A well-studied model of partition is that of the *E. coli* sex factor, F plasmid. We reconstituted its partition system by introducing fluorescent SopA, SopB and plasmid (encoding sopC) into a DNA-carrying flowcell (nucleoid biomimetic) and visualized the system dynamics using TIRFM. We found SopA-ATP dynamically bound the DNA carpet, and the steady-state carpet density was lowered by SopB. When all components were infused onto the DNA carpet with ATP, the plasmids transiently bound the carpet with colocalized SopA and SopB. Over time, the static plasmids released SopA, began to wiggle, and finally popped off when little to no SopA remained. The results suggest that plasmid movement and release are coupled to SopB-stimulated ATP-hydrolysis by SopA. The tethered particle motion of some wiggling plasmid clusters produced SopA craters on the DNA carpet, suggesting that plasmid-bound SopB not only releases SopA on the plasmid but also releases nearby SopA on the DNA carpet. We propose SopB communicates with both SopA on the plasmid and nucleoid to control the transport of plasmid via an ATP-driven diffusion-ratchet mechanism.

Study of this unique transport system is vital because surface-mediated patterning as a means for cargo carrying is inherently different from the classical motor protein or the actin/microtubule-mechanisms of transport.

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Dynamic Self-Organization of Bacterial DNA Segregation Machinery in a Cell-Free Reaction
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Bacterial plasmids have evolved segregation machineries to partition replicated DNA to the daughter cells at cell division. P1 phage lysogenizes as a low-copy-number plasmid in Escherichia coli. Its partition system consists of three components, a centromere-like region, parS, an adaptor protein that binds to the centromere, ParB and a partition ATPase, ParA. In general, it is known that a ParR/parS partition complex is formed when ParB oligomerizes onto the centromere. This large nucleoprotein complex interacts with ParA and is thought to couple ATP hydrolysis to drive the movement and segregation of plasmids to opposite cell-halves. To understand ATP-driven DNA segregation, we reconstituted the P1 plasmid partition system in a cell-free reaction and visualized the spatiotemporal dynamics using TIRF microscopy. We coated a flow cell surface with non-specific DNA to mimic the bacterial nucleoid surface and flowed in the three-component reaction system. We found that ParA coats the artificial nucleoid creating a reference scaffold for plasmid movement. ParA assembles onto the ParB/parS complexes and anchors them onto the ParA-coated nucleoid surface. ParB stimulates ParA disassembly leading to vigorous Brownian motion of the plasmid as the complex loses bridging interactions with the nucleoid. The plasmid detaches from the nucleoid surface leaving a hole devoid of ParA, which is refilled rapidly with ParA rebinding onto the nucleoid. FRAP experiments demonstrate the dynamic exchange of proteins on the nucleoid surface and the partition complex. We present a Par partition model of ParB-stimulated ParA assembly/dissassembly triggering dynamic instability leading to plasmid segregation and movement.

Neutron & X-Ray Scattering
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Accurate Structures, Conformations, and Assemblies of Macronormolecules in Solution by X-Ray Scattering (SAXS) using Quality Control Parameters John Tainer.

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Conformations and assemblies of proteins, DNA, and RNA, plus their detailed structural chemistry, encode key information needed to define biological outcomes in cell biology. We are developing SAXS combined with crystallography as a premiere tool for defining macromolecular conformations and connections suitable to join proteins to pathways and at the proteomic scale. Crystallography supplies unsurpassed structural detail for mechanistic analyses. Yet, advances in SAXS are making this technique increasingly powerful and robust for efficiently examining complexes in solution, as aided by interfaces allowing molecular insight to these experiments. 3. Structures of flexible filaments like the XLF vs XRC4C1-Ligase IV-5; the conformational and flexible Rad50 link to Mre11, as well as of DNA-PKcs in complex with Ku and DNA7 support the promise of SAXS for examining the assemblies and conformations of dynamic complexes in solution. In principle, SAXS can provide reliable solution data on small and large macromolecules. 2. In practice, SAXS can be limited by problems in samples and analyses, which can be reduced or avoided. SAXS has not been perceived as a legitimate structural technique largely due to previous limitations from data collection and interpretation. Our emerging results show that SAXS has great potential to provide accurate shapes, conformations, and assembly states in solution and inform biological functions in fundamental ways. Our results furthermore show that many SAXS limitations can be overcome by improved sample characterization, quality control parameters and methods to assess model accuracy.