Traversing the Unknown Within Mycobacterial Horizontal Gene Transfer by Assessing Recipient Mating Identity Protein Function

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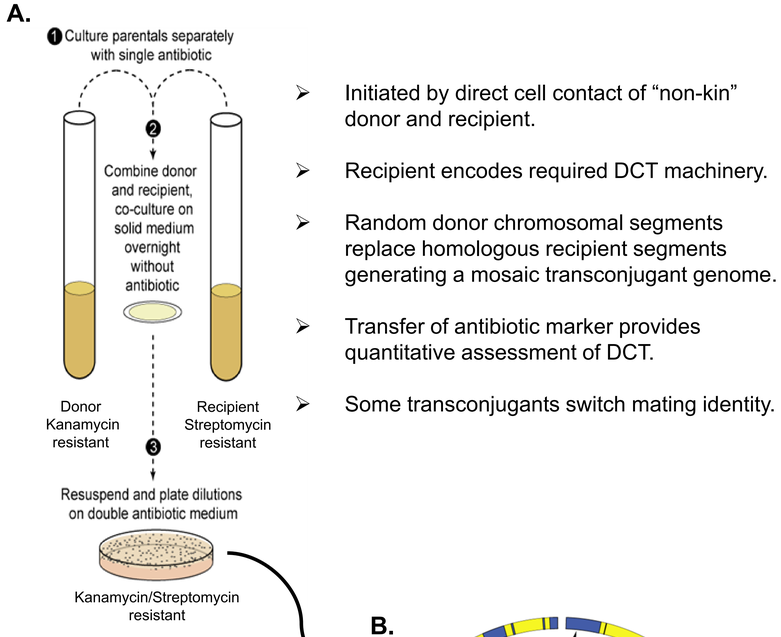
Introduction

Mycobacteria pose a substantial challenge globally due to the emergence of drug-resistant strains. Distributive Conjugal Transfer (DCT) is a unique form of horizontal DNA transfer, resulting in diverse progeny, that has been described in mycobacteria. DCT requires cell-to-cell contact and DNA is transferred from a donor to a recipient cell that are genetically distinct. DCT is permitted by the recipient mycobacteria's ability to distinguish contacting mycobacteria as "kin" or "non-kin" using hypermorphic proteins encoded by genes in a mating identity locus known as *mid*. Utilizing *Mycobacterium smegmatis* (*M. smegmatis*) as a model organism, previous studies have shown that the MidA protein determines "kin" identity in the donor partner. This research aims to determine the roles and mechanisms of the MidA protein in the recipient partner when responding to contact by a "kin" or "non-kin" donor.

Distributive Conjugal Transfer (DCT)



- Initiated by direct cell contact of "non-kin" donor and recipient.
- Recipient encodes required DCT machinery.
- Random donor chromosomal segments replace homologous recipient segments generating a mosaic transconjugant genome.
- Transfer of antibiotic marker provides quantitative assessment of DCT.
- Some transconjugants switch mating identity.

Figure 1. Schematic of a DCT assay and a transconjugant genome. (A) The DCT assay. DCT mating identity, recognition and response all occur during coculture in step (2). (B) DCT generates genomes with donor segments (non-yellow) replacing homologous recipient chromosomal regions. Random transfer of an antibiotic marker from the donor strain allows an estimation of DNA transfer efficiency by colony enumeration in step 3 of (A).

mid Locus

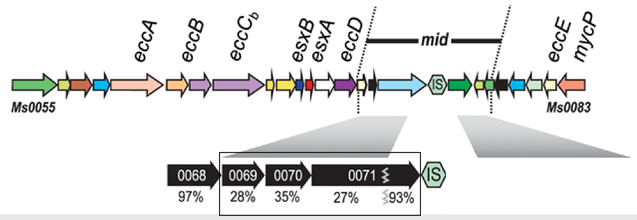


Figure 2. *mid* locus map. The *mid* locus comprises three genes *msmeg0069* (*espJ*), *msmeg0070* (*midA*), and *msmeg0071* (*espK*). These three genes within the *mid* region encode proteins that have low amino acid identity among orthologs found in other *M. smegmatis* strains (Clark et al.). We hypothesize that this amino acid sequence diversity in the *mid* locus is key to "kin" or "non-kin" recognition between contacting donor and recipient cells, determining whether DCT will occur.

MidA Structural Predictions

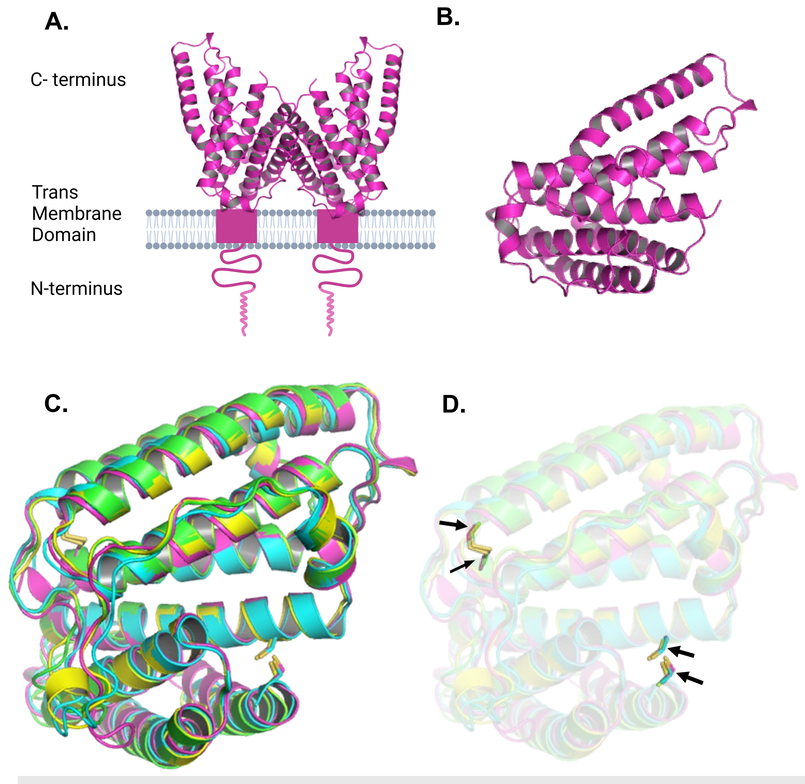
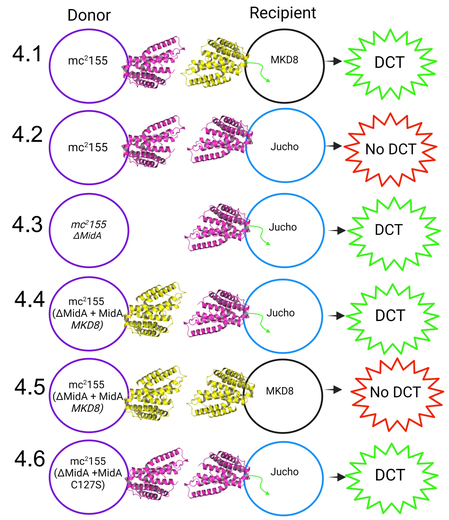


Figure 3. Structural predictions and features of MidA proteins. (A) Hypothesized structure of MidA, shown as a putative dimer. The C-terminal domain is predicted to be extra-cytoplasmic and is anchored in the membrane by a trans-membrane domain. The cytoplasmic N-terminal domain is ≈60 amino acids and is unstructured. (B) AlphaFold structure of the MidA extra-cytoplasmic C-terminal domain. (C) Superimposed MidA C-termini of hypermorphic homologs show structural conservation despite amino acid sequence divergence. (D) Identified conserved cysteines in C-terminus of MidA that are positioned to form disulfide bonds and suggest a possible role for signal transduction of "kin" recognition.

MidA Interaction Model



4.1 Wildtype donor and MKD8 recipient with non-identical MidA proteins identify as "non-kin".

4.2 Wildtype donor and Jucho recipient with identical MidA proteins identify as "kin".

4.3 Deletion of MidA protein in donor removes kin identifier allowing the Jucho recipient to recognize it as "non-kin". Compare to 4.2.

4.4 Swapping the MidA of donor with a MidA from MKD8, identifies it as "non-kin" to the Jucho recipient. Compare to 4.2.

4.5 Mating the swapped MidA donor with MKD8 such that they now have identical MidA, "kin", prevents DCT. Compare to 4.1

4.6 Mutating the conserved cysteine in donor MidA inactivates MidA- "kin" function allowing DCT with Jucho. Compare to 4.2.

Figure 4. Cells present different MidA hypermorphic proteins (purple or yellow) on their surface and interact to distinguish "kin". Recipients that detect "non-kin" will trigger DCT transcriptional responses (green arrows). Jucho and mc²155 encode identical (purple) MidA proteins.

Future Plans

Characterizing the *mid* gene operon in recipient.

- Generate precise deletions of each of the three *mid* genes in the recipient and determine whether they are required for DCT.

Dissecting the interacting hypermorphic surfaces of the C-terminus of MidA.

- Create C-terminal domain-swapped MidA proteins to map functional interfaces using DCT as a functional assay.

Define MidA protein-protein interactions.

- Perform protein pulldown assays using affinity tags to test whether MidA interacts with itself, with non-kin MidA, or with proteins encoded by flanking *mid* genes.

These findings will aid in unveiling how mycobacteria interact with one another by revealing new pathways and activities that have been hidden in monoculture studies.

References and Acknowledgements

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