The Leading Edge: How Plasmids Overcome Bacterial Immunity

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Horizontal gene transfer (HGT), largely mediated by conjugative plasmids, plays a crucial role in bacterial evolution and the spread of antibiotic resistance. While bacteria have evolved defense systems like CRISPR-Cas and restriction-modification (R-M) to counteract invading mobile genetic elements (MGEs), plasmids have developed counter-strategies to evade these defenses. My thesis investigates the critical role of the plasmid leading region, the DNA segment first transferred during conjugation, in overcoming host immunity. A comprehensive computational analysis revealed that leading regions harbor a remarkable diversity of anti-defense systems, including anti-CRISPRs, anti-restriction proteins, SOS-response inhibitors, and other counter-defense mechanisms. These genes often cluster into "anti-defense islands" and frequently possess ssDNA promoters, facilitating their rapid expression from the transferred single-stranded DNA before its conversion to double-stranded form. Experimental validation using the F plasmid and a targeted CRISPR-Cas9 system in *E. coli* confirmed the significance of localization of anti-defense mechanisms in the leading region for efficient conjugation. These findings underscore the strategic importance of the leading region in plasmid dissemination. Focusing research on this region offers promising avenues for discovering novel anti-defense mechanisms and developing efficient conjugative delivery systems for various biotechnological applications, including targeting antibiotic-resistant bacteria and modifying natural microbial communities.