De Novo Selection of Peptides That Confer Antibiotic Resistance

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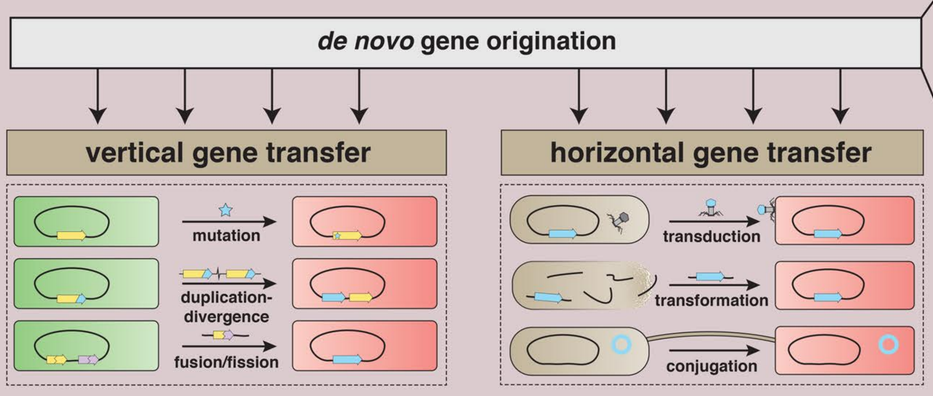
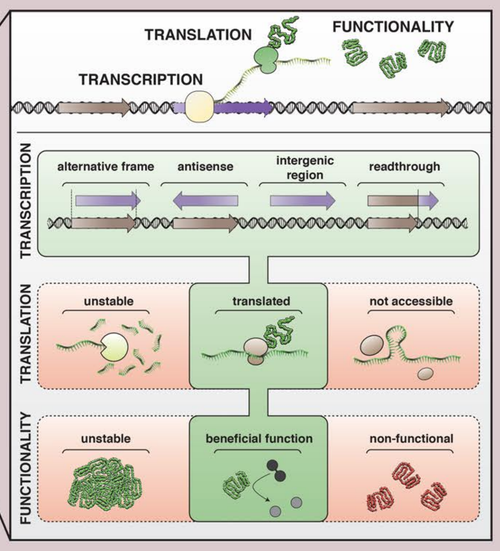
Question

How can new genes originate *de novo* (from random DNA sequences) ?





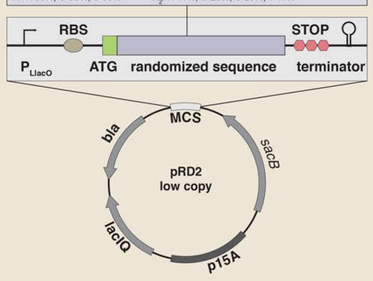
Conclusion

- Random sequences can encode functional peptides at a frequency that can be experimentally assessed
- These peptides confer resistance levels similar to chromosomal mutations
- The resistance mechanisms rely on pre-existing cellular functions
- The isolated peptides are highly hydrophobic and are predicted to interact with the membrane

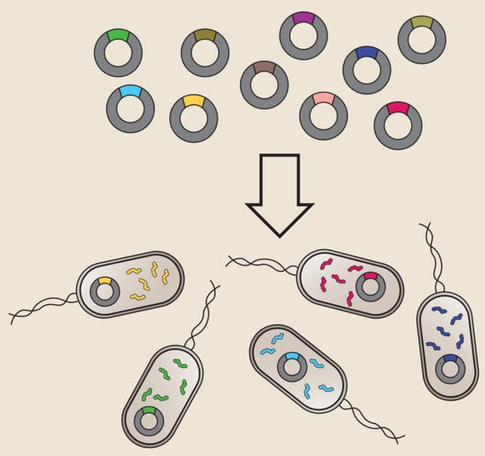
Library construction

name	size (aa)	diversity (x10^6)	sequence	#Stop	bias
rnd 10	10	1.34	..(NNB)..	1	none
rnd 20	20	1.42	..(NNB)..	1	none
rnd 50a	50	1.71	..(NNB)..	1	none
rnd 50b	50	1.04	..(VNY)..	0	primordial
rnd 50c	50	0.31	..($X_1X_2X_3$)..	<1	hydrophilic

N: A 25% G 25% C 25% T 25% Y: C 50%, T 50% X_1: G 56%, C 22%, T 22%
B: G 50% C 25% T 25% X_2: A 30%, G 31%, C 25%, T 14%
V: A 33%, G 33%, C 33% X_3: A 41%, G 23%, C 25%, T 11%



Transformation



Selection

Antibiotic resistance

Rescue of auxotrophies

Rescue of thermosensitivies

Utilization of novel carbon sources

Interaction with RNA regulation

Characterization

Is the phenotype caused by the mRNA or the encoded peptide?

Is the phenotype limited to the expression system, plasmid and/or strain selected in?

How does the resistance level compare to spontaneous resistance mutants?

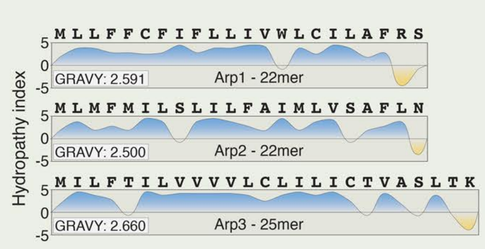
What is the mechanism of action?

Experimental Set-Up

Results

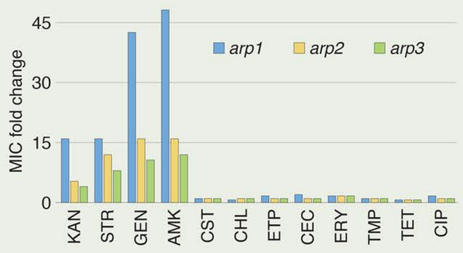
Aminoglycoside Resistance

Sequence analysis



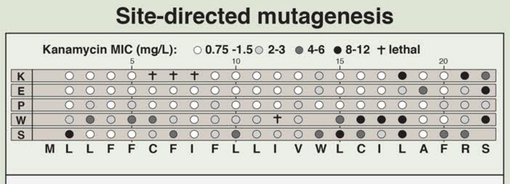
All three identified peptides (Arp1-3) are short, highly hydrophobic and predicted to be transmembrane helices

Cross-resistance



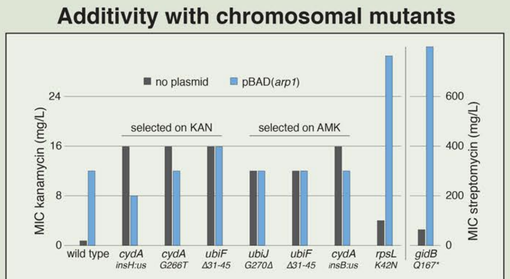
Arp1-3 confer resistance to all tested aminoglycosides, but no other antibiotics

Site-directed mutagenesis



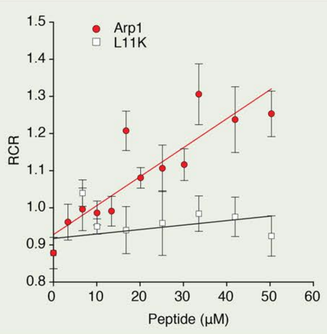
Changes to charged aminoacids (K/E) or proline (P) cause a complete loss of function in most cases

Additivity with chromosomal mutants



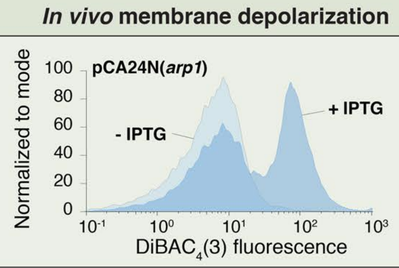
Arp1 shows additivity with target alteration mutations, but not with membrane-potential defective mutants

In vitro membrane depolarization



Arp1 causes in vitro depolarization of membrane vesicles
(Collaboration with Prof. Pia Ädelroth, Stockholm University)

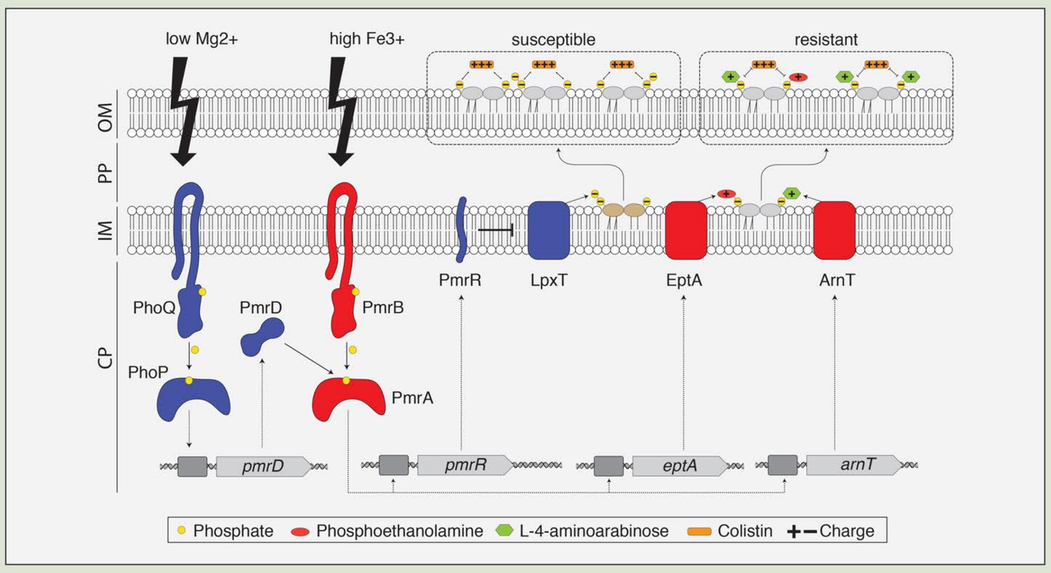
In vivo membrane depolarization



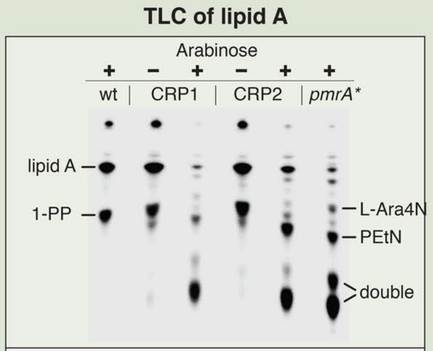
Arp1 causes an increased uptake of the membrane-potential sensitive probe DiBAC4(3) *in vivo*

Colistin Resistance

- Colistin is a last-resort antibiotic for treatment of multi-drug resistant *Pseudomonas*, *Klebsiella* and *Acinetobacter* species
- Resistant mutants typically show an overactivation of the PmrAB two-component system resulting in Lipid A modifications
- Only one plasmid-borne resistance gene has been described (mcr), an enzyme that also causes Lipid A modifications

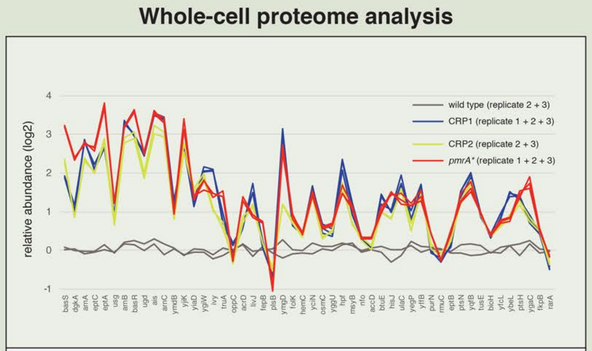


TLC of lipid A



CRP expression induces Lipid A modifications
(Collaboration with Prof. Stephen M. Trent, University of Georgia)

Whole-cell proteome analysis



Protein abundance changes caused by CRP expression are identical to a mutant with constitutive PmrA activation

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REFERENCE:

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