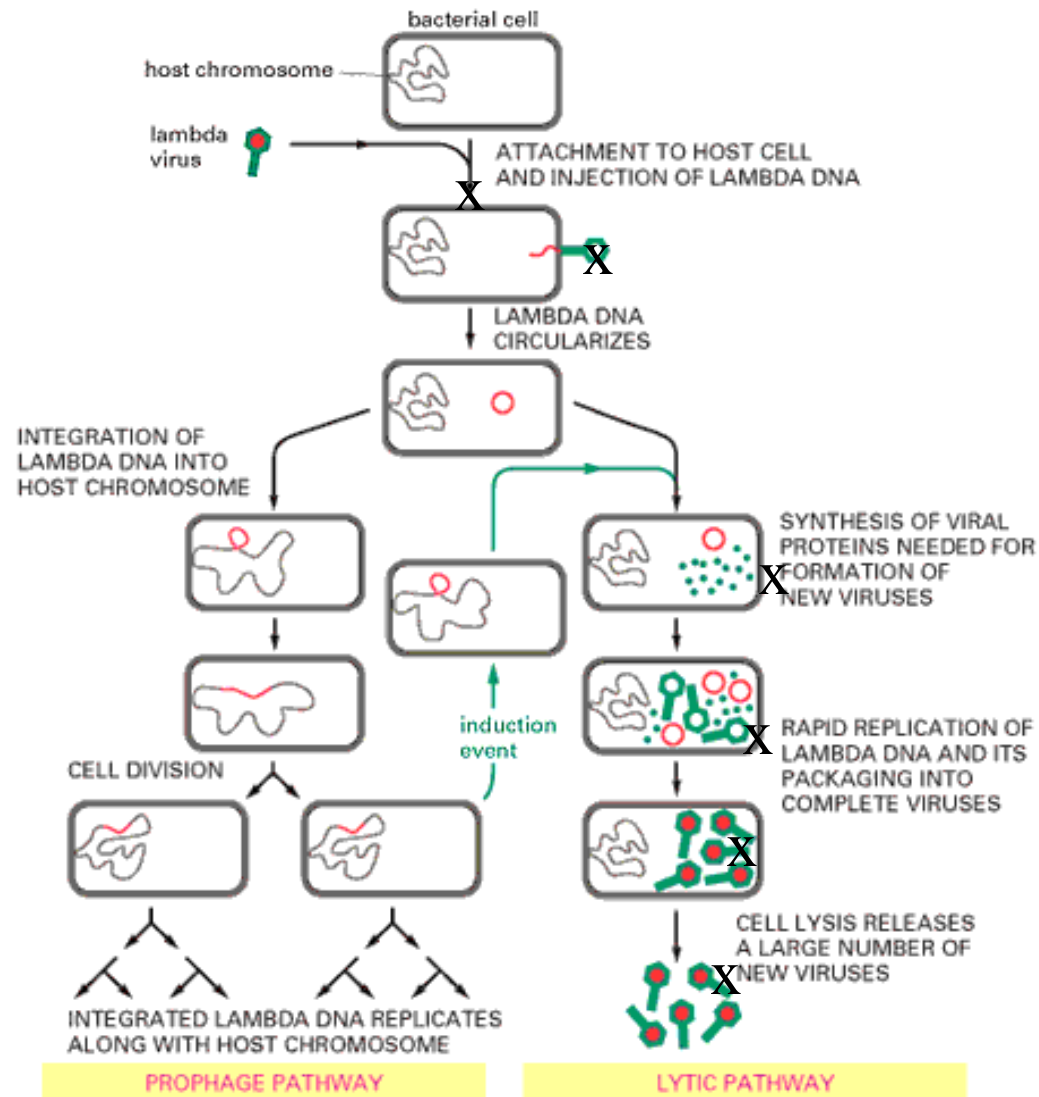


Bacteriophage life cycle



Practices to minimize phage infection

- Strain with resistant mechanisms
 - Adsorption, R/M, abortive infection
- Combination of multiple defense systems
- Bioengineering strains with new approaches
 - Phage-encoded resistance
 - antisense
- Strain rotation

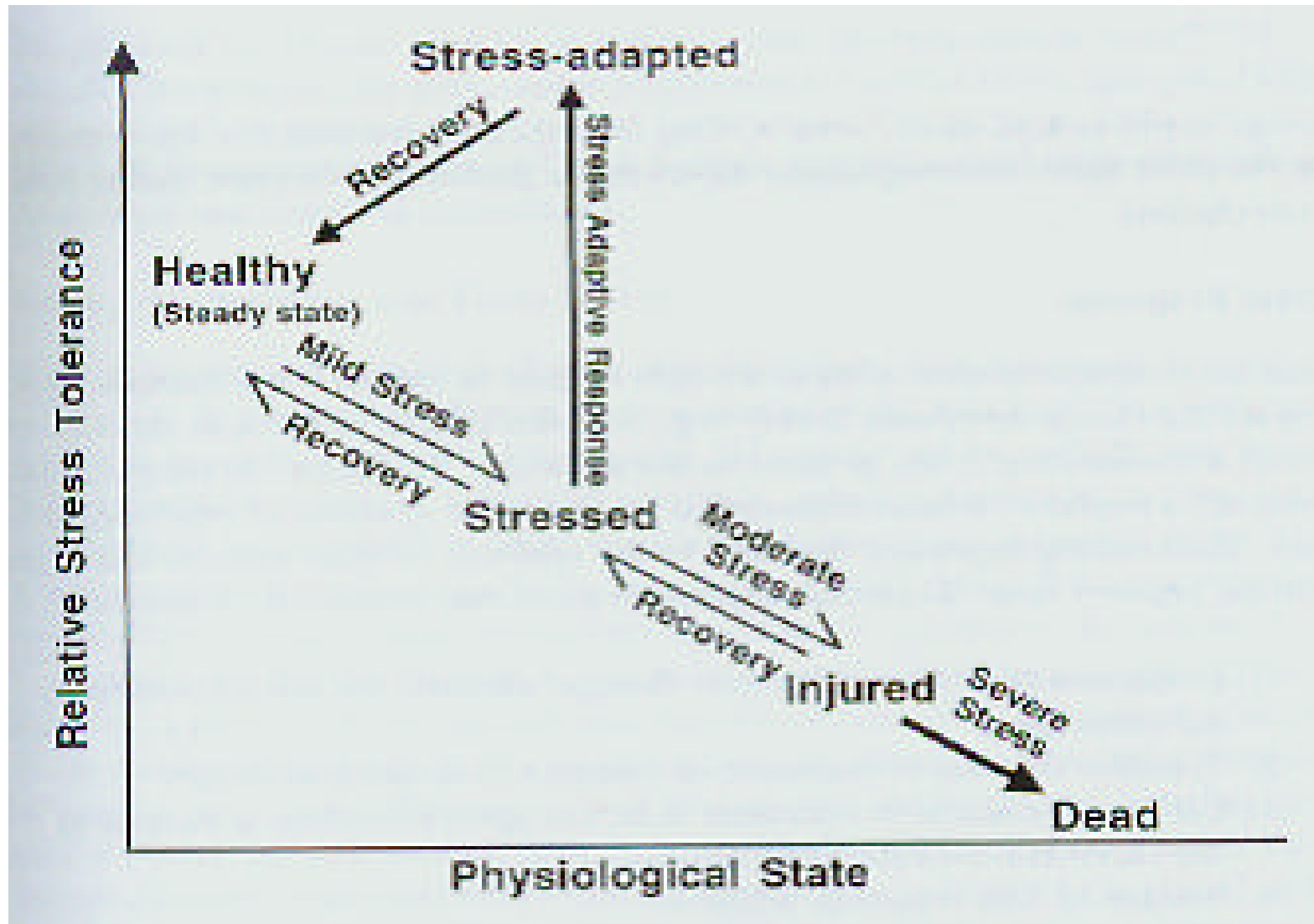
Stress Responses (I)

Definitions

- Stress: any deleterious factor or condition that adversely affects microbial growth or survival.
 - Heat, cold, acid, chemicals, oxidation, etc.
- Stress responses: biological changes following the sense of stress.
 - Production of proteins for repair and protection; transient increase in resistance or tolerance; transformation to a dormant state, etc.

Definitions

- **Adaptation and tolerance:** the stress responses can increase the organism's tolerance to the same or different type of stress, at the same or elevated levels.
 - Heat shock cross-protection; acid adaptation
- **Injury:** damage to cellular components by stresses may impair the ability of microorganisms to multiply or may sensitize the cells to mildly deleterious factors



Proposed interrelations among physiological states of microbial cell subjected to different stresses (Yousef and Courtney, 2003)

The function and regulation of heat
shock proteins in *Escherichia coli*

Heat Shock and Stress Response

- Respond to unfavorable conditions at the cellular level
- Response, survival and protection
- Activate the expression of a small number of specific genes (heat shock genes)
- The expression of most other genes are inhibited
- Heat shock protein groups

Heat shock response

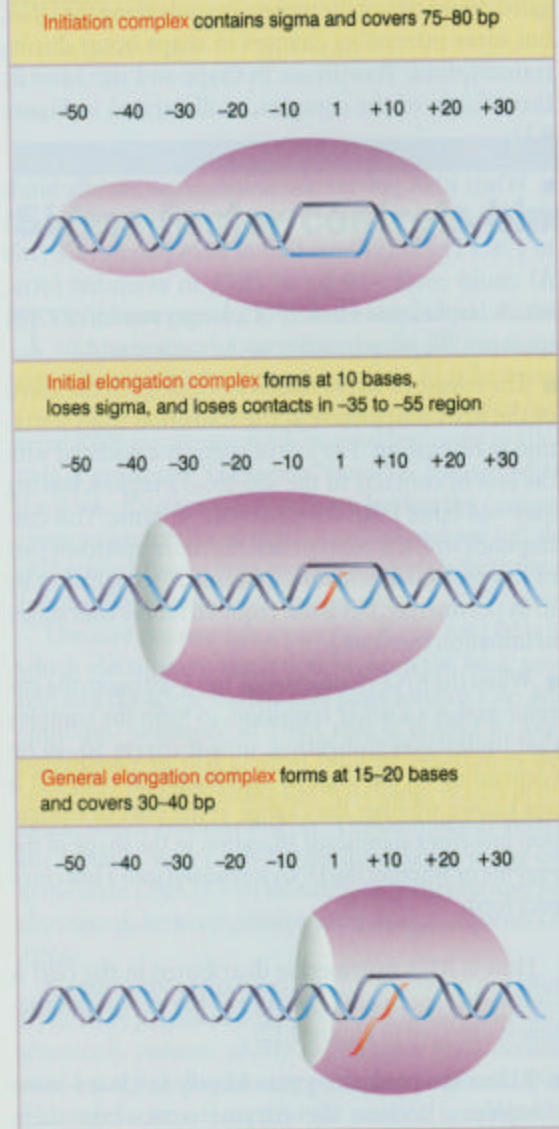
- Multiple copies of these genes are present in eukaryotes
- Only single copy presents in prokaryotes
- These genes are involved in essential cell functions –constitutive expression required
- How to respond to stress?

Transcription (I)

- Catalyzed by RNA polymerase
- Bacterial RNA polymerase (holoenzyme): core enzyme ($\alpha_2\beta\beta'$) + sigma (σ) factor
- Eukaryotic RNA polymerases are multisubunit and depend on various transcription factors
- Transcription unit: DNA between promoter and terminator



Figure 9.11 RNA polymerase initially contacts the region from -55 to +20. When sigma dissociates, the core enzyme contracts to -30; when the enzyme moves a few base pairs, it becomes more compactly organized into the general elongation complex.

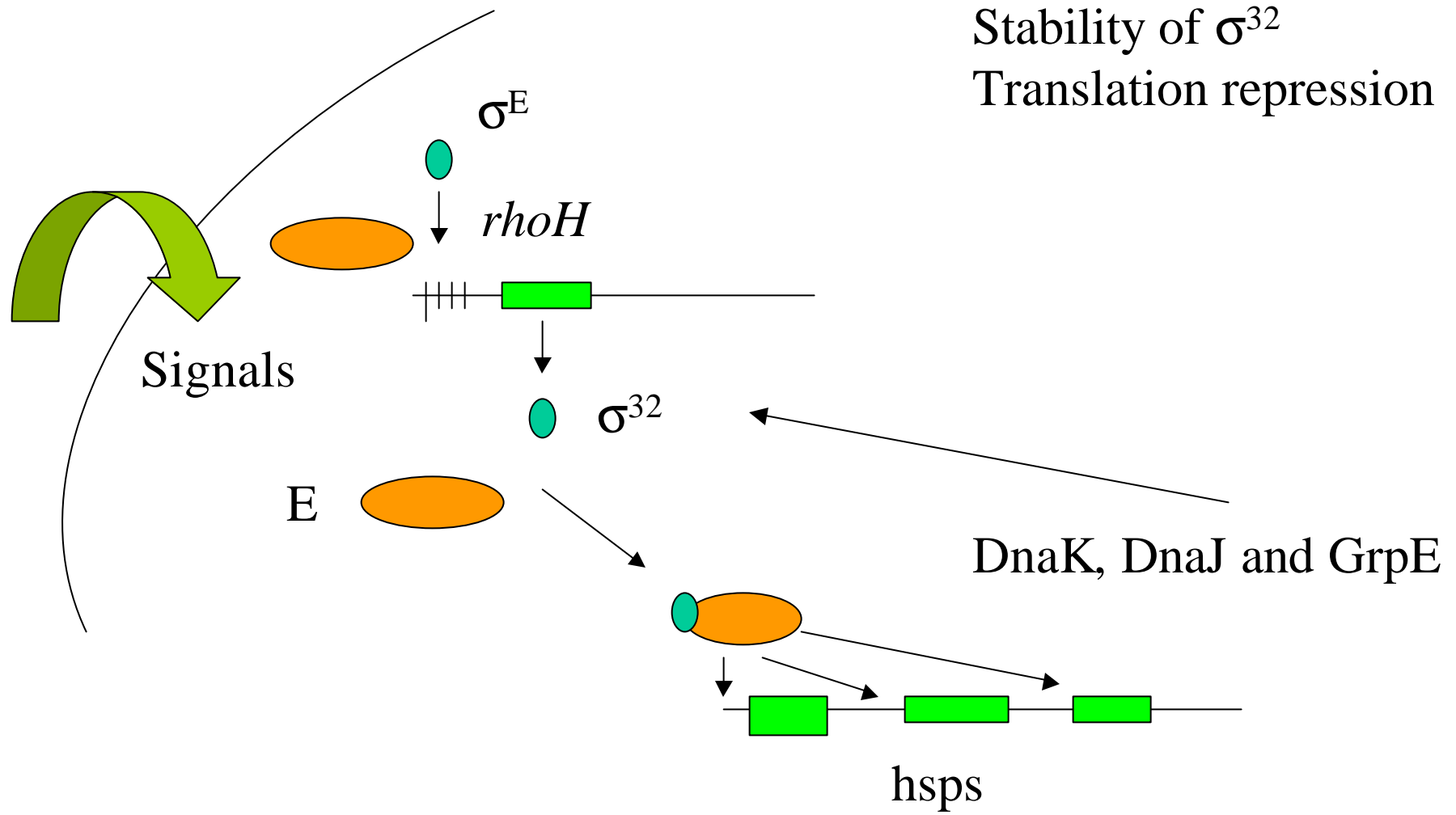


Heat shock regulon

- A small group of proteins show a large but transient increase in synthesis
 - About 20 proteins
 - Homology to eukaryotic hsps
 - Induced also by DNA-damaging agents, amino acid analogs, starvation for aa, phage λ infection, ethanol, etc.
- Biosynthesis positively regulated by *rpoH* gene encoding σ^{32}

Function of the heat shock proteins

- Required for growth
- Ratio of hsps expression at low temp and high temp (20% at 46°C)
- Inactivated *rpoH*, strain lacks σ^{32} viable only at low temp, no adaptation
- Inactivating other hsp genes such as GroE and GrpE affects growth at low temp too
- Some of the Hsps involved in prt-prt interaction and proteolytic processing



Mutant analysis

- A *rpoH* mutant expressed Hsps at low temp but no heat-inducible transcription of heat shock genes
- Purified, identified as σ^{32}
- The promoters of Hsp genes recognized by σ^{32} instead of σ^{70} –regulation distinct from other genes

Transient accumulation of σ^{32}

- Hsps increased synthesis in minutes
- Rifampicin-inhibitor of transcription
- Affecting the synthesis and the rate of accumulating
- σ^{32} with increased stability; synthesis rate change

Transcription of the *rpoH* Gene

- Has multiple promoters, activities vary with temp.
- P1 and P4 recognized by $E\sigma^{70}$ for transcription under regular conditions
- P5 weak, by $E\sigma^{70}$ without glucose or with ethanol
- P3 strong, by $E\sigma^{24(E)}$ at high temp

Multiple pathways

- Transcription of σ^{32} is controlled to provide the proper expression under a variety of steady-state conditions
- σ^{32} not the only stress response pathway
- Other alternative σ factors involved

Transient accumulation of σ^{32} mediates the heat shock response

- The synthesis of hsps increased 10- to 20-fold within 5 min after temp shift, then decline to new steady-state
- Increased synthesis of hsp mRNAs
- By increased transcription initiation at promoters recognized by σ^{32}
- Increased stability of σ^{32} contribute to accumulation

Transient induction of hsps

- σ^{32} present in limited amount in regular conditions (negative repression)
- Temp shift, stability and synthesis increase
- Increased transcription of hsps

Regulation loop for hsp expression control

- Some hsps are involved in the negative regulation of σ^{32} synthesis and stability, thereby regulate the expression of their own and other hsps
- Mutant over-express the hsps at 30C, fail to shut off the heat shock response after temp shift

Mechanism for cross protection?

E. coli

