Mutations: essential genes & synthetic phenotypes

Revised reading (last Friday and Today):
pp 109-114 (*new background*); 614-620

Reading for Global Regulation Wednesday 11 Mar 09:
pp574-583

Micro/GS411

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Mal^R selection for SS mutants

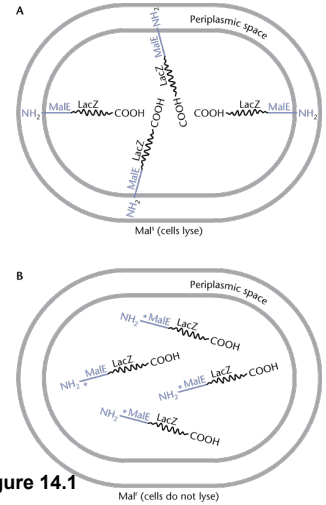


Figure 14.1

Mal+ strains also containing a translational fusion of MalE/LamB-LacZ are Mal^S on maltose (healthy w/o maltose). Selection of Mal^R mutants (on maltose) allowed identification of important features of secretion targeting signals signal sequences (SS); showed the hydrophobic core of the SS has primary importance.

LacZ$^+$ selection & Sec mutants

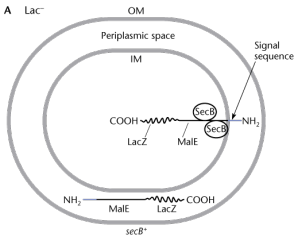
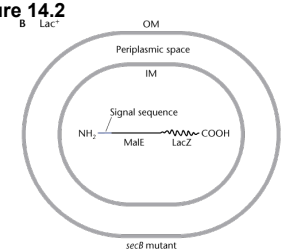


Figure 14.2

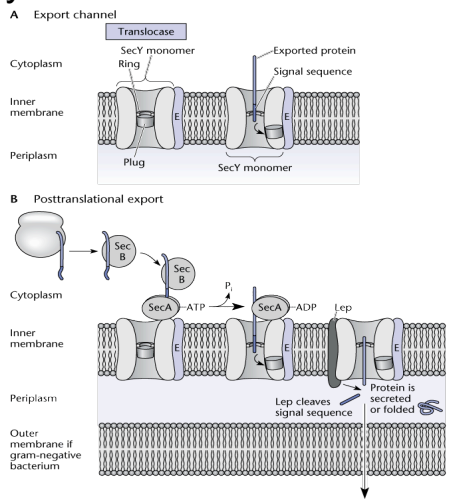


Note: not an all or nothing phenomenon, as shown here….

- MalE-LacZ fusion can make strain Mal^s, but targeting to Sec leads to low LacZ activity (LacZ gets stuck?)
- In conditions w/o Mal^S, can ask for Lac$^+$ activity of fusion, leading to conditional (leaky) Sec mutants; LacZ fusion stays in cytoplasm and folds into active state

Mutants of the essential secretion machinery

Figure 2.40 A/B



Lac$^+$ selection at 30°C uncovered *secA$_{ts}$*. Mutant had defective localization of MalE, LamB, PhoA, OmpF.

Variations on screen gave several other *sec* mutations. Mutants of SecY SecE were *cold sensitive*.

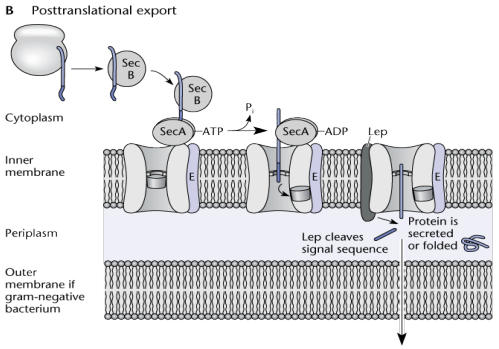
Homologues of SecEY are ubiquitous. However, SecA is only found in Bacteria.

Mechanism of secretion

Selection for ***gain of function*** mutants via suppressor analysis: took LamB mutants with SS defects (SS*LamB) and selected for growth on maltodextrins (Dex+), which requires LamB porin (these mutants also acquire λ^S).

Strong PrlA4 mutant (Dex+) is dominant and suppresses many different SS* mutants. Some other Prl mutants show strong SS* allele specificity for suppression.

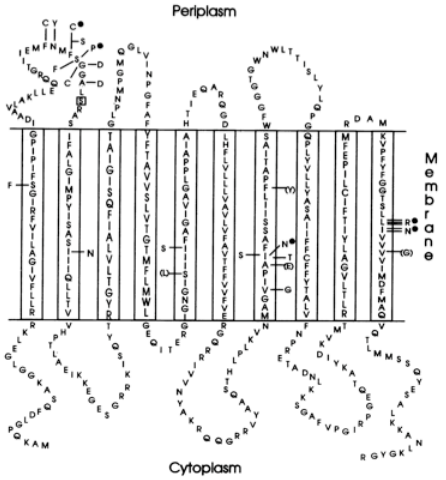
Figure 2.40 B



PrlA mutants of SecY

100+ *prlA* alleles (SS* suppressors) of SecY analyzed: PrlA4 mutant and few others have two changes in *secY* gene; several mutations isolated 2-4 times.

The distribution of Prl mutants of SecY shows strong clustering in particular regions of the protein. Also see this in SecE.



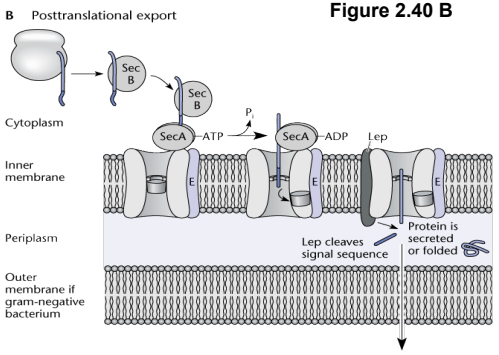
Prl mutants & synthetic lethality

Clustering of changes in some Prl mutants is consistent with SecYE interaction with SS.

Prl mutants in SecA, SecY, and SecE suppress localization defects of SS* mutants, but they do not have significant growth phenotype otherwise.

However, particular combinations of different *prl* alleles result in cell death (**synthetic lethality**), which is *strong evidence for protein-protein interactions*.

Figure 2.40 B



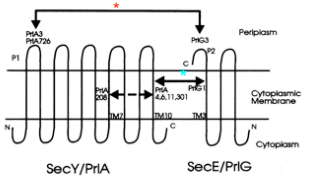
Synthetically lethal interactions:
SecE (*prlG*) & SecY (*prlA*)

Lethal interactions noted in table, mapped onto the SecY/SecE proteins

Table 1. Complementation assay for synthetic phenotypes

prlA allele[a]	Domain[b]	pBAD18[c]	*secE*$^{+}$[c]	*prlG1*[c]	*prlG2*[c]	*prlG3*[c]	*prlG8*[c]
secY^{+}	NA	−	+	+	+	+	+
prlA1	TM7	−	+	+	+	− *	+
prlA3	P1	−	+	+	+	+	+
prlA4	TM10,TM7	−	+	− *	+	+	+
prlA6	TM10,TM5	−	+	−	+	+	+
prlA7	TM10,TM7	−	+	+	+	+	+
prlA9	P1	−	+	+ *	+	+	+
prlA11	TM10,TM10	−	+	−	+	+	+
prlA200	TM5	−	+	+	+	+	+
prlA202	TM7	−	+	+	+	+	+
prlA205	P1	−	+	+	+	+	+
prlA208	TM7	−	+	− *	+	+	+
prlA300	P1	−	+	+	+	+	+
prlA301	TM10	−	+	− *	+	+	+
prlA302	P1	−	+	+	+	+	+
prlA303	TM7	−	+	+	+	+	+
prlA304	TM2	−	+	+	+	+	+
prlA306	P1	−	+	+	+	+	+
prlA666	P1	−	+	+	+	+	+
prlA726	P1	−	+	+	+	− *	+/−
prlA8911	TM1	−	+	+	+	+	+
prlA8913	P1	−	+	+	+	+	+
prlA8914	P1	−	+	+	+	+	+

Assays were performed as described in Materials and methods. Results shown are at 23°C, in the presence of arabinose.
[a]Chromosomal *prlA* suppressor allele.
[b]Domain where the PrlA mutation is located. NA indicates not applicable.
[c]Plasmid encoded *prlG* allele that was introduced into the *prlA* strain. + indicates complementation of the cold-sensitive defect, − indicates no complementation, +/− indicates poor complementation.



Directed knock-out mutants

- Can use PCR to synthesize a linear dsDNA with homology (*ca* 50 bp) to any gene and a cassette for drug resistance.
- These construct can be transformed into cell and undergo homologous recombination (works well with λ recombination functions).
- Selection for drug resistance leads to gene replacement and knock-out.
- Now have libraries of non-polar disruptions of non-essential genes: *E. coli*, *P. aeruginosa*, *etc.*

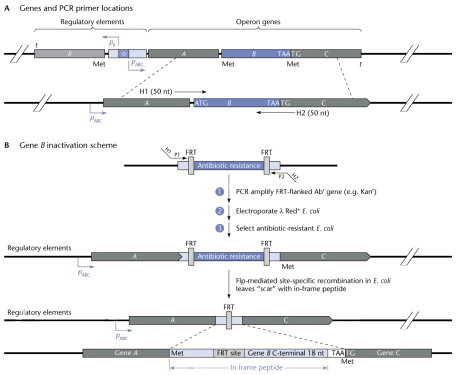
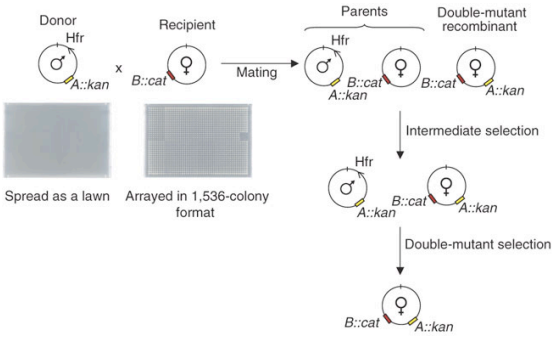


Figure 12.29

Genetic interactions in *E. coli*

- *E. coli* single gene disruption library (Baba *et al*, 2005): attempted 4288 targeted disruptions, recovered 3985 knock-outs; 303 proposed essential genes (under conditions used).
- 1755 uncharacterized ORFs: can previously undetected interactions suggest function?
- Effort to establish strategies to efficiently combine all combinations of double mutants for any particular gene of interest is daunting endeavor. We aren't there yet, but progress.…

Synthetic phenotypes in *E. coli*: Hfr mating to the rescue!!!



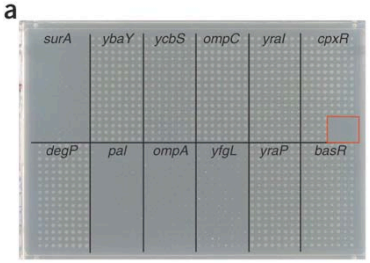
2008: Two groups showed that small number of Hfr's in gene can move a particular disruption into large number of recipients in F-based Hfr matings on plates (long incubation).

Tested recovery and growth of Cm^R and Kan^R recombinants.

Synthetic lethals (no growth) **and** positive interactions (better growth) detected

Test case: cell envelope interactions

Typas *et al* did 12x12 matrix: Here, *pal::kan* Hfr crossed with *gene B::cat*. Scored both the viability and growth of recombinants on rich and minimal media.



Significant synthetic interactions

Pairs	Interaction
DegP-SurA*	Lethal
Pal-SurA	Lethal
Pal-YfgL	Lethal
Pal-OmpA	Sick/Lethal
DepP-YfgL*	Sick
CpxR-Pal	Sick
Pal-YraP	Positive
OmpA-SurA	Positive
CpxR-OmpA	Positive
OmpA-YraP	Sick

Limitation: Closely linked markers (<20kb) not well tested via this analysis.

* = previously known interactions