

Programmed Cell Death in Bacteria

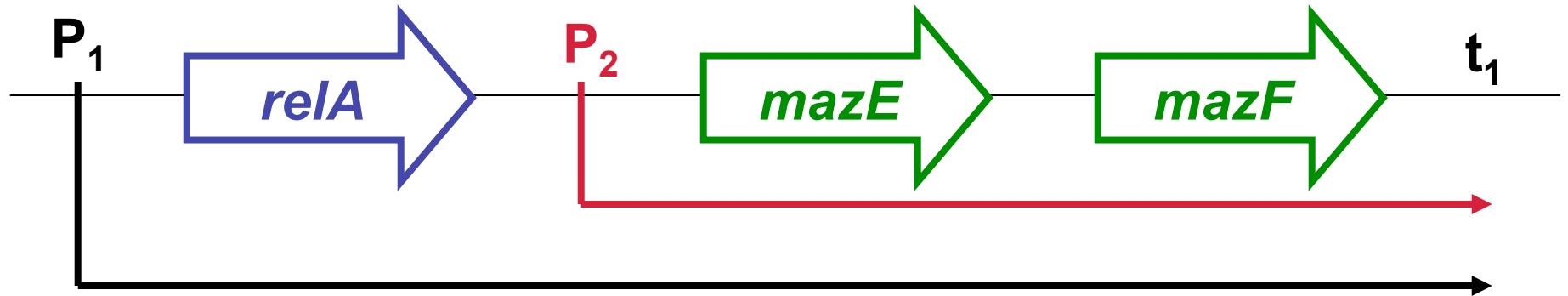
Combination of stringent control and toxin-antitoxin systems to kill off starving cells in an altruistic manner

Stringent control

- Under AA starvation, an uncharged tRNA enters the ribosome signaling RelA to produce ppGpp
- If the carbon source is limiting, [ppGpp] level also increases because SpoT is inactive and does not degrade ppGpp
- ppGpp associates with RNAP and reduces the transcription of genes whose products are involved in metabolic processes

➤ rRNA/tRNA operons

mazE and *mazF* Chromosomal Encoded Addiction System



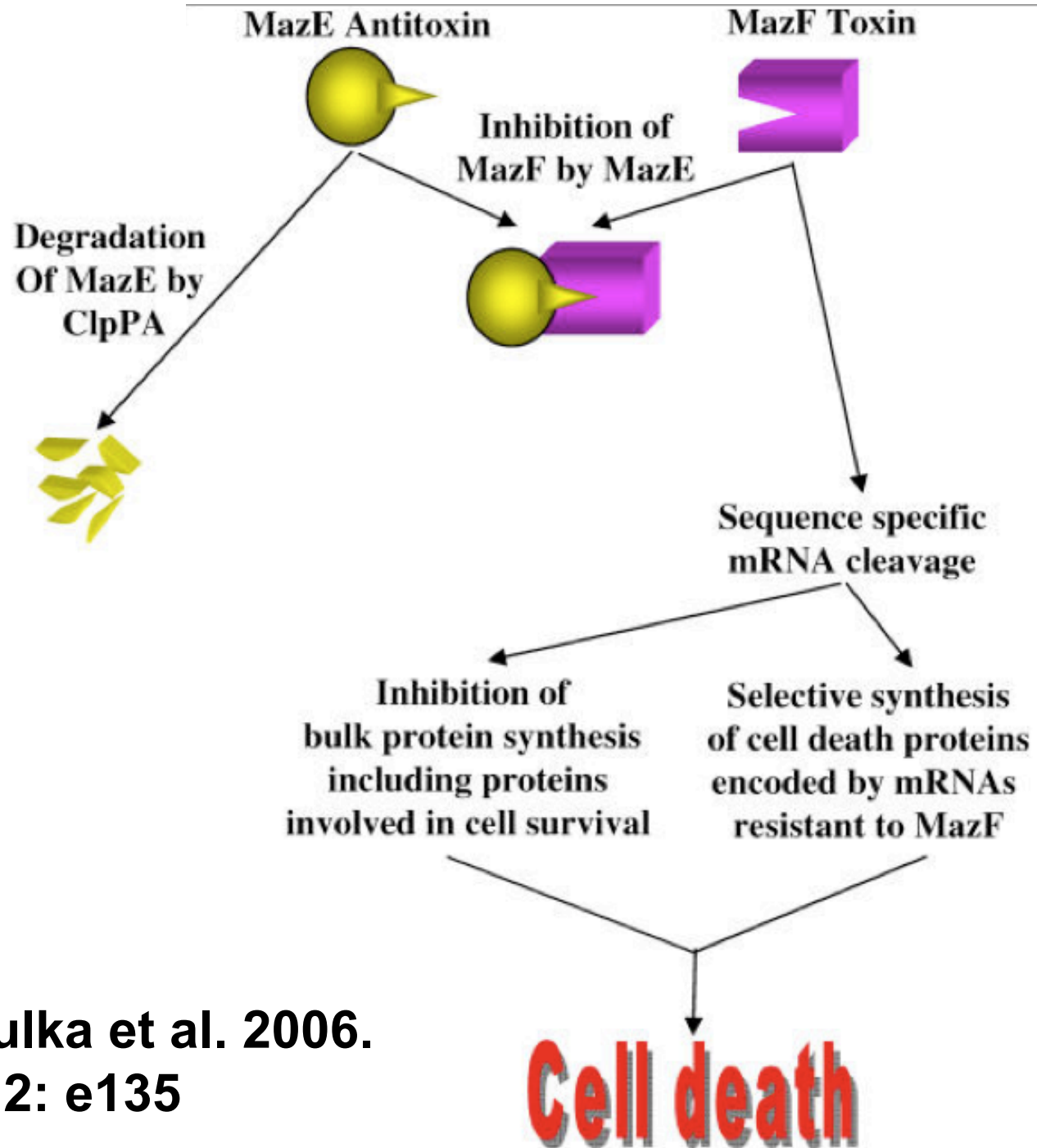
- **RelA:** produces ppGpp when cell is starved for AA
 - ppGpp represses txn of genes under stringent control
- **MazE:** antitoxin that has a short-half life
 - Degraded by the protease ClpPA
- **MazF:** toxin that has a long half-life

MazF mode of action: endoribonuclease that targets mRNAs and tmRNA at 'ACA' sequences

How could this leads to cell death?

- **mRNAs whose products are necessary for survival may contain an abundance of 'ACA' motifs**
- **Certain mRNAs that encode products for cell death may not contain an abundance of 'ACA' sequence and thus would be translated at a higher frequency due to increased ribosome availability**
- **tmRNA would not be available to tag errant proteins for degradation which would be a burden to the cell**

If MazE can quickly stop the cascade, newly produced tmRNAs can target the TLN products from degraded mRNAs for degradation



Engelberg-Kulka et al. 2006.
PLoS Genet. 2: e135

Under normal conditions

➤ More MazE is produced than MazF due to differential translation

2) Enough MazE is present to overcome both degradation by ClpPA and the toxicity of MazF

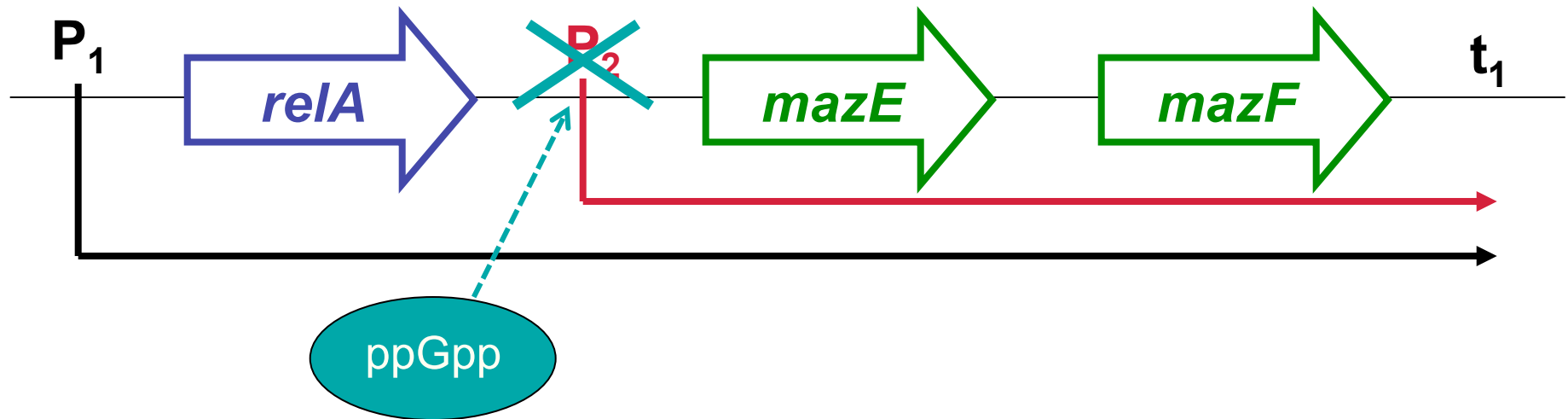
Under starvation conditions

1) RelA production of ppGpp represses transcription from P_2

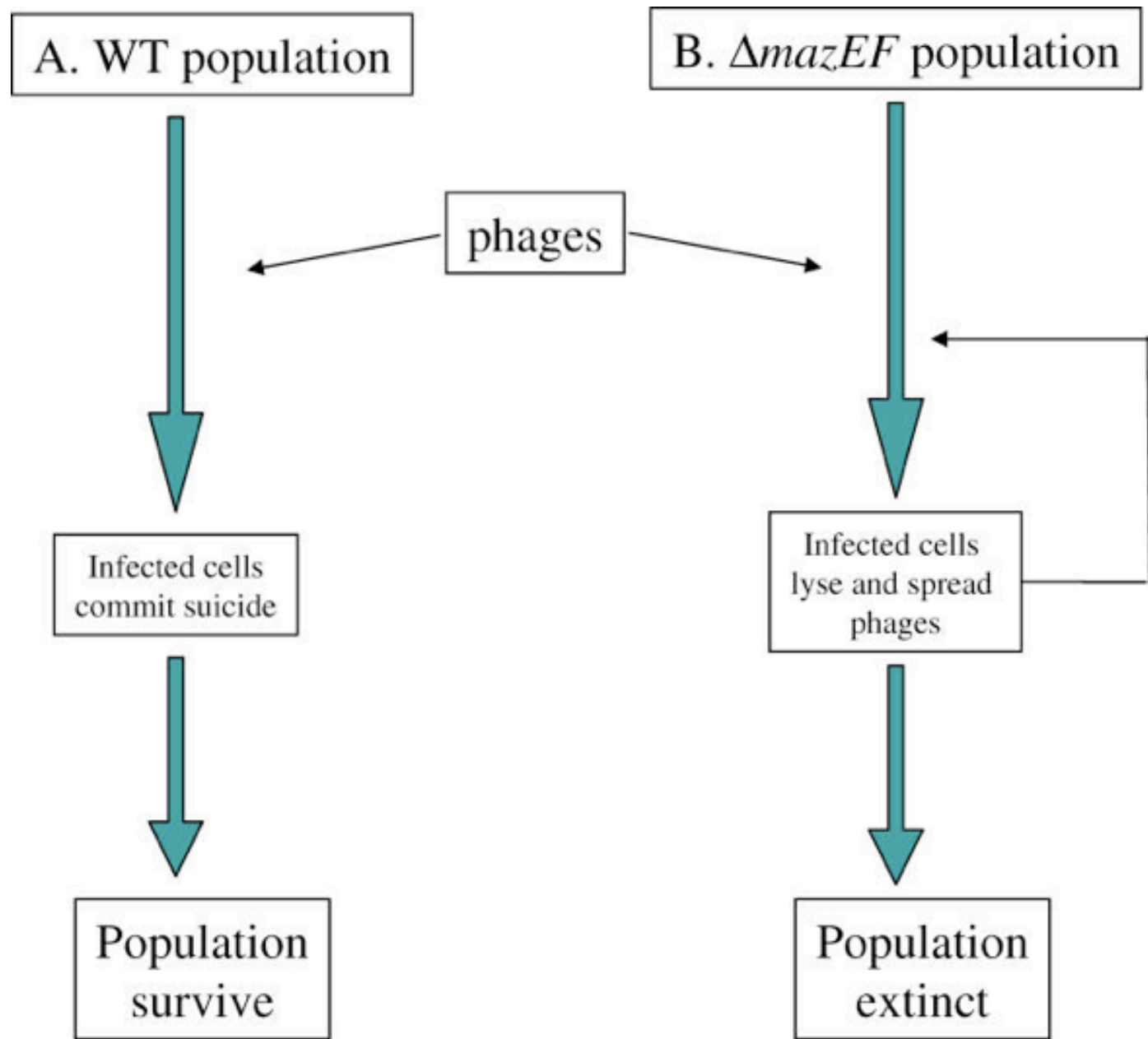
2) [MazE] present in the cell decreases quickly since it is short-lived and degraded by ClpPA

3) Free MazF exerts its toxic effect and kills the cell

Under starvation conditions



- **RelA:** produces ppGpp when cell is starved for AA
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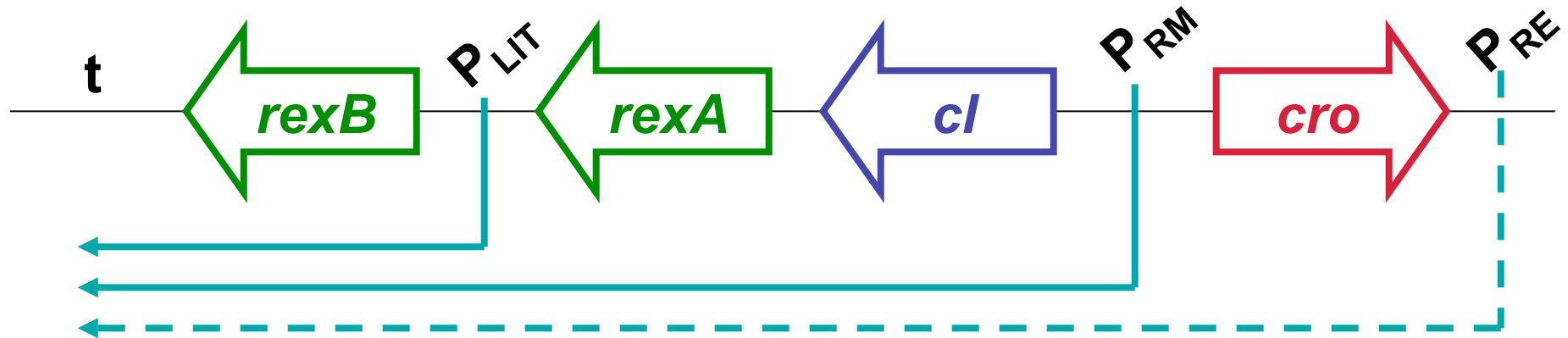


Engelberg-Kulka et al. 2006. PLoS Genet. 2: e135

Lambda and Anti-Cell Death Gene

λ lysogens inhibit programmed cell death of host

rex operon of lambda:



- **RexAB:** involved in exclusion of other phage such as T4
- ***rexB*:** co-expressed from P_{LIT}
 - Besides exclusion of other phage, RexB also inhibits the protease ClpPA which degrades Phd and MazE antitoxins

Lambda and Anti-Cell Death Gene

- In λ lysogens, RexB inactivates ClpPA allowing for the antitoxins Phd and MazE to exist at the same concentration as their respective toxins, Doc and MazF
- If lysogen encounters nutrient stress, programmed cell death will not occur regardless of the presence of ppGpp

Expression of *mazEF* will decrease, but MazE will no longer be degraded by a protease and will prevent the toxic activity of MazF



Antibiotics and Programmed Cell Death

Antibiotics that affect transcription or translation:

- **Chloramphenicol: inhibits translation by interacting with the 50S subunit of the ribosome and blocking peptidyl transfer**
- **Spectinomycin: inhibits translation by affecting tRNA translocation**

Bacteria treated with these antibiotics are not killed instantly, but seem to go into a state of hibernation prior to death

Sat et al. 2001. JBAC. 183: 2041-2045

Proposed that antibiotics that affect TXN and TLN induce programmed cell death

- **Wild type cells exposed to rifampin, chloramphenicol, and spectinomycin will not grow, but do not lyse immediately**
- **Strains with *mazEF* or *clpP* deleted, go dormant after treatment with the above antibiotics but do NOT lyse over time**
 - **Mutants are not antibiotic resistant, because protein synthesis and cell growth rates are reduced**
- **Mutant strains are still susceptible to the antibiotic ampicillin which kills cells by inhibiting cell wall synthesis**

Findings suggested that antibiotics that target transcription and/or translation may lead to increased levels of ppGpp

Comparisons between wild type cells treated with antibiotics and cells untreated indicate:

- **Comparable levels of MazF in both treated and untreated cells**
- **Lower levels of MazE in treated cells**

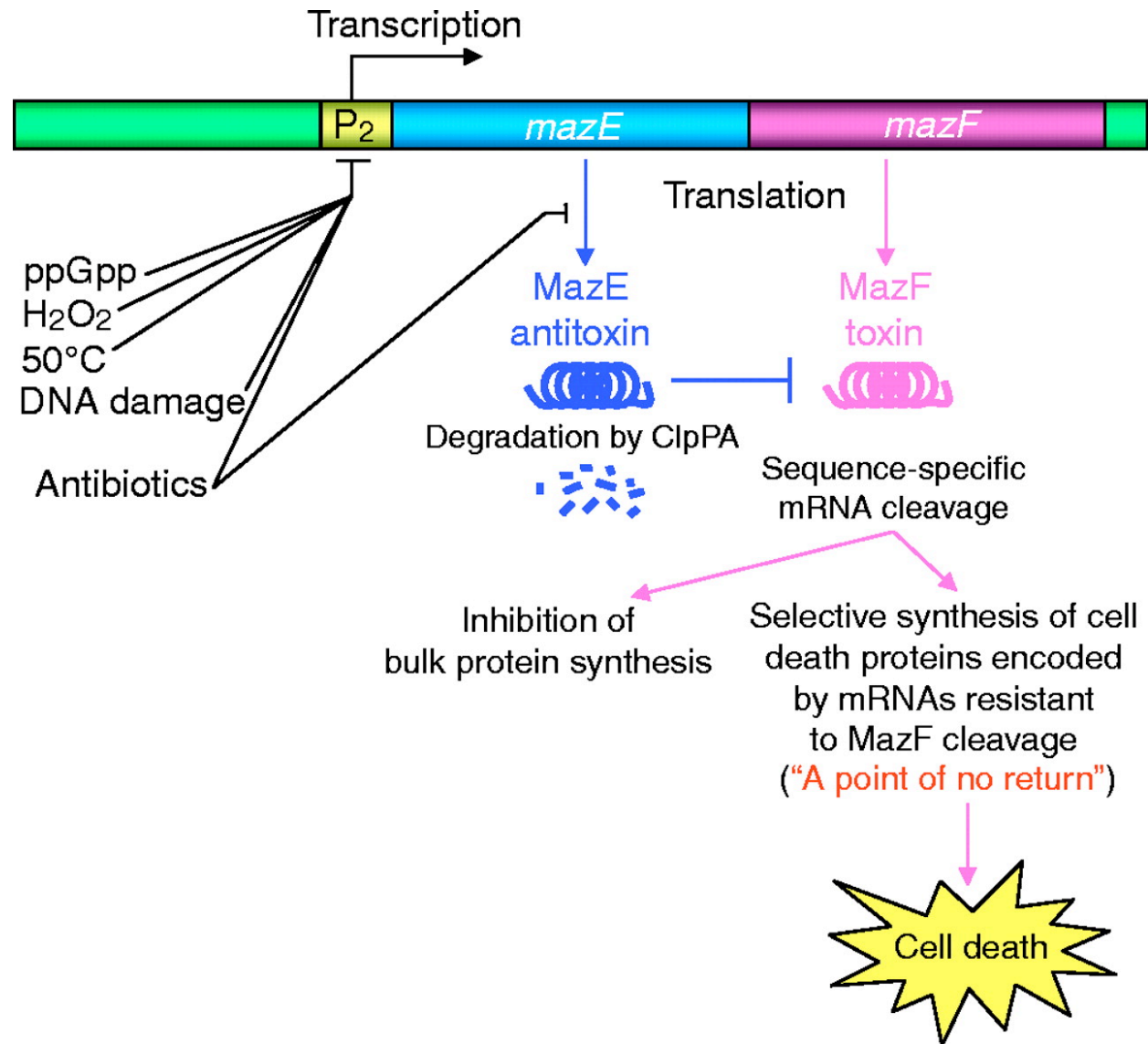
➤ **MazF induced death**

Analysis of a ppGpp production mutant

- **What sort of mutant should be made?**

Programmed cell death may be the mode of action of certain antibiotics

A schematic representation of the *E. coli* mazEF-mediated cell death pathway (for details, see text)



Engelberg-Kulka, H. et al. J Cell Sci 2005;118:4327-4332