Traxler/411 March 6, 2009

Revised reading: pp 109-114 (*new background*); 614-620

Outline:

1. Analysis of essential activities
   - RNA pol
   - The Mal proteins and the secretion apparatus for protein localization (*sec* genes)
     - Signal sequences
     - Sec apparatus
2. Synthetic lethality

Recap: Analysis of *E. coli* RNA pol

Chromosomal markers: *metB-*, rif^S, $lacZ_{am}$, *recA-*; KLF10: rif^R, *metB+*.

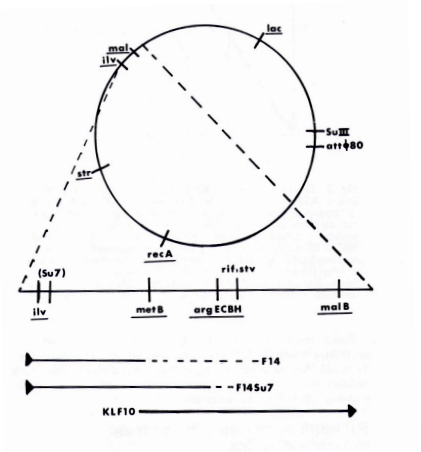
The Rif mutants had to keep the F' for growth on minimal glucose (no Met)

Class 1 rif^R/rif^R (14/144): can switch KLF10 for F'*lac* on minimal lactose + Met

Class 2: rif^-/rif^R (90/144):

class 2A: cannot lose KLF10 (must stay merodiploid; no Lac+) 82/90

class 2B: cannot lose KLF10 for F'*lac* but can lose KLF10 for F'14*su7* (and become Lac+) 8/90 are rif_{am}



Lessons of *rif* analysis

- *rif* is an essential gene (1 copy/chromosome)
- Mutations leading to Rif resistance are rare (only about 2x more common than those leading to suppressible amber mutations)
- Su7 tRNA inserts Gln at the amber codon. 4 amber mutations checked for *su*III supression, which inserts Tyr: 1/4 ambers produced functional protein.
- Data is consistent with "DNA blockage" model for recessive nature of rif^R; demonstrated that rif^S/rif^R strains contain both sensitive and resistant forms of RNA pol (equal amounts)

Problem (1978): Localization of proteins to different cellular compartments

After synthesis in cytoplasm, about 25% of all proteins made in a cell are destined to different cellular compartments. How is targeting and localization to these compartments accomplished?

- Cytoplasmic proteins seem to be without particular targeting signals (default localization)
- Brute force biochemistry had demonstrated that secreted proteins in eukaryotic model system are initially synthesized with N-terminal patches composed primarily of hydrophobic amino acids, cleaved during translocation into membrane vesicles
- Known that several periplasmic and outer membrane proteins in *E. coli* also made as "precursors" with N-terminal extensions.

What is importance of N-terminal extensions? How similar is localization process in bacteria and euks?

LacZ fusions and molecular genetics

Beckwith & colleagues (1976) had developed genetic tools for making transcriptional and translational fusions to *lacZ*. Early application was to dissect gene regulation and identify genes.

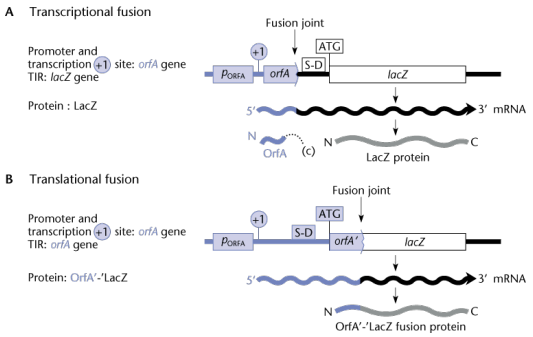


Figure 2.44

Maltose uptake & utilization

Important players:
✓LamB: OM porin, required for maltodextrin transport
✓MalE: maltose binding protein in periplasm
✓MalFGK: CM transporter
✓ MalT: + regulator

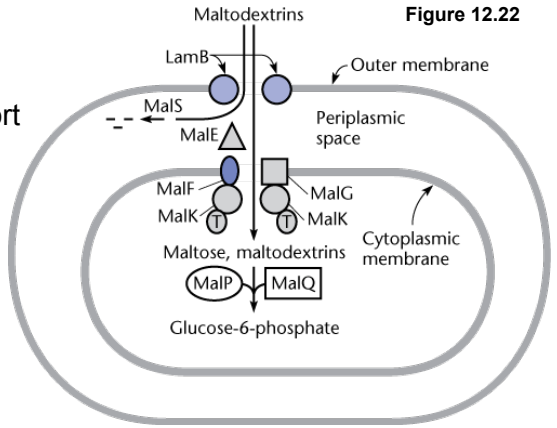


Figure 12.22

Mal^R selection

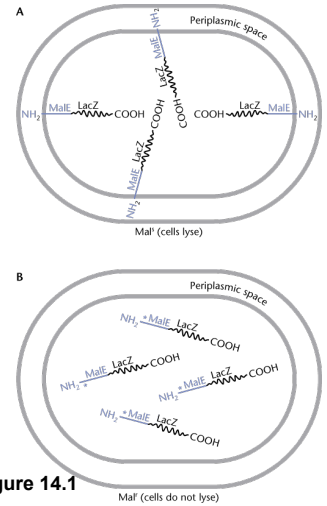


Figure 14.1

Geneticists reasoned that one could isolate mutations in important features of secretion targeting signals by **selection for growth** of MalE/LamB-LacZ strains on maltose

Variation in signal sequences

Protein	Signal Sequence
LamB (OM)	Met **Lys** Ala Thr **Lys** Leu Val Leu Gly Ala Val Ile Leu Gly Ser Thr Leu Leu Ala Gly ↓ (20/**2**+/14)
MalE (peri)	Met **Lys** Ile **Lys** Thr Gly Ala **Arg** Ile Leu Ala Leu Ser Ala Leu Thr Thr Met Met Phe Ser Ala Ser Ala Leu Ala ↓ (26/**3**+/17)
βla (peri)	Met Ser Ile Gln **His** Phe **Arg** Val Ala Leu Ile Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala ↓ (23/**2**+/15)

Analysis of signal sequences for protein secretion

N-terminal extensions on proteins destined to cross the cytoplasmic membrane for final localization outside the cell (or in the periplasm/outer membrane of Gram neg. bacteria) have diverse amino acid sequences. Mutational analysis was important is showing what was important.

Bacteria and euks with same general properties for these signal sequences (SS, or leader sequences).

Putting a SS on a normally cytoplasmic protein leads to targeting to secretion machinery.

The first secretion mutation: *secA*

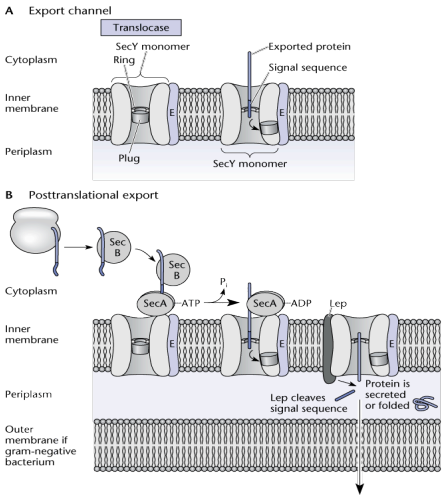
Oliver and Beckwith constructed a strain that was *mal*$^{+}$ and had a *malE-lacZ* fusion (LacZ-).

They screened for spontaneous Lac+ at 30°C and identified 80 candidates.

2/80 Lac+ mutants did not grow at 42 °C (therefore are *temperature sensitive*) on any media; they are still ts when cured of the *malE-lacZ* fusion.

An essential function: Protein secretion

Figure 2.40 A/B



Oliver & Beckwith found *secA*. SecA needed for localization of MalE, LamB, PhoA, OmpF.

Hfr mapping: *ts*+ of $secA_{ts}$ at 0-7'

Variations on screen gave several other *sec* mutations.

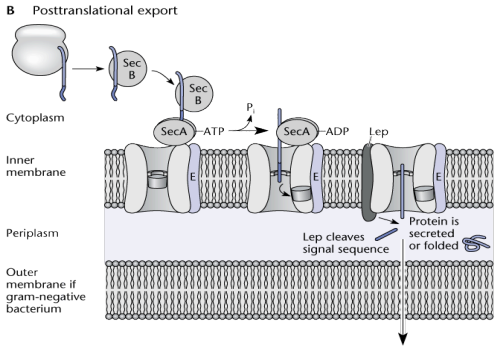
Mechanism of secretion

Figure 2.40 B

SS associates directly with SecA and SecYE at different stages.

Selection for ***gain of function*** mutants via suppressor analysis: started with LamB-LacZ SS mutant (Lac-) and selected for Lac+.

Prl mutants have minor defects in Sec proteins that are more tolerant of bad signal sequences.



Prl mutants & synthetic lethality

- Prl mutants of SecE and SecY map near plug of Sec channel, probably facilitate its opening.
- Combining two particular *prl* mutations into same strain = cell death. This called ***synthetic lethality.***