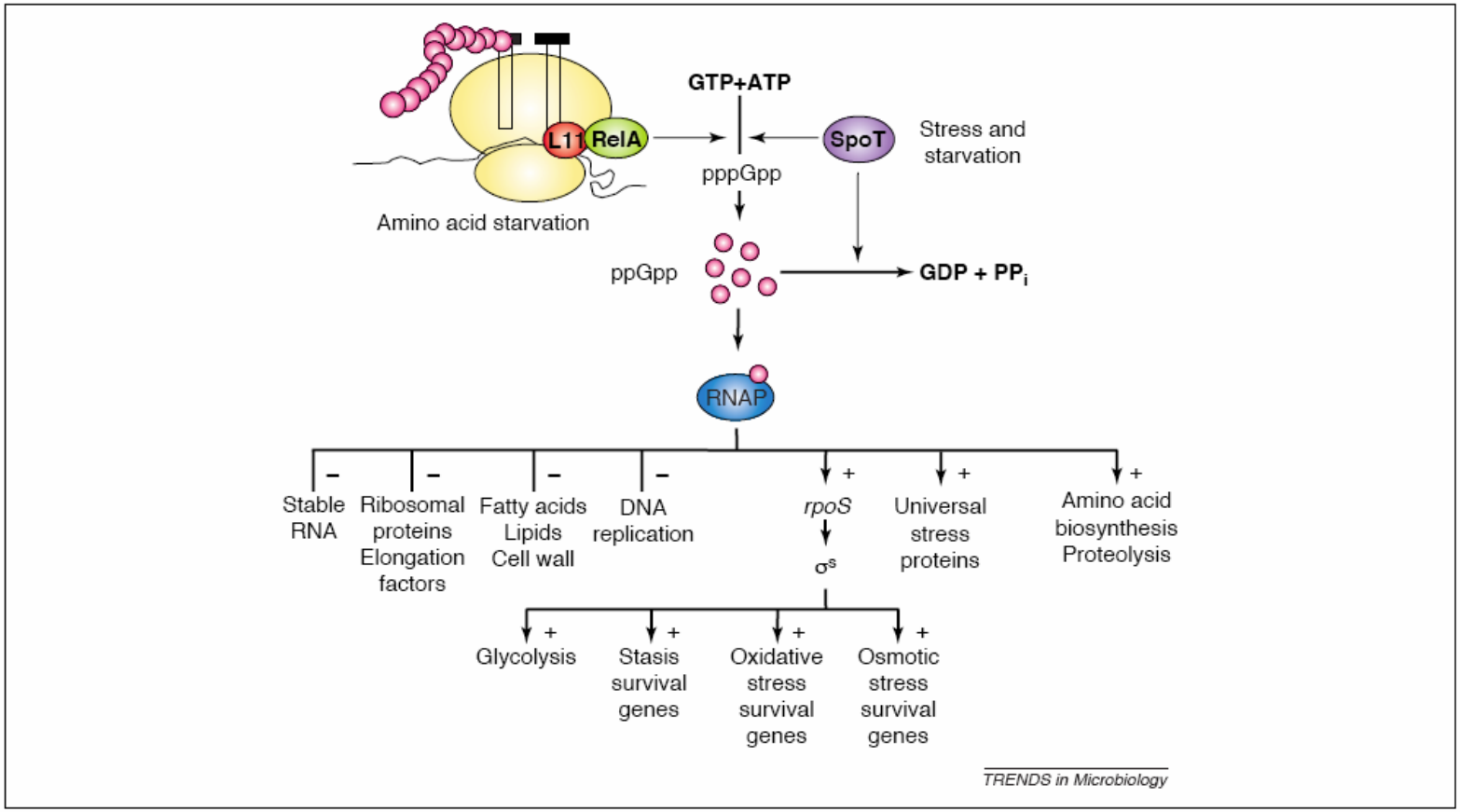


Figure 1. The stringent response. pppGpp is produced from GTP and ATP by two parallel pathways in response to starvation and stress and is subsequently converted to ppGpp [3]. ppGpp binds RNAP and redirects transcription from growth-related genes to genes involved in stress resistance and starvation survival. SpoT is also responsible for hydrolyzing ppGpp.

ppGpp binding to RNAP prevents the complex from opening helix. The ppGpp molecule binds to the site where the polymerase would form the open complex. The mechanism of ppGpp then is to prevent the open complex formation that would lead to transcription.

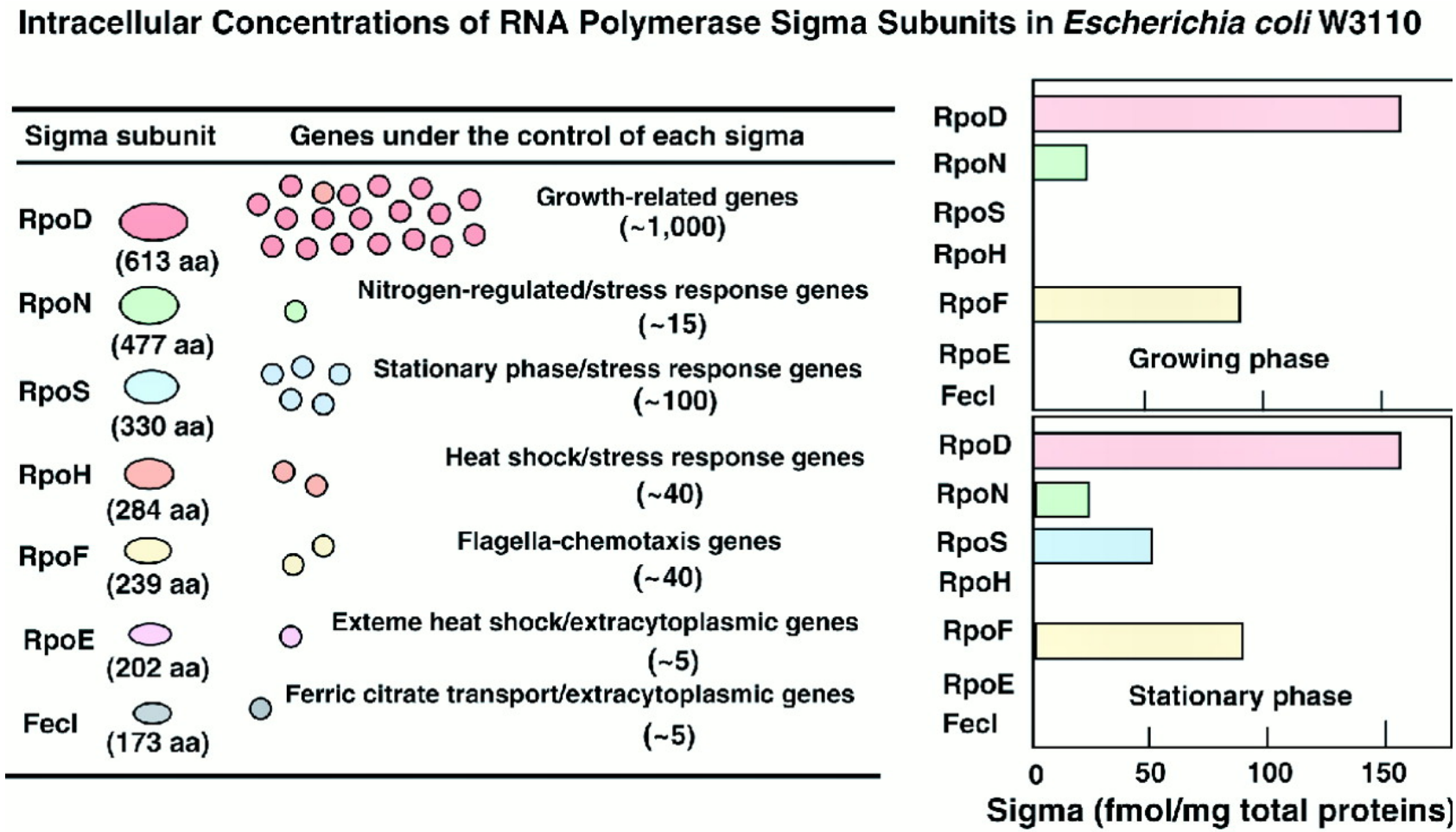
Consequences of *relA* mutation in non-pathogens and pathogens

Table 1. The ppGpp-deficiency phenotype

Phenotype	Mechanism	Organism	Refs
No growth on minimal media without amino acids	Expression of amino acid biosynthesis operons dependent on ppGpp?	*E. coli*	[3]
Filament formation	Possibly due to involvement of ppGpp in cell division	*E. coli*	[3,7]
Decreased survival	Unknown	*E. coli*	[47]
Decreased virulence	Virulence gene expression (SPI I)	*S. typhimurium*	[9,49]
Decreased virulence	Decreased biofilm formation	*E. coli*	[50]
Decreased virulence	Unknown	*P. aeruginosa*	[51]
Decreased expression of σ^S-dependent genes	RpoS depend on ppGpp both for expression and function	*E. coli*	[19,43]
Increased mistranslation during amino acid starvation	Unknown	*E. coli*	[47]
Decreased σ^{32} function	Unknown	*E. coli*	[23,45,46]
Decreased σ^{54} function	Unknown	*E. coli*	[20,23,44]
Failure to induce spherical cell shape	Decreased expression of the σS-dependent bolA gene	*E. coli*	[47]
Lower glycogen content	Unknown	*E. coli*	[55]
Sporulation deficiency	Expression of A-signal is dependent on ppGpp	*Myxococcus xanthus*	[56,57]
Reduced production of antibiotics	Unknown	*S. coelicolor; S. antibioticus*	[56]

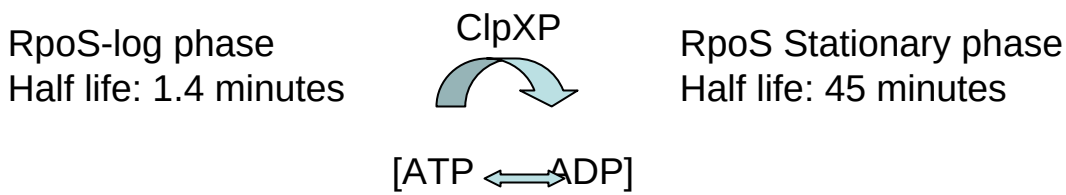
Growth phase vs Stationary phase regulation

Sigma factors control different sets of genes at different phases of growth



Regulation of RpoS levels to control stationary phase genes

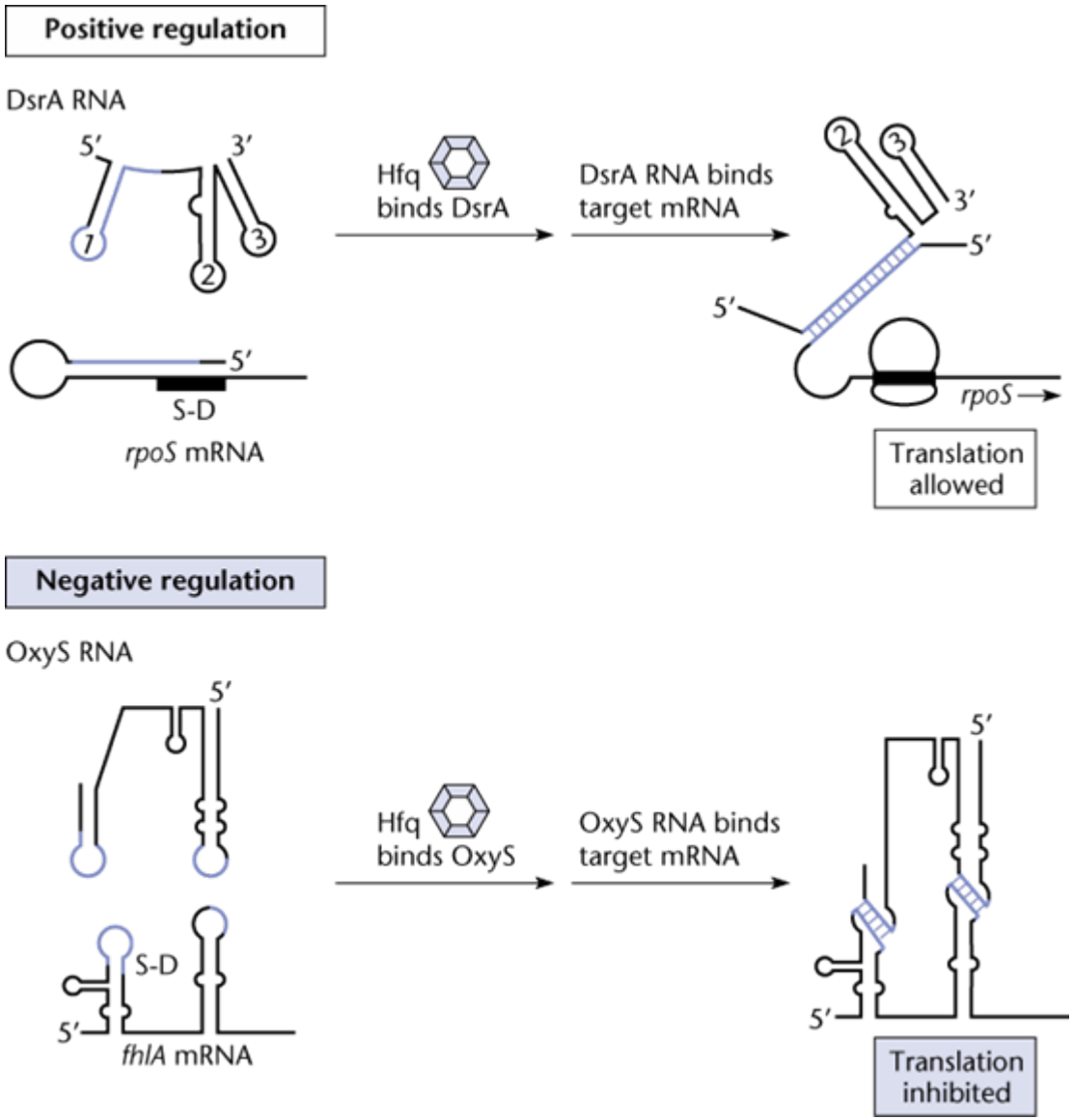
1. Transcription rate in log phase is 10-fold lower than in stationary phase
2. Effects of ClpXP in log vs stationary phase



The activity of the protease is proportional to the amount ATP in the cell
-log phase cells have more
-ClpXP is also needed to degrade misfolded proteins
-DsrA a regulatory RNA is needed for optimal function. This regulatory RNA binds to the ribosome binding region of the *rpoS* mRNA and either blocks or facilitates translation

Box 13.5

Posttranslational regulation of RpoS



Regulatory RNAs
-the function of the regulatory RNA are to affect mRNA translation.
-an inactive mRNA is a target for degradation

-Concentration of RpoS in the cells remains constant during logarithmic phase and increased during stationary phase.
-Yet, RpoS regulated genes are not translated during log phase.
-The regulatory RNAs account for the translational regulation

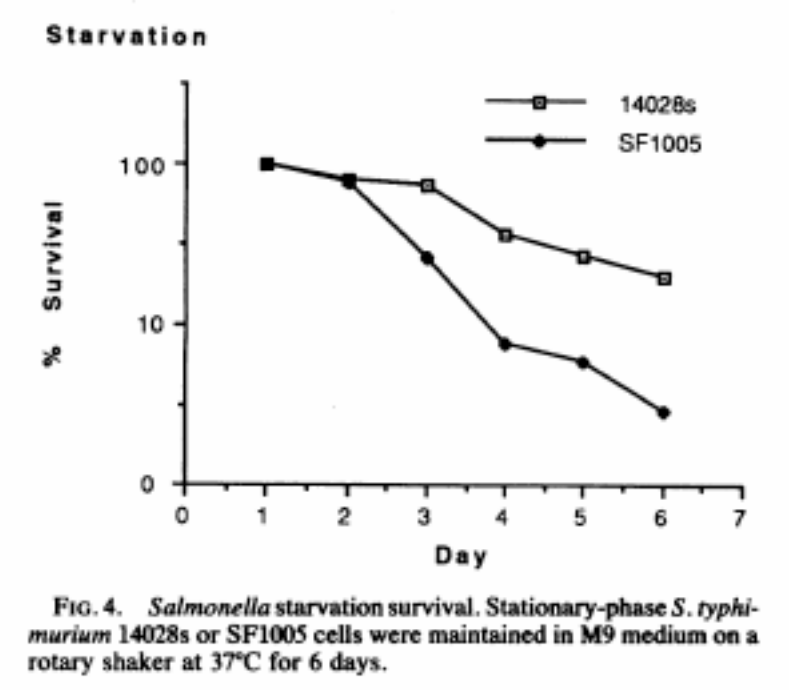
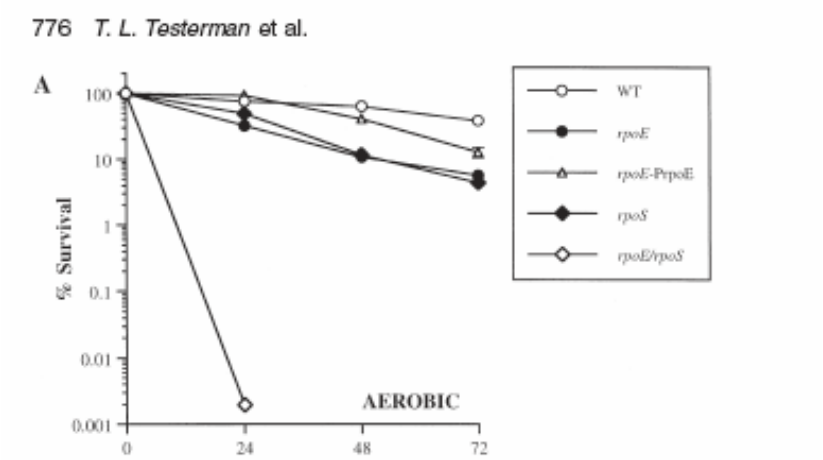


FIG. 4. *Salmonella* starvation survival. Stationary-phase *S. typhimurium* 14028s or SF1005 cells were maintained in M9 medium on a rotary shaker at 37°C for 6 days.

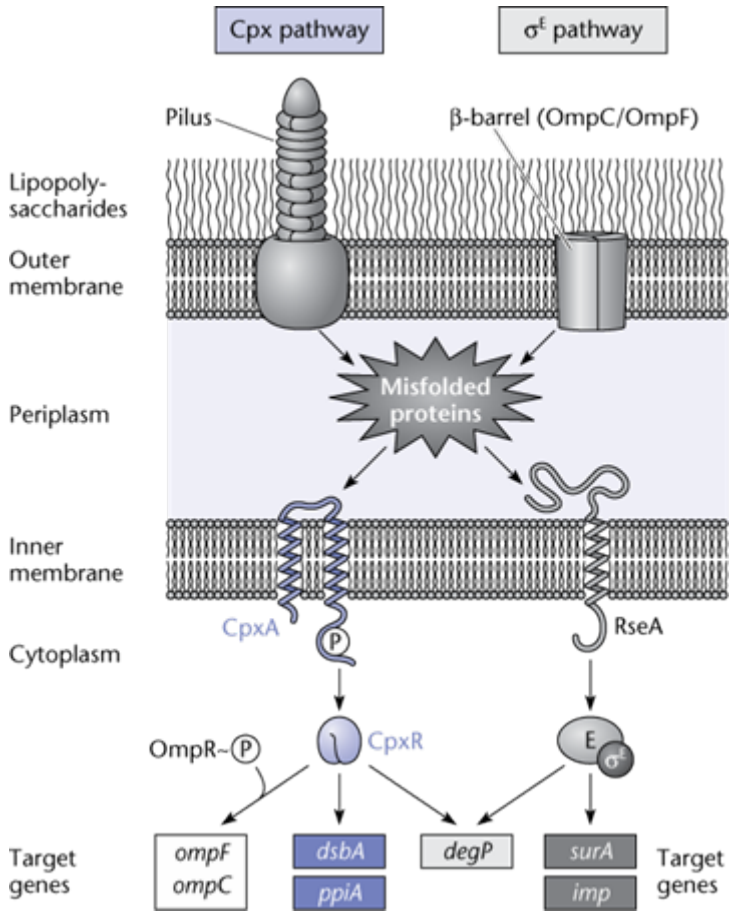


rpoE is needed for stationary phase survival also.

rpoS is needed for stationary phase survival in E.coli and Salmonella
-controls stationary phase gene expression: 10% of genes chromosome
-stationary phase genes are not expressed during log phase
-level of rpoS is controlled by proteolysis in log phase and becomes stable in stationary phase

Figure 13.15

Extracytoplasmic Stress Sensing



RpoE-the other stationary phase-stress sigma factor.

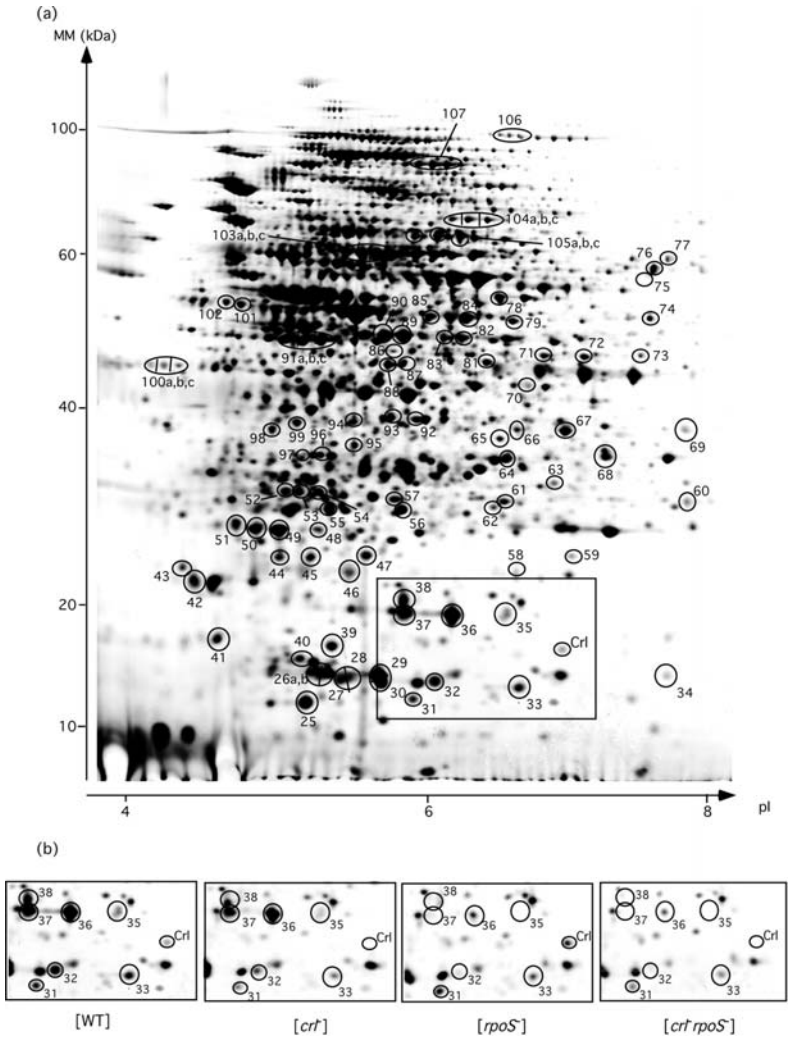
1. RpoE regulated genes needed to respond to periplasmic stress and mis-folded proteins in the periplasm and outer membrane.
2. RpoE is teathered to the inner membrane by a protein called RseA. This is also called an antisigma because it sequesters the sigma factor.
3. When a periplasmic stress is sensed by proteases in the periplasm, RseA is cleaved by DegS in the periplasmd and YaeL degraged the transmembrane domain and the sigma is released.
4. In E.coli, DegS, and YeaL mutants are lethal as are RpoE mutants. Salmonella can tolerate these same mutations.
5. The CpxA-R pathway seems to be specific to pilus assembly and is a method to keep the periplasm from filling with pilin subunits. The CpxA-R is another 2-component pathway.

How to measure global change in gene expression

Transposon mutagenesis with some reporter fusion *lac* or *phoA*
Problems with the method:

1. Lots of colonies are needed to cover the genome~50,000 to be sure.
2. It is difficult to replicate a particular culture environment on a agar plate ie pH, expensive compounds, etc
3. Transposon insertions creates mutants. The insertion mutants could encode regulatory factors that now inactivated alter the screen
4. The transposon may cause unwanted down-stream affects: polarity or regional affects on expression. Transposon insertions may cause changes in expression by transcription out the ends.
5. Proteomics: Examine the proteins being made in a cell by gel electrophoresis. What are the limitations of this method?
6. DNA microarrays.

2D gel electrophoresis of the WT strain



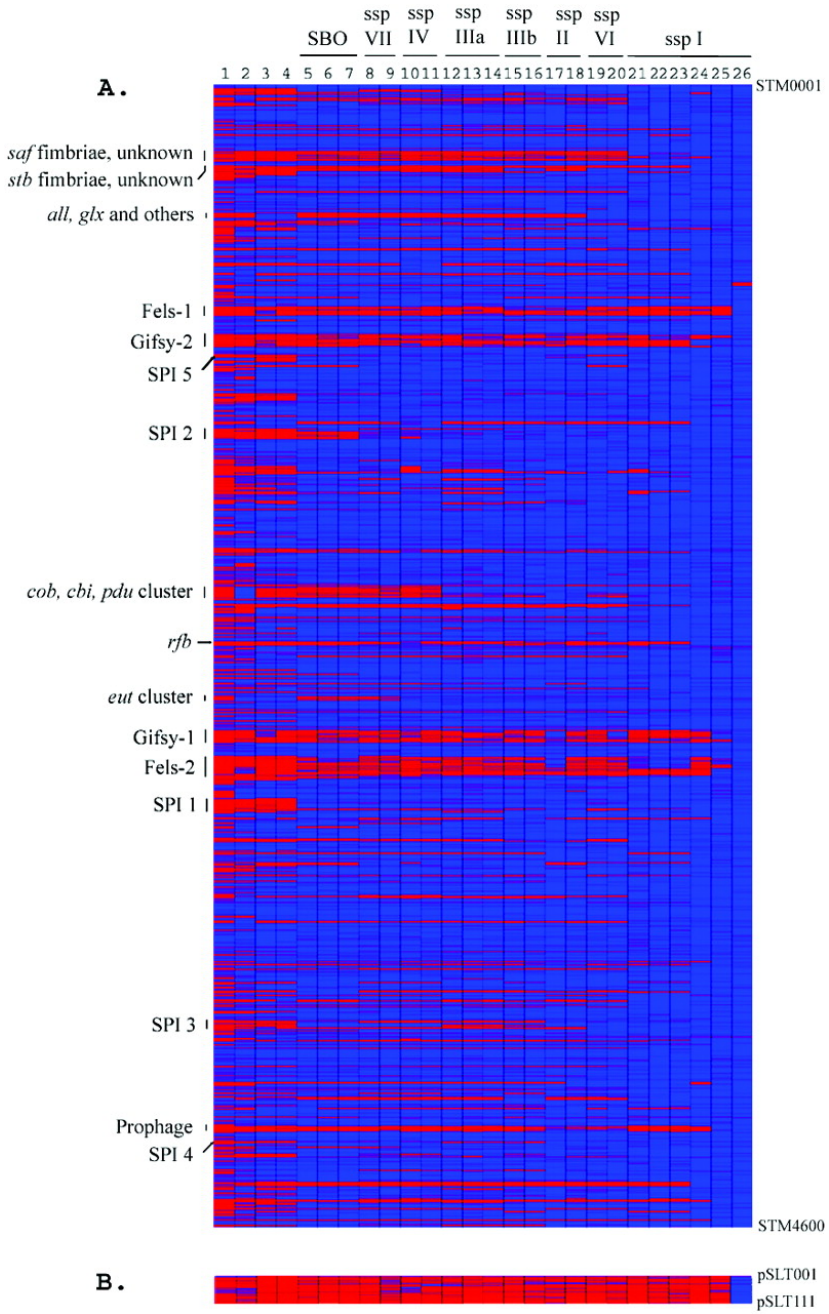
Lelong, C. (2007) Mol. Cell. Proteomics 6: 648-659

1. Grow cells
2. Extract proteins
3. Separate on the basis of charge first
4. Separate on the basis of size secondly

Each spot represents the steady state amount of protein in the cell at the time sampled.

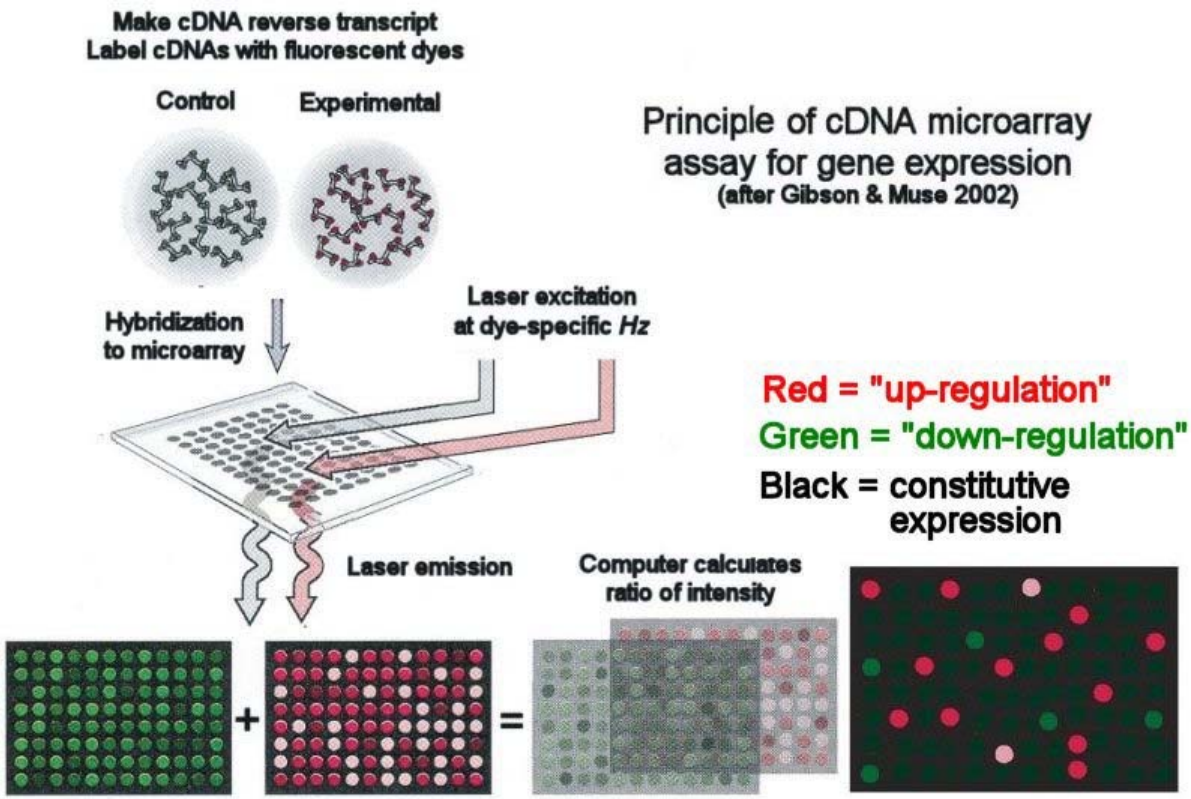
It does not tell you anything about the expression of the gene that encoded it.

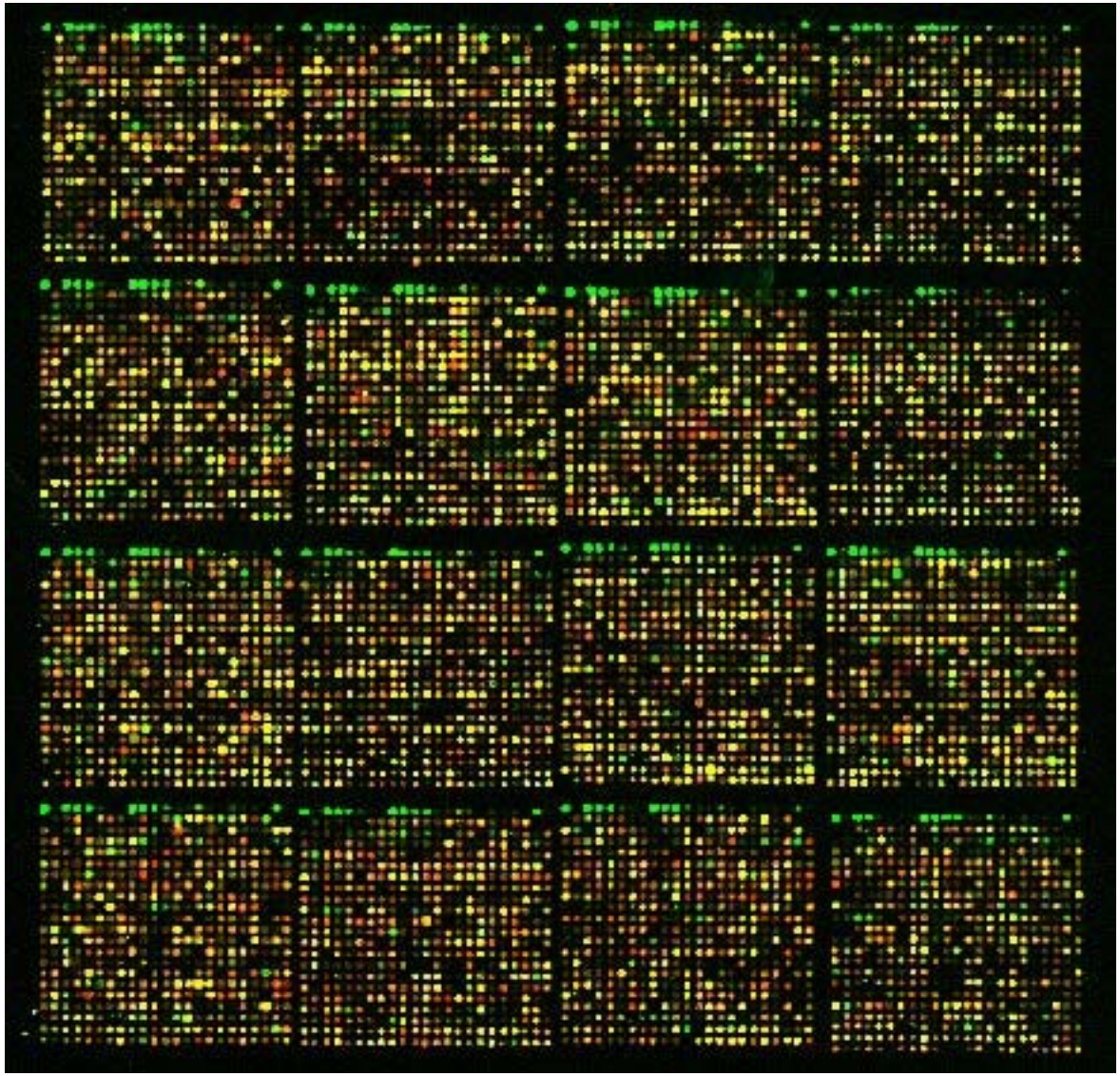
Porwollik, Steffen et al. (2002) Proc. Natl. Acad. Sci. USA 99, 8956-8961



1. DNA microarrays can be used to look at the genomic content of cells and determine genetic make up.
2. In this case, the 7 major groups of Salmonella were examined for genes that are present and those that are absent among the different groups.
3. Red lines are the pathogenicity islands blue are house keeping. What is obvious is that some groups do not have the same sets of “virulence” genes are do others.
4. This kind of analysis would have been done by Southern hybridization in the old days!

Microarray methods





An expression array of an entire chromosome from Salmonella!
More on Wednesday!