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)?

Vertical gene transfer
- Transformation
- Transcription
- Translation

Horizontal gene transfer
- Gene duplication
- Recombination
- Horizontal gene transfer
- Translocation

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
Transformed in E. coli expressing relevant receptor
Selection
- Mixed culture of E. coli and expressing receptor pellet

Characterization
- Antibiotic resistance
  - Rescue of auxotrophs
  - Rescue of thermosensitivities
  - Utilization of novel carbon sources
  - Interaction with RNA regulation

Aminoglycoside Resistance Results Colistin Resistance

Sequence analysis
- All three identified peptides (Ayr-1, Ayr-2, Ayr-3) are short, highly hydrophobic and predicted to be transmembrane helices

Site-directed mutagenesis
- Chang to charged amino acids (K/S), or proline (P) cause a complete loss of function in most cases

In vivo membrane depolarization
- Ayr1 causes in vivo depolarization of membrane vesicles (collaboration with Prof. Pål Assholt, Stockholm University)

Cross-resistance
- Ayr1-3 confer resistance to all tested aminoglycosides, but no other antibiotics

Additivity with chromosomal mutants
- Ayr1 shows additivity with target alteration mutants, but not with membrane-potential detecting mutants

In vivo membrane depolarization
- Ayr1 causes an increased uptake of the membrane-potential sensitive probe DIOC(5). In vivo

Colistin resistance
- Colistin is a last-resort antibiotic for treatment of multidrug-resistant Pseudomonas, Klebsiella and Acinetobacter species
- Resistant mutants typically show an overactivation of the PomAB two-component system resulting in LipA modifications
- Only one partial/mild resistance gene has been described (per), an enzyme that also causes LipA modifications

Experimental Set-Up

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REFERENCE: