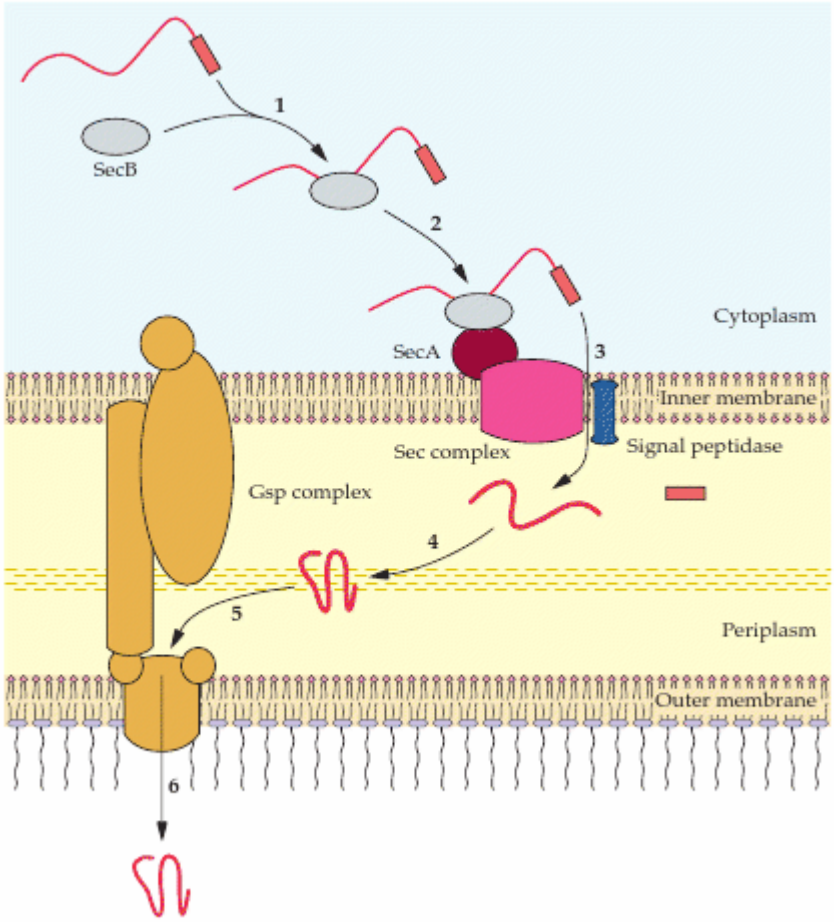
Engineering cell factories: Prokaryotes

Protein folding and secretion in *E. coli*

Lecture 7

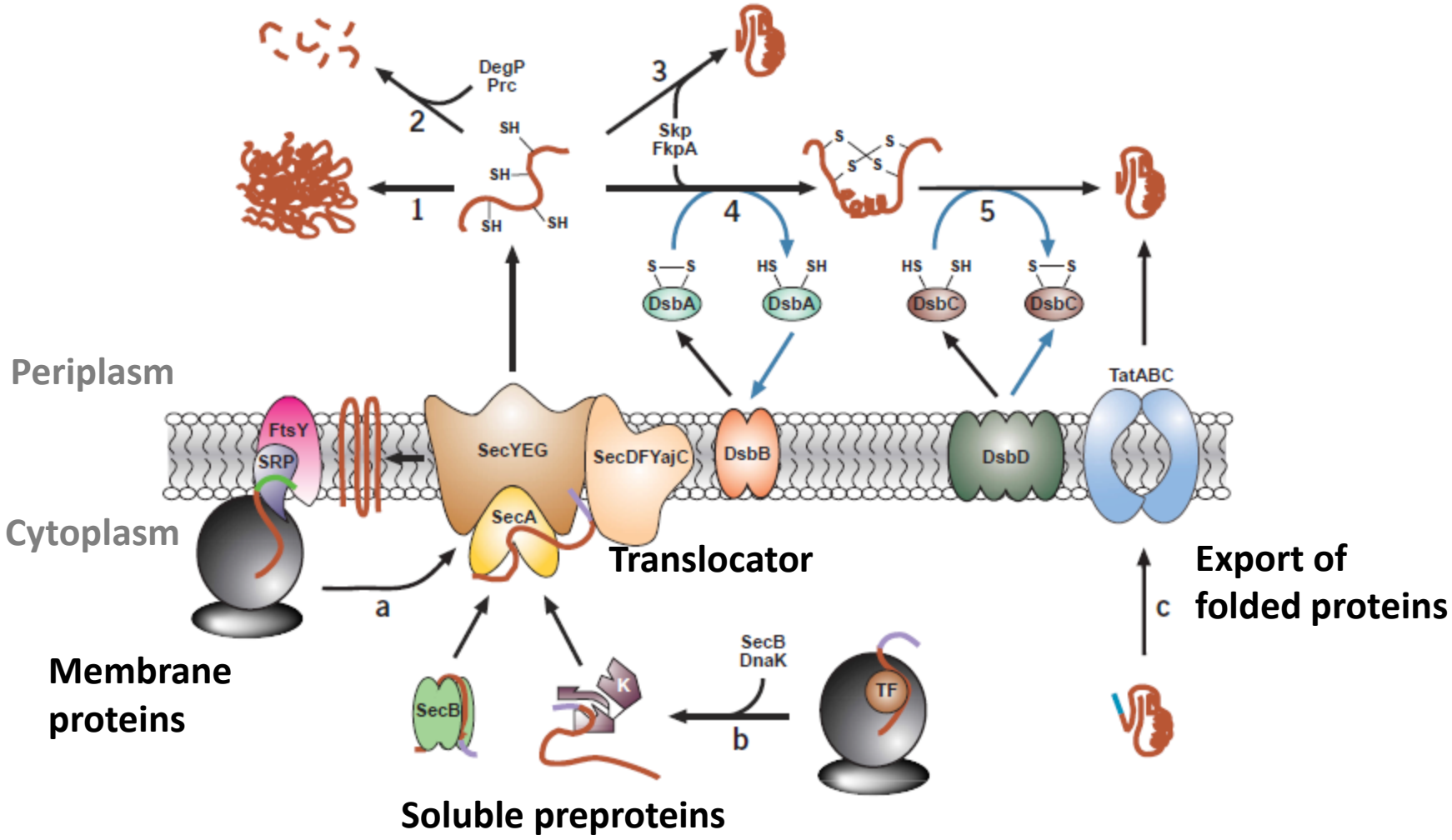
Schematic representation of a type II secretion pathway in gram-negative bacteria

1. The SecB protein binds to a secretory protein in the cytoplasm
2. SecB attaches to the SecA protein that is part of the Sec complex of the inner membrane
3. the secretory protein is translocated through the inner membrane
4. A signal peptidase removes the signal peptide, and the secretory protein is properly folded in the periplasm
5. the secretory protein combines with the Gsp complex
6. it is translocated to the external environment



Glick, Bernard J. (Author). Molecular Biotechnology : Principles and Applications of Recombinant DNA (4th Edition). Washington, DC, USA: ASM Press, 2010

Export and periplasmic folding pathways in *E. coli*



Baneyx and Mujacic, 2004, Nature Biotech. 22:1399

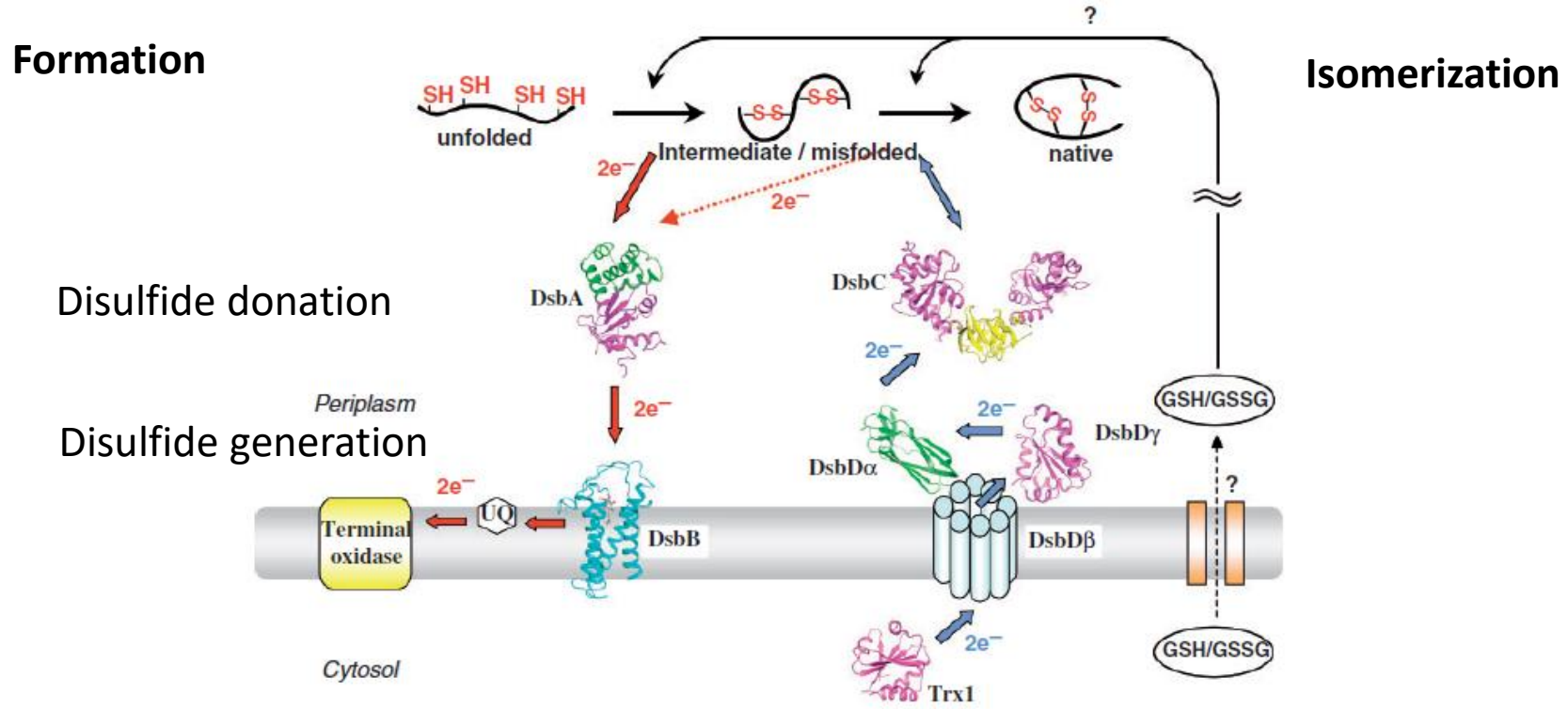
Periplasmic folding modulators

Table 2 Periplasmic folding modulators

Classification	Protein	Substrates
Generic chaperones	Skp (OmpH)	Outer membrane proteins and misfolded periplasmic proteins
	FkpA	Broad substrate range
Specialized chaperones	SurA	Outer membrane proteins
	LolA	Outer membrane lipoproteins
	PapD (and its family)	Proteins involved in P pili biosynthesis
	FimC	Proteins involved in type 1 pili biosynthesis
PPIases	SurA	Outer membrane β-barrel proteins
	PpiD	Outer membrane β-barrel proteins
	FkpA	Broad substrate range
	PpiA (RotA)	Unknown
Proteins involved in disulfide bond formation	DsbA	Reduced cell-envelope proteins
	DsbB	Reduced DsbA
	DsbC	Proteins with nonnative disulfides
	DsbG	Proteins with nonnative disulfides
	DsbD	Oxidized DsbC, DsbG and CcmG
	DsbE (CcmG)	Cytochrome *c* biogenesis
	CcmH	Cytochrome *c* biogenesis

Baneyx and Mujacic, 2004, Nature Biotech. 22:1399

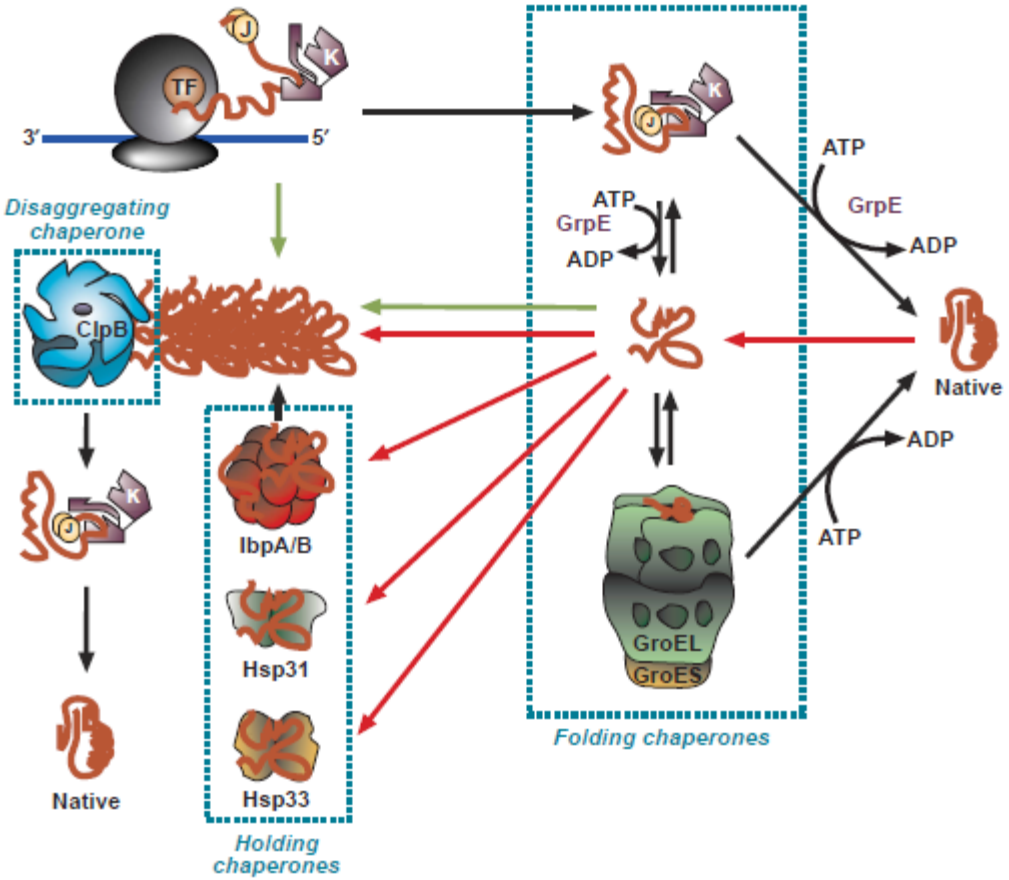
The disulfide-introducing and disulfide-isomerizing pathways in *E. coli*



- Dsb enzymes involved in the oxidative system are shown as ribbon representations.
- The thioredoxin domains of the Dsb enzymes are shown in magenta
- Oxidized and reduced glutathione possibly contributes to oxidative protein folding, either directly or indirectly.

Chaperone-assisted protein folding in the cytoplasm of *E. coli*

A fraction of proteins (especially proteins with conformationally complex structures) do not reach their native conformation and aggregate as insoluble deposits named inclusion bodies.
Protein release from inclusion bodies is mainly controlled by DnaK, ClpB



Baneyx and Mujacic, 2004, Nature Biotech. 22:1399

Cytoplasmic chaperones

Table 1 Cytoplasmic chaperones

Family	Name	Cofactors	Function	Substrate specificity	ATP requirement
Hsp100 (AAA+)[a]	ClpB		Disaggregase	Segments enriched in aromatic and basic residues	+
Hsp90	HtpG		Possible folding/secretory chaperone	Unknown	+
Hsp70	DnaK	DnaJ, GrpE	Folding chaperone	Segments of four to five hydrophobic amino acids, enriched in leucine and flanked by basic residues	+
	HscA	HscB	Iron-sulfur cluster protein assembly	LPPVK motif in iron-sulfur cluster protein assembly IscU	+
	HscC	YbeV, YbeS	σ^{70} regulation	Unknown	+
Hsp60	GroEL	GroES	Folding chaperone	α/β folds enriched in hydrophobic and basic residues	+
Hsp33	Hsp33		Holding chaperone	Unknown	–
DJ-1 superfamily	Hsp31		Holding chaperone	Unknown	–[b]
Small Hsps	IbpA, IbpB		Holding chaperone	Unknown	–[c]
PPIase	TF		Holding chaperone, PPIase	Eight amino acid motif enriched in aromatic and basic residues	–
SecB	SecB		Secretory chaperone	Nine amino acid motif enriched in aromatic and basic residues	–

[a]AAA, ATPases associated with a variety of cellular activities. [b]ATP binding negatively regulates the chaperone activity of Hsp31 at high temperatures[23]. [c]ATP binding to certain small Hsps triggers conformational changes and substrate release[17].

No protein disulfide isomerase activity in cytoplasm, only chaperone and PPIase

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Hatahet *et al. Microbial Cell Factories* 2010, **9**:67
http://www.microbialcellfactories.com/content/9/1/67

MICROBIAL CELL FACTORIES

RESEARCH Open Access

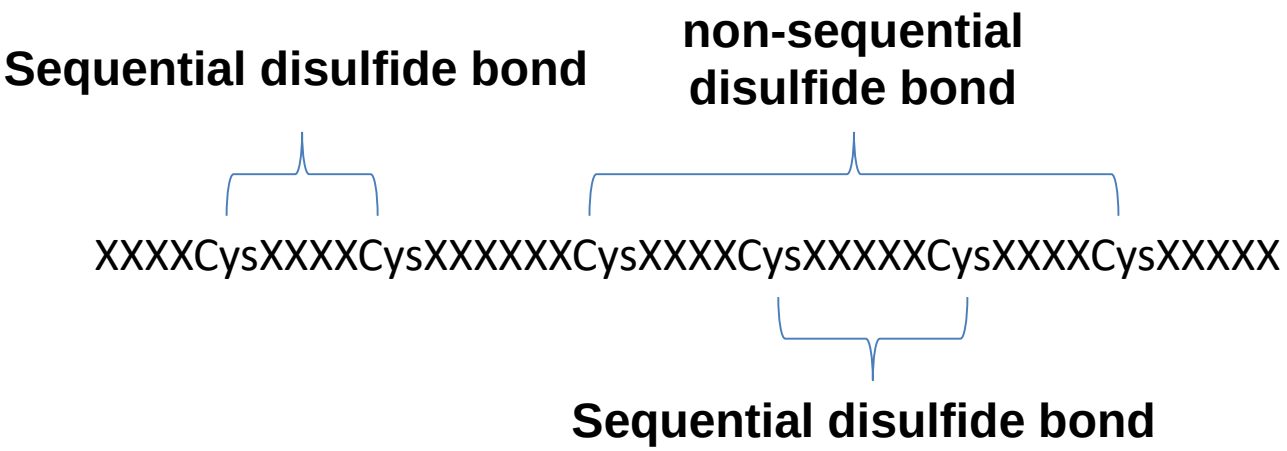
Disruption of reducing pathways is not essential for efficient disulfide bond formation in the cytoplasm of *E. coli*

Feras Hatahet, Van Dat Nguyen, Kirsi EH Salo, Lloyd W Ruddock*

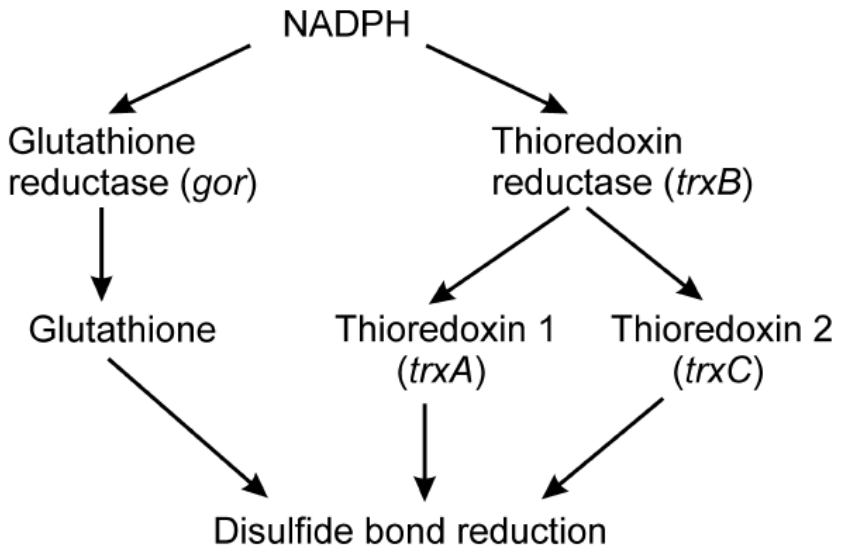
Model proteins

- PhoA: simple model substrate, protein folds, though is **not active, in the absence of disulfide bond formation, disulfide bonds are sequential**

- AppA: E. coli periplasmic protein. It **contains four disulfide bonds one of which is non-sequential**



Cytosolic disulfide bond reduction in *E. coli*

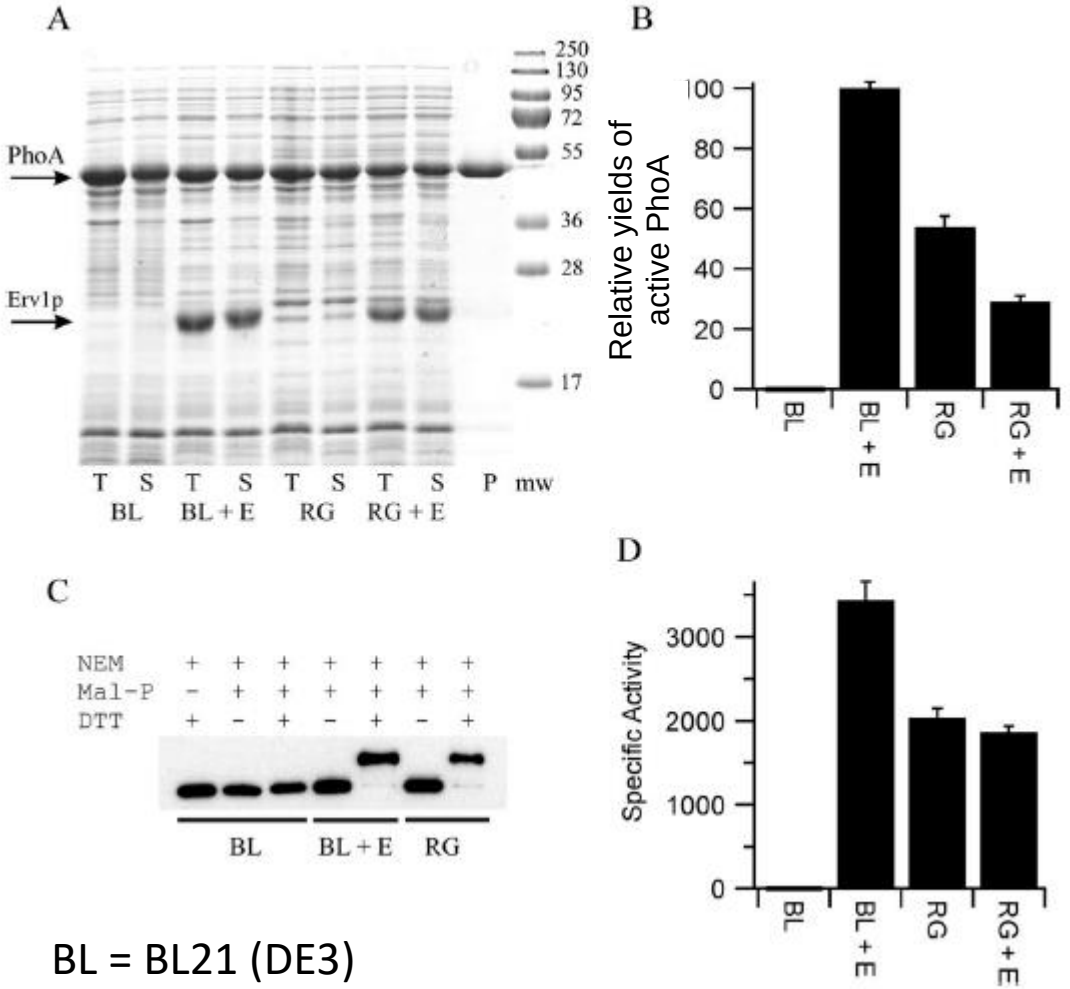


- Cytoplasmic disulfide reduction pathways
- Inactivation of *gor* and *trxB* genes enables S-S bond formation in the cytoplasm, but not S-S bond isomerization (See list of *E. coli* strains, Lecture 2)

- Erv1p is a sulfhydryl oxidase and FAD-dependent catalyst of disulfide bond formation found in the inter membrane space of mitochondria
- Introduction of Erv1p allows the efficient formation of S-S bonds in the cytoplasm of *E. coli*
- *E. coli* DsbC is a periplasmic S-S bond isomerase

Hatahet et al., 2010, Microb Cell Fact. 9:67

Production of PhoA in the cytoplasm of *E. coli*



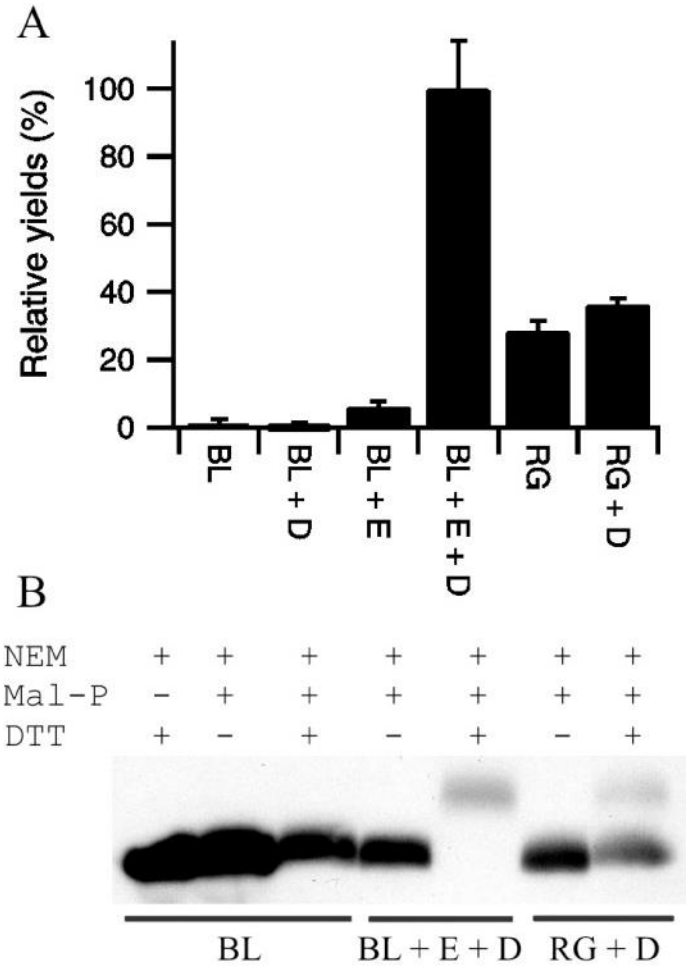
Questions:
How can a periplasmic protein be expressed in the cytoplasm?

Why do we see identical amounts of PhoA in the gel (panel A) but very different activity levels in panel B?

BL = BL21 (DE3)
RG = Δ*gor* Δ*trxB (no isomerization possible)*
E = Erv1p

Hatahet et al., 2010, Microb Cell Fact. 9:67

Production of AppA in the cytoplasm of E. coli



Questions:
What is the difference between the two model proteins used (PhoA and AppA) in terms of disulfide bonds?

Which other accessory protein function is introduced here in addition? Why?

Hatahet et al., 2010, Microb Cell Fact. 9:67

Summary protein folding

- Protein folding in pro- and eukaryotes shares many similar functions -> S-S bond formation, chaperones, PPI
- S-S bond formation is a highly conserved process