

# Chapter 13: Global Regulation of Ribosomal Proteins, rRNAs, and tRNAs

One of the major ways cells conserve energy is by regulating synthesis of their ribosomes and stable RNAs

- Rapidly growing *E. coli* cells contain up to 70,000 ribosomes, while slower growing cells contain around 20,000
- It is important to maintain tight control on the synthesis of translation components
- However, the cell has to retain the ability to quickly compensate for changes in growth conditions

# Mechanisms of regulation

**1) Translational autoregulation: ribosomal proteins can bind mRNAs of other ribosomal proteins at the RBS**

**3) Stringent Control: amino acid starvation leads to decrease in rRNA/tRNA synthesis**

➤ **When a cell becomes starved for any amino acid, it can turn off synthesis of translational components**

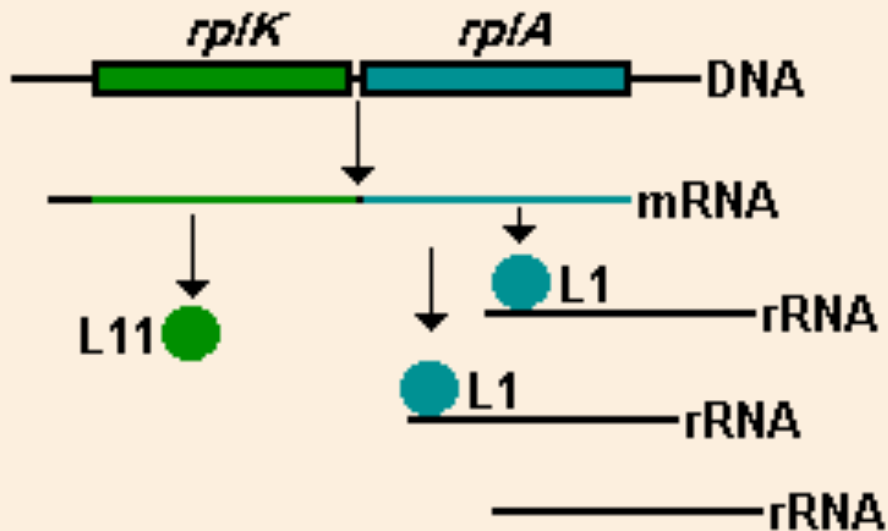
**4) Growth rate regulation: promoters for tRNA/rRNA operons form short-lived complexes with RNAP**

# Translational autoregulation

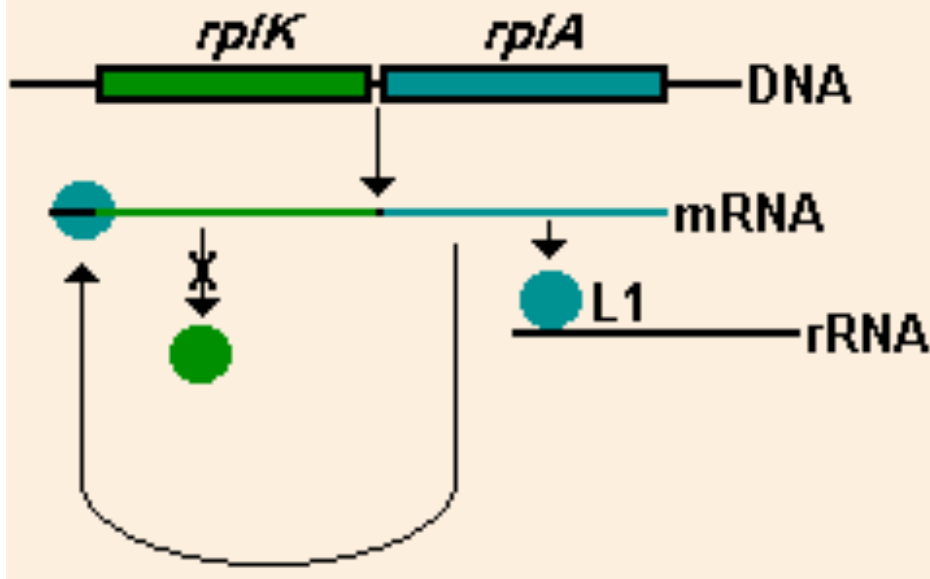
**There are a total of 53 genes for ribosomes in *E. coli* and they are all organized in operons**

- **One ribosomal protein of each operon binds to the mRNA of that operon.**
- **If lots of rRNA is present in the cell, most of the protein is bound to rRNA and so the mRNA is translated to produce more ribosomal protein.**
- **However, if there is little rRNA in the cell, the protein will bind to the mRNA and prevent translation.**

### High level of rRNA



### Low level of rRNA



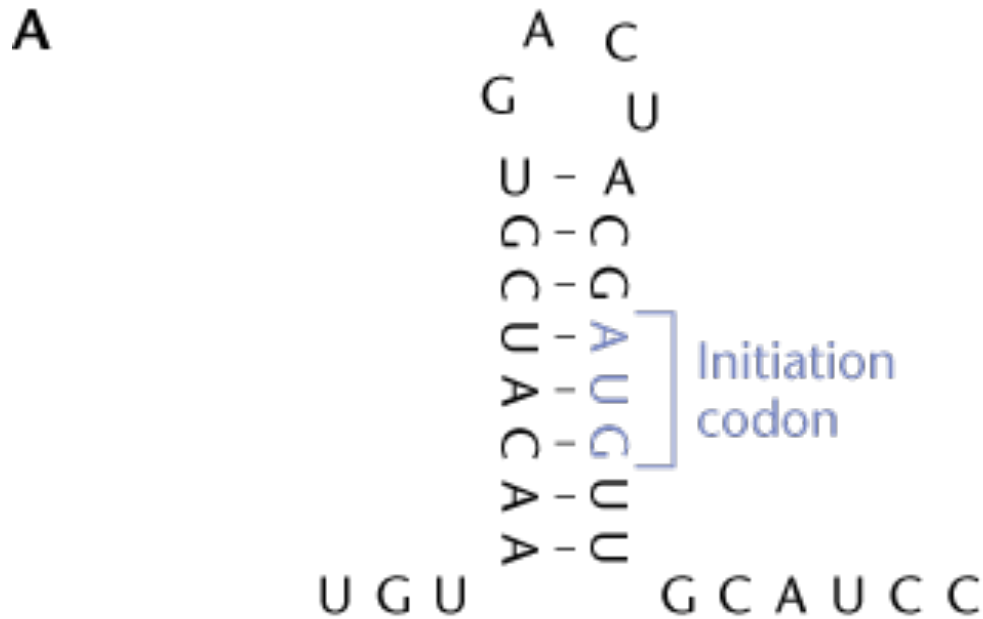
### High level of rRNA

- Ribosomal proteins bind to rRNAs allowing for translation of the *rplK* and *rplA* RNA

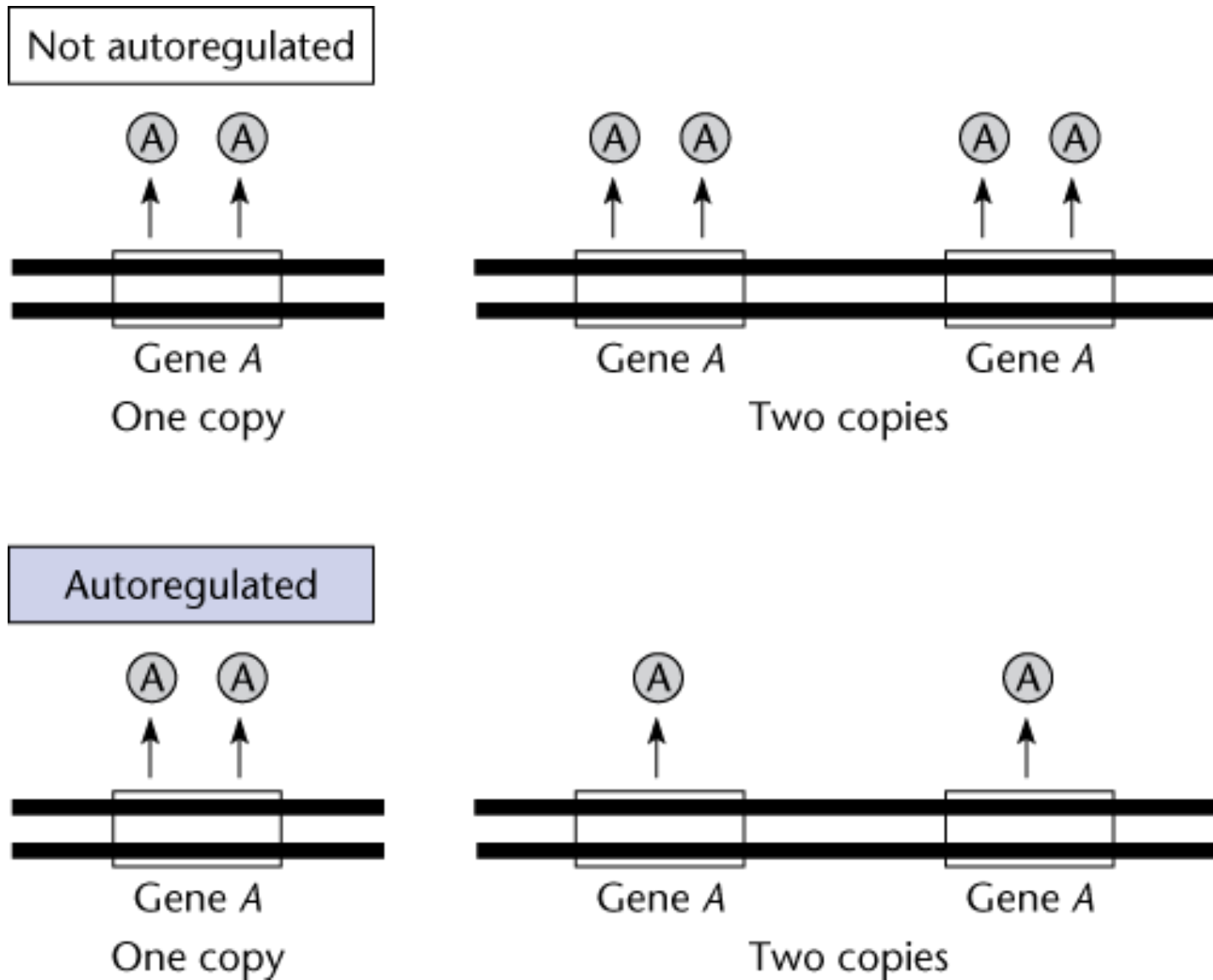
### Low level of rRNA

- L1 binds to the RBS of the *rplK* mRNA preventing translation → no synthesis of L11 protein
- While L1 does not bind to the RBS of *rplA* mRNA, translation of L11 and L1 are coupled → no synthesis of L1

# Genes for ribosomal proteins are susceptible to translational coupling



## Autoregulation of genes can be determined by gene dosage experiments



# Gene Copy Number

➤ 5S, 16S and 23S rRNAs are all transcribed as a single molecule (which also contains several tRNAs) that is then processed to give the individual components.

↳ This arrangement ensures that the rRNAs are produced in a 1:1:1 ratio.

• To increase the amounts in the cell, there are multiple copies of the rRNA (or tRNA) operon in the genome.

• High rate of transcription because operons have strong promoters.

➤ UP elements upstream of promoter help recruit RNAP



- Operons also have antitermination sequences located immediately downstream of the promoter and in the 16S-23S spacer region to prevent RNAP pausing.

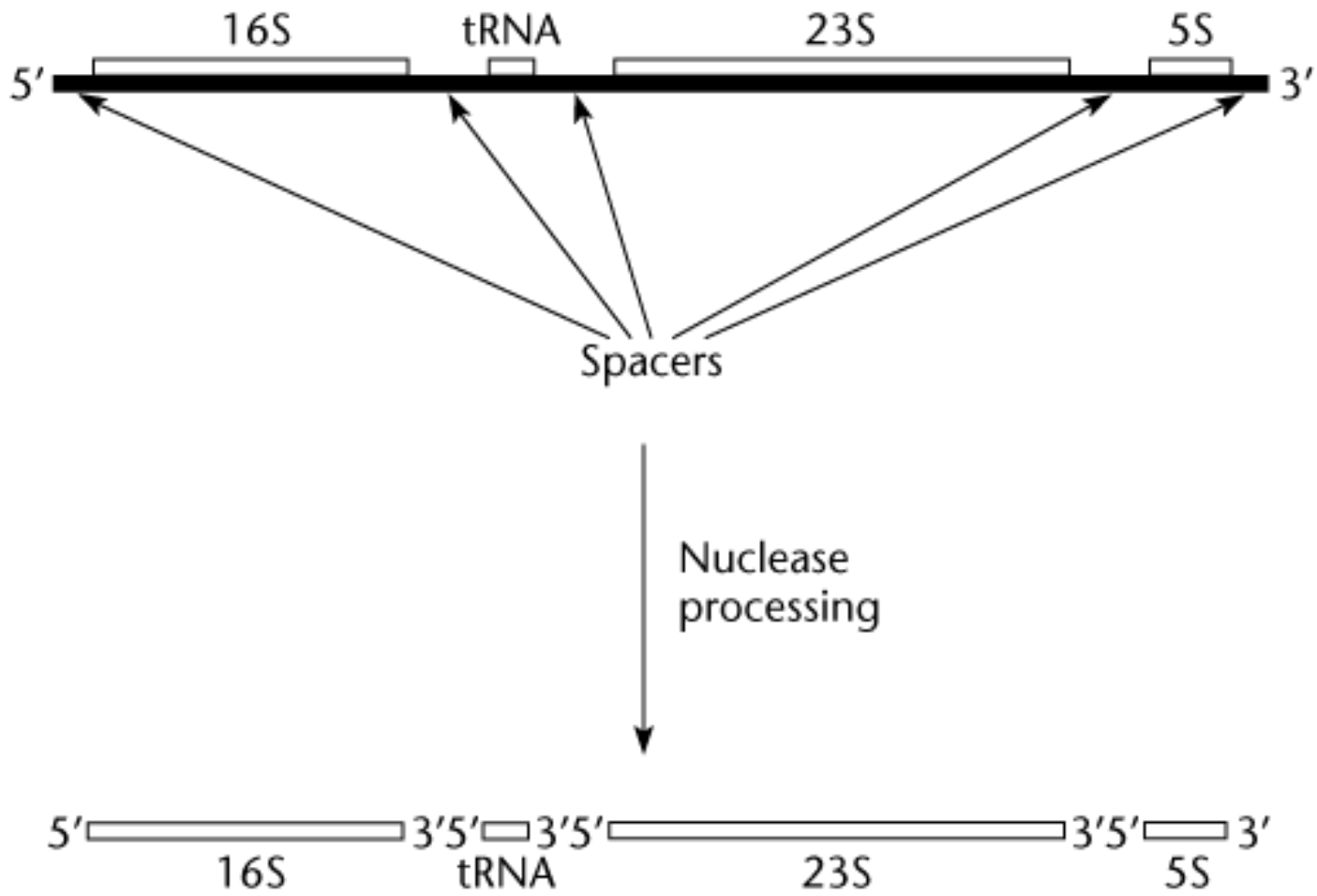
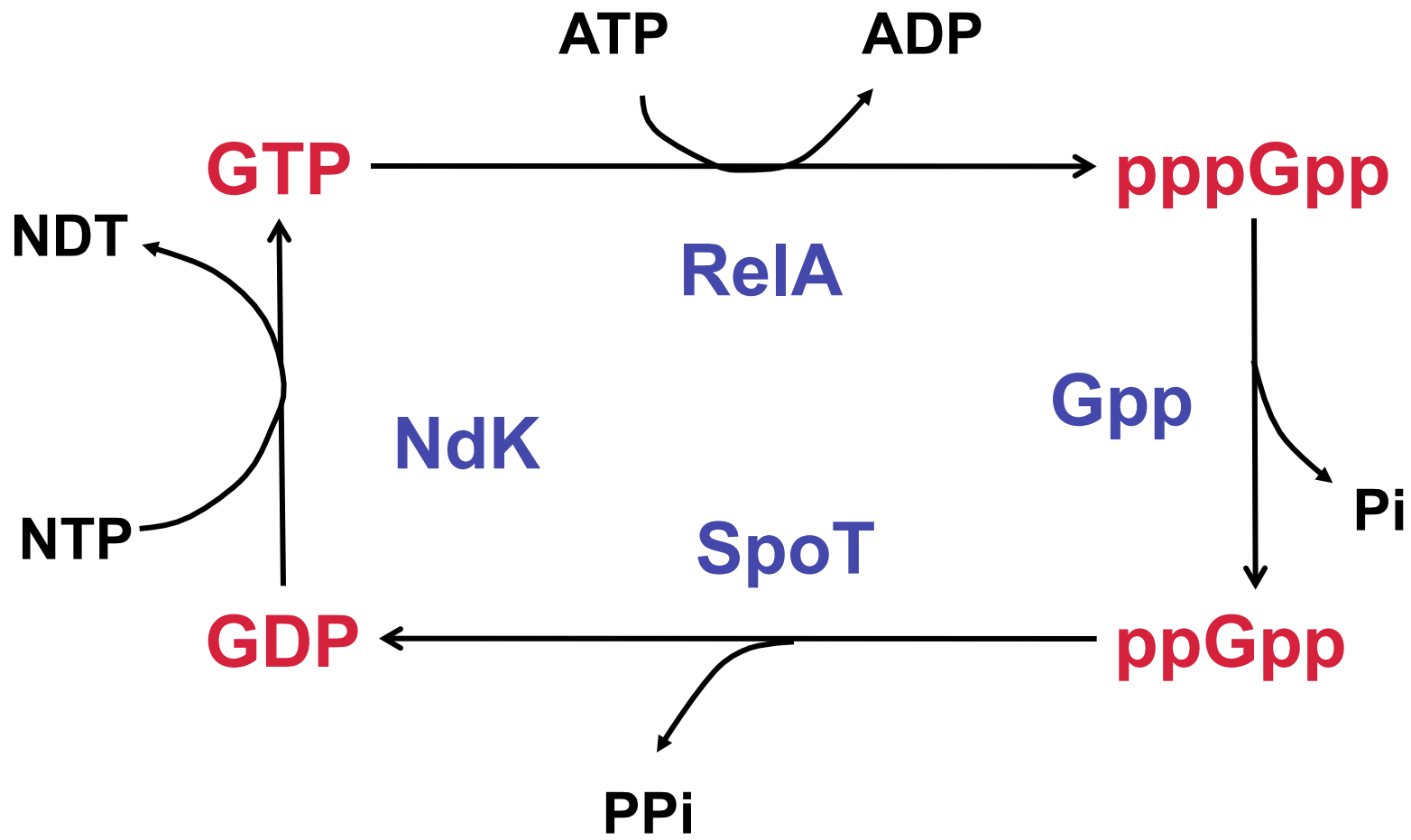


Fig. 2.20

## Stringent Control- takes affect during starvation

- **Why transcribe rRNAs and tRNAs if all the amino acids are not available?**
- **Cell couples the rate of rRNA/tRNA synthesis to that of protein synthesis so that transcription of rRNA/tRNA does not occur if the cell is starved for an amino acid**
- **RelA: (Relaxed control gene A) enzyme associated with ribosomal protein L11 that mediates stringent control**
  - **Transfers two phosphates from ATP to the 3' end of GTP yielding pppGpp**
    - ↳
    - ↳ **Presence of pppGpp and/or ppGpp is a signal of extreme starvation**

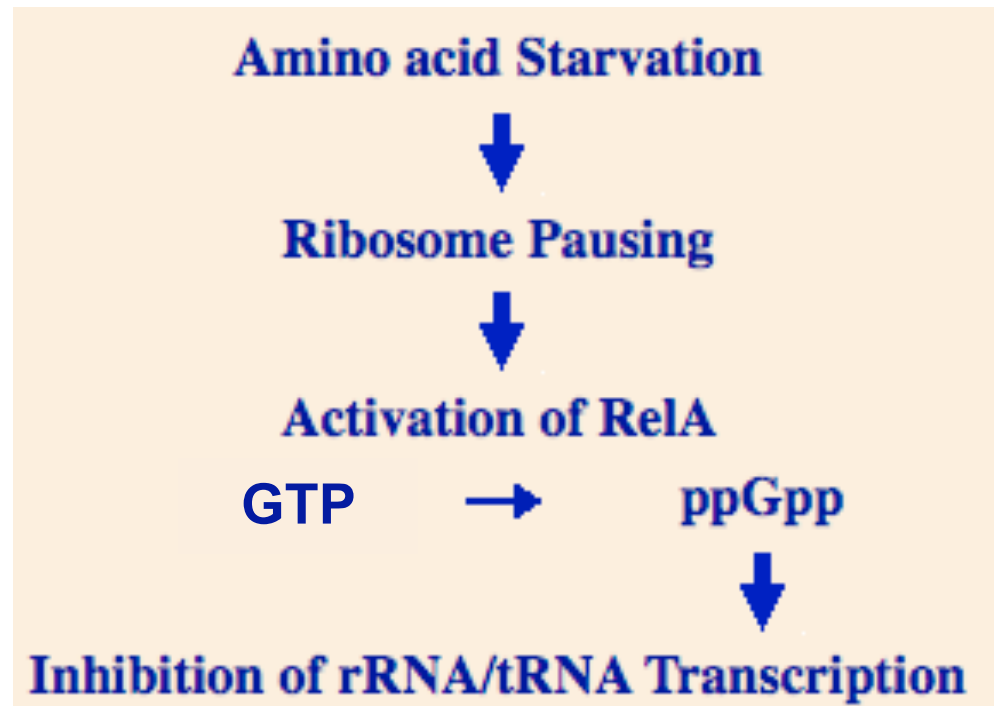


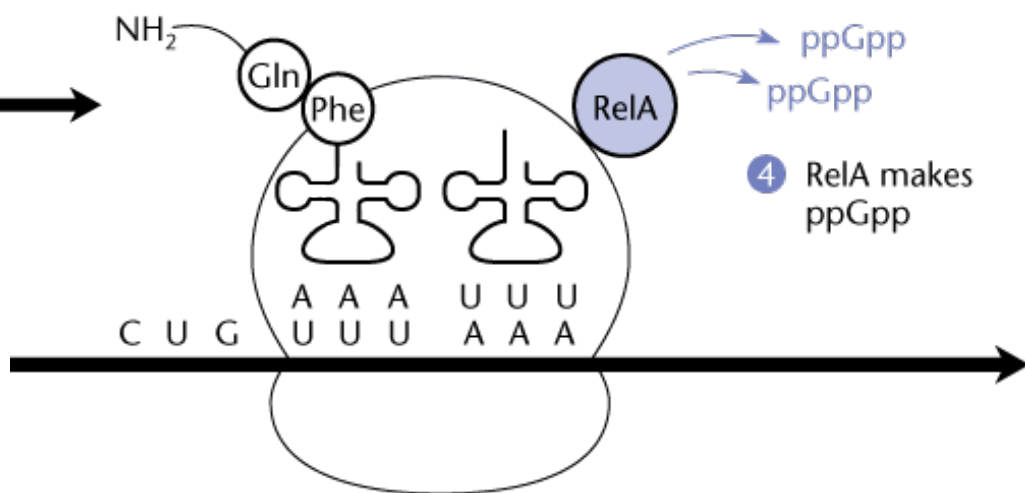
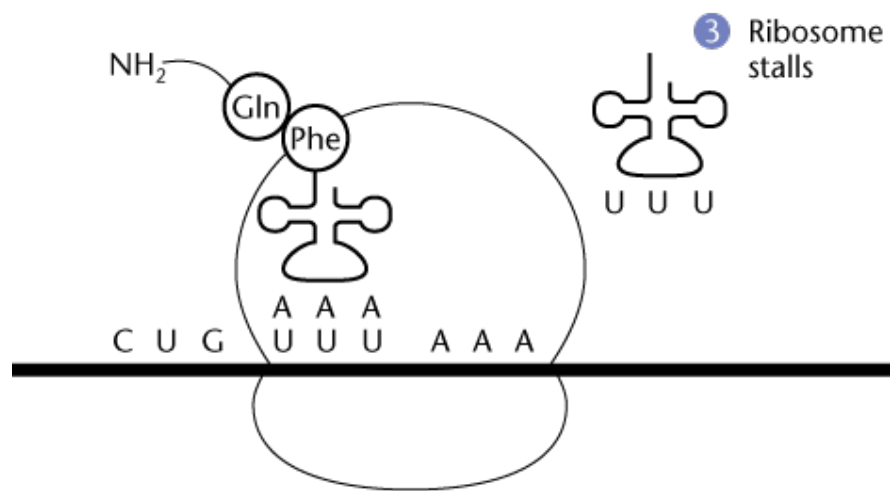
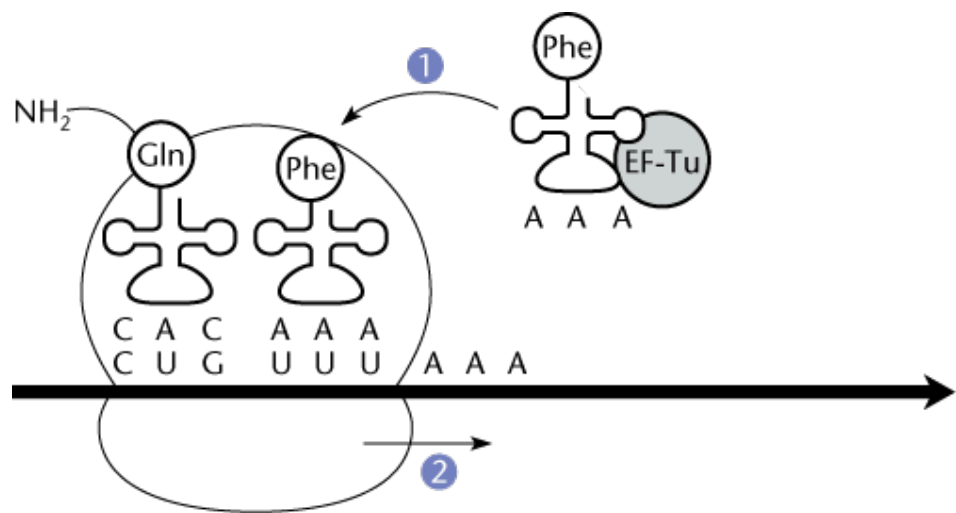
## Sequence of Events During Stringent Control

1) Amino acid starvation leads to ribosome stalling due to lack of charged tRNAs

3) Presence of uncharged tRNA in A-site stimulates RelA to produce ppGpp

4) Presence of ppGpp leads to inhibition of transcription of the tRNA and rRNA operons





## Regulation of ppGpp levels

- **SpoT protein degrades ppGpp under normal conditions**

- **Protein is inhibited by amino acid starvation**



- **If cell encounters an AA source, SpoT will degrade ppGpp**



## Presence of ppGpp decreases transcription of rRNA/tRNA operons

- **Transcription start site of many rRNA/tRNA operons begin with a G**

- **ppGpp may compete with rGTP**

# Multiple Roles of Stringent Response

## Inhibits:

- 1) tRNA/rRNA synthesis
- 2) Replication initiation
- 3) Cell division
- 4) Active transport

## Stimulates:

- 1) Amino acid synthesis
- 2) Stationary phase sigma factor

## Growth Rate

- **Cells growing at a fast rate have many more ribosomes and a higher concentration of tRNAs than slow growing cells**
- **Rate of transcription initiation is dependent on the concentration of initiating nucleotide**
- **tRNA/rRNA operon promoters are unusual in that they have a high affinity for RNAP, but form short-lived open complexes**
  - **If ribonucleotide does not enter initiation complex immediately, RNAP will disassociate from promoter**

- For reasons unknown, the [rATP] and [rGTP] are lower than the [rCTP] and [rUTP] in slow growing cells
- If the initiator nucleotide is not incorporated as soon as RNAP binds to the promoter of tRNA/rRNA operons, the RNAP will fall off and bind to another promoter
  - The [ppGpp] is also higher in slow growing cells than fast growing cells, so may compete with rGTP



# Modes of Regulation