Visualizing the structure and dynamics of the horizontal gene transfer during bacterial conjugation.

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Bacterial conjugation is a sophisticated process that facilitates horizontal gene transfer between bacterial cells and is also responsible for the proliferation of antibiotic resistance genes among the bacterial population. Conjugation is a process of unidirectional transfer of single-stranded DNA from donor cells to recipient cells either via a contact-dependent (tight transfer) manner or through pilus lumen in physically distant cells. The conjugational transfer of DNA is mediated by the Type IV Secretion System (T4SS), one of the most versatile and elaborate secretion systems in bacterial cell envelopes. T4SS assembles highly dynamic filamentous structures (T4SS pilus) that undergo cycles of extension and retraction. The T4SS pilus plays a major role in bringing the donor cell and recipient cell together to form a stable mating junction and it is also shown to serve as a conduit for DNA transfer in physically distant cells[1,2]. Several studies have attempted to unravel the biochemical, molecular, and structural framework of conjugation. However, none of these studies could provide a comprehensive account of the transfer of DNA over the four membranes from the donor cytoplasm to the cytoplasm of the recipient. Here we use genetics, fluorescence microscopy, cryo-light microscopy(cryo-LM), and cryo-electron tomography (cryo-ET) to investigate the molecular and structural basis of conjugation. In this study, we have successfully established cryo-light microscopy and cryo-ET workflow for contact-dependent conjugating mating pairs. Cryo-ET of the mating pair showed the tight interaction/tight junction between donors and recipient outer membranes and densities corresponding to T4SS, densities connecting outer membranes. This workflow will allow us to visualize the structure and dynamics of active DNA transfer during active bacterial conjugation.

Couturier, A. et al. Real-time visualisation of the intracellular dynamics of conjugative plasmid transfer. Nat Commun 14, 294 (2023).
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