

# Phage encoded plasmid addiction

Reference list and Chapter 4- Box 4.4

## Addiction Modules

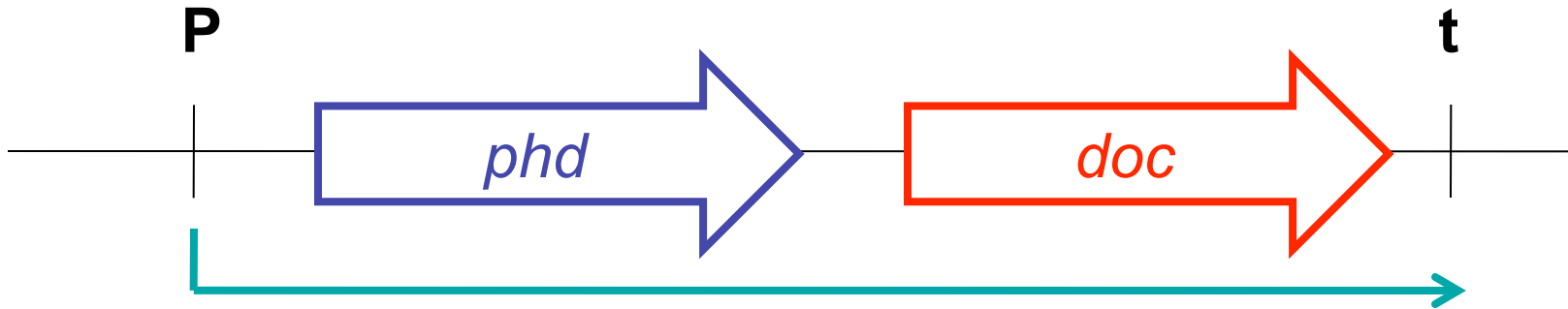
- **Encode genes for toxin – antitoxin systems**
  - **Toxin protein is generally more stable than antitoxin**
  
- **Present in extra-chromosomal elements such as phage and plasmids**
  
- **Prevent host bacterial cell from losing the modules**
  - **Progeny cells that do not contain the extra-chromosomal element do not survive**

## **P1 phage**

- **Temperate phage that exists as a plasmid in a lysogen**
- **Low copy number in cell that is tightly regulated**
- **Encodes its own partitioning system**
- **Possesses a region of DNA that frequently inverts**
  - **Orientation of the phage tail encoding DNA determines host range**

# Phd-Doc Plasmid Addiction System

Phage P1 genome region



- **Doc (Death on curing):** toxin that kills the bacterial cell by blocking translation
- **Phd (Prevents host death):** antitoxin that binds to and inactivates Doc
  - If bacterial cell is expressing *doc* must also express *phd* to avoid death
  - *phd* and *doc* are expressed from same promoter, but the Phd protein is translated at a higher rate



## Differential translation of polycistronic mRNA

- mRNAs contain target sites for ribonucleases, thus RNA decay can cause differential levels of corresponding proteins

- It is believed that ORFs at the beginning of an operon are translated at a higher level due to coupled transcription and translation

↳ While RNAP is actively transcribing a polycistronic mRNA, ribosomes can be actively translating the 5' end of the transcript

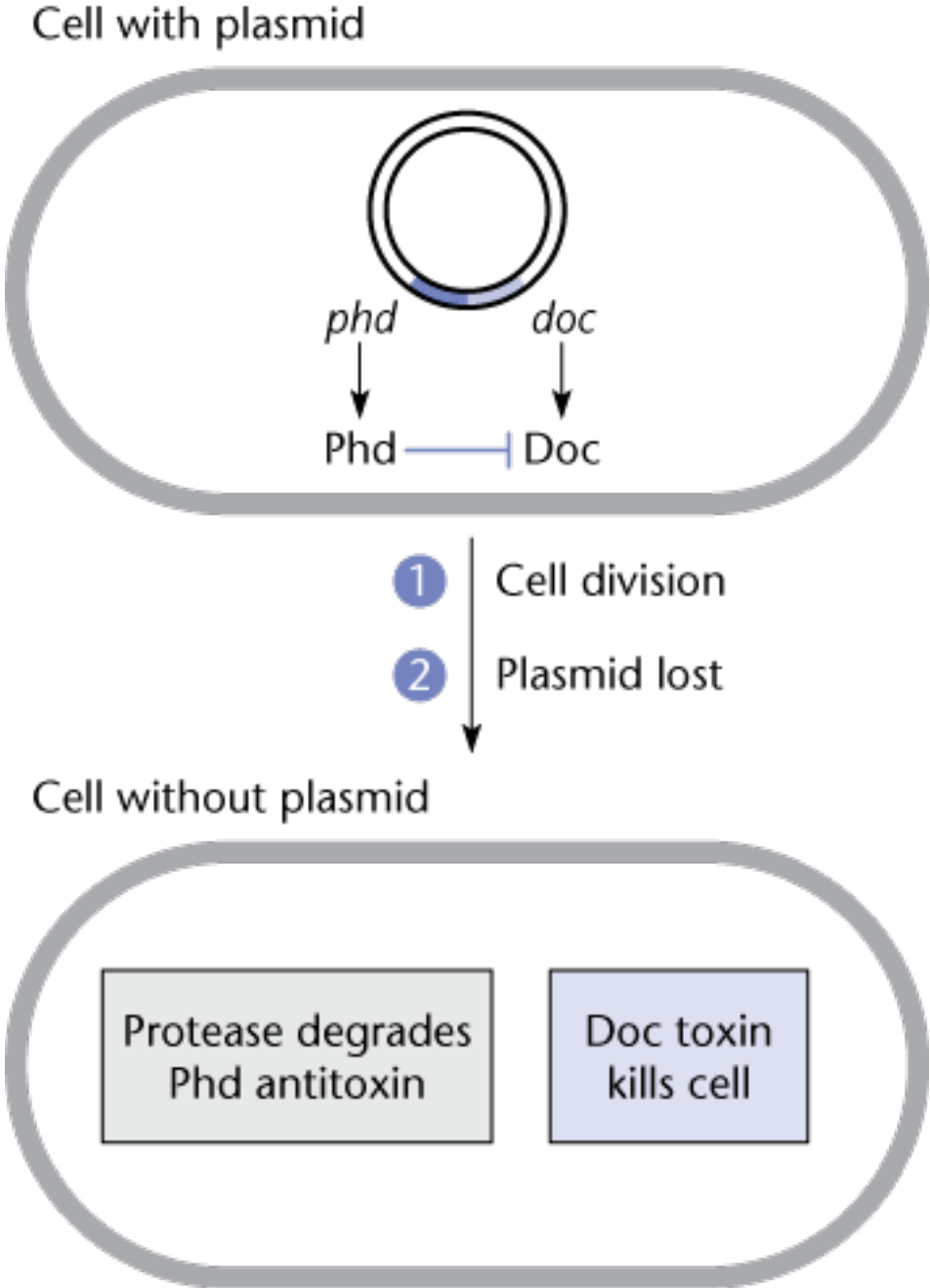
## If bacterial host is cured of the P1 DNA:

- Since the P1 DNA is no longer present, transcription of *phd* and *doc* stop.
- Phd and Doc protein products are still present in the host cell from earlier expression
- Protein turnover of Phd is must faster than Doc

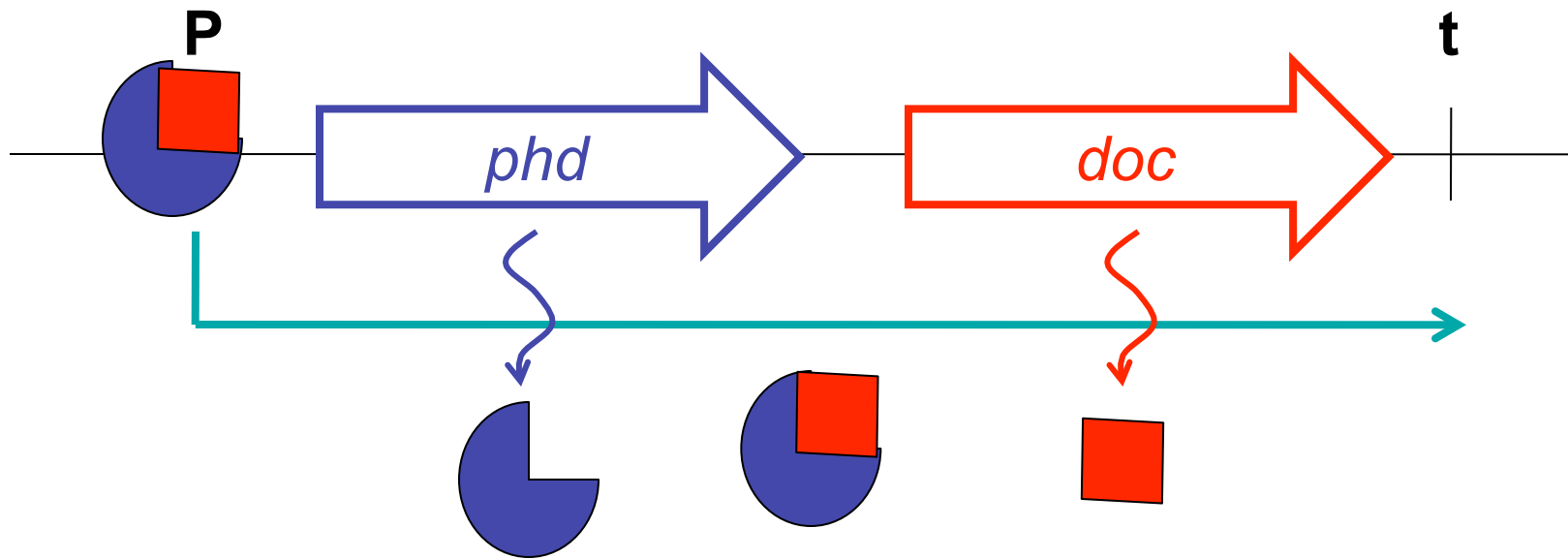


- Doc remaining in the cell following P1 DNA loss and Phd degradation kills the cell
  - Host cell must retain P1 plasmid to keep expressing enough *phd* to outcompete proteases and prevent death by Doc

**Box 4.4 pg. 223**



## Regulation of Phd-Doc



- Since Phd is synthesized faster than Doc, excess Phd negatively autoregulates the operon through repression
- Phd+Doc bound to the promoter region represses transcription even more

The negative autoregulation buffers the cell against fluctuations in P1 plasmid copy number

# Plasmid Addiction System of *E. coli*

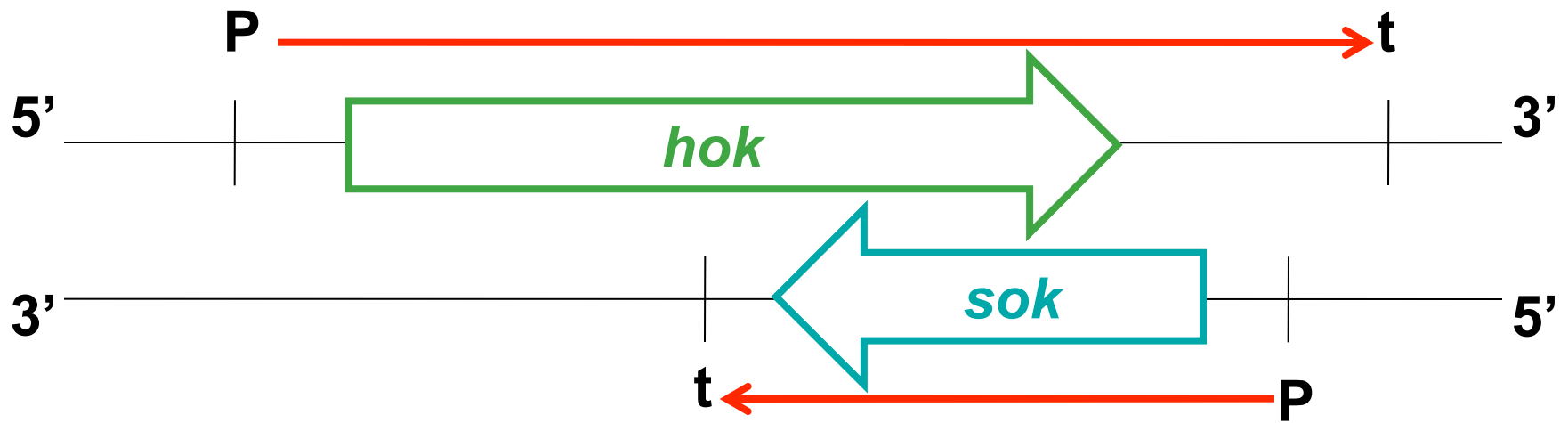
## Plasmid R1

**R1: low copy number plasmid that relies on a type II segregation system to ensure vertical transfer**

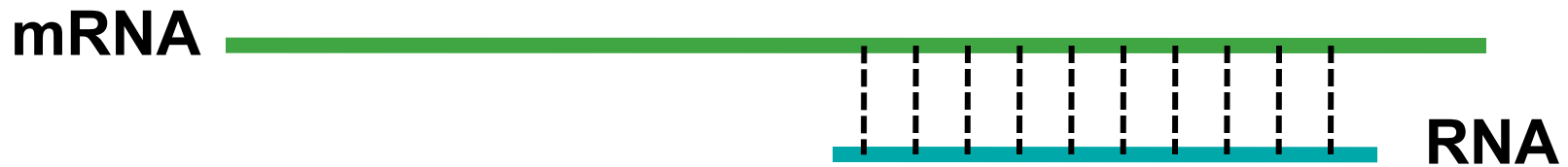
- **Possesses the *hok-sok* addition system**

- **Hok (host killing): protein that destroys cellular membrane potential causing loss of cellular energy**

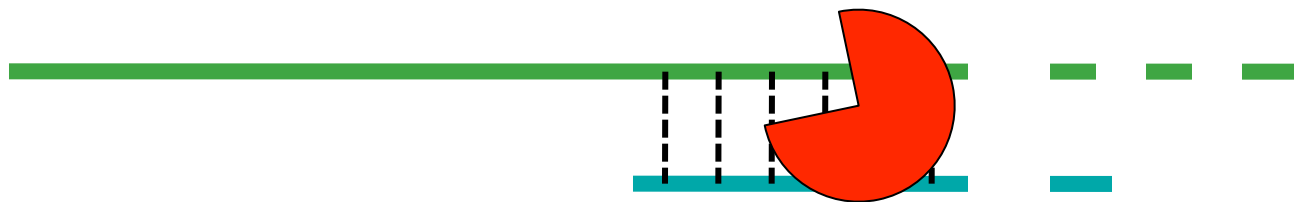
- ***sok* (suppressor of killing): gene that encodes a sRNA that binds to *hok* mRNA preventing translation of the toxin**



Products of TXN



dsRNA is target for RNases



# *hok-sok* System Model

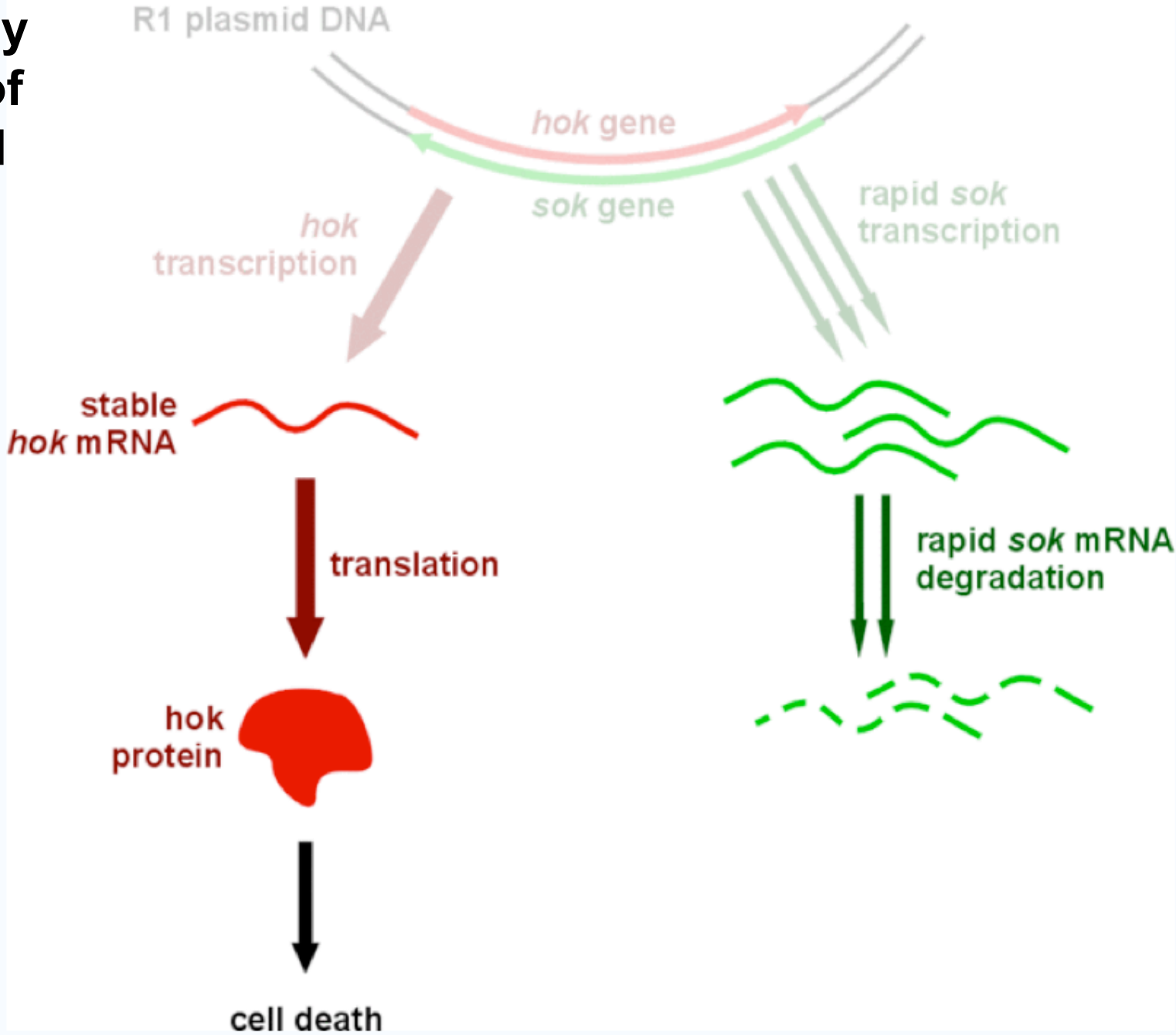
Based on differential decay of transcripts

- *hok* mRNA: very stable, does not turnover quickly in the cell
- *sok* RNA: is transcribed more quickly, but is rapidly degraded

If *E. coli* is cured of R1 plasmid:

- *sok* RNA in the cell will be degraded quickly
- *hok* mRNA will be translated into Hok protein without *sok* RNA present

# Immediately after loss of R1 plasmid



# Overview of Plasmid Addiction Systems

- **Systems can be dependent on protein-protein interactions or RNA-RNA interactions**
  - **The addiction systems are a selective pressure for the plasmids to be retained since progeny cells that do not obtain a plasmid copy are killed post-segregation**
  - **The addiction systems may also be a method for excluding competing plasmids that use the same segregation systems**
- **Progeny cells that only contain the competing plasmid without a addiction module (psk-) after segregation would be killed**