

## **In Vivo Dynamics of Type II Plasmid Segregation Systems**

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The R1 Par operon is a self-contained plasmid partitioning system composed of three parts: parC, ParR and ParM. parC is a stretch of DNA consisting of 10 sequential repeats, each of which binds ParR. The ParR/parC complex in turn binds the actin homolog ParM. Previous studies have shown that ParM forms filaments nearly identical to those of eukaryotic actin filaments and that ParM filament bundles appear to position plasmids at each end of a rod-shaped cell. We recently demonstrated that ParM filaments are dynamically unstable and can elongate bidirectionally in vitro. These observations led to a model in which ParM filaments continually search the cytoplasm and eventually capture a ParR bound parC region on a plasmid. Insertional polymerization at the ParM/ParR interface will then push the plasmids to opposite ends of the cell and hold them in place until cell division, ensuring that each daughter cell receives a copy. To test this model directly in live cells, we used GFP labeled lacI to visualize lacO sites present on a plasmid containing a functional R1 Par operon. We observe that the plasmids make both diffusive and directed movements wherein two plasmids move away from each other very rapidly. These directed movements occur many times during a single cell division, indicating that segregation is a very dynamic process. In addition, we have visualized GFP labeled ParM filaments and observe very similar dynamics were filaments polymerize, undergo catastrophe, and rapidly depolymerize in less than a minute. We also observe the formation and dissolution of plasmid clusters. These clusters have reduced dynamics and are more frequently localized towards the center of the cell. Our results indicate that plasmid partitioning by ParM is a very dynamic process and suggest a new model for polymer-based plasmid segregation.