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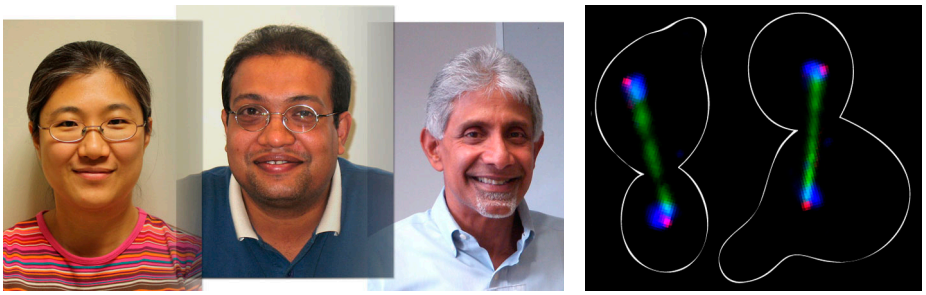
A plasmid goes motoring

Study suggests that parasitic DNA hitchhikes to its nuclear address on motor protein.

Focal Point



(L-R) Hong Cui, Santanu Ghosh, and Makkuni Jayaram are probing how the 2 micron plasmid gets into position near a chromosome before segregation. In this image of yeast cells in anaphase, one copy of the plasmid (pink) has traveled from the mother cell to the daughter. Stretching along the spindle between the cells is a chain of Kip1p motor proteins (green). The researchers' findings indicate that the protein carries the plasmid to its destination close to a chromosome.

Talk about a parasite getting a free ride from its host. As Makkuni Jayaram and colleagues show, a yeast plasmid hops aboard a motor protein that appears to truck it to a location near a chromosome. After such a move, the selfish DNA circle is poised to tag along with the chromosome during mitosis.

The 2 micron plasmid—named for its length when stretched out—resides and reproduces in most *Saccharomyces* yeast strains (1). "We call it the benign plasmid element," says Jayaram, because it strives to be a good houseguest. For example, the DNA loop minimizes the burden on its host by limiting its numbers to around 40–60 copies. Cells coerced to harbor more plasmids sicken and die (2).

During mitosis, plasmids shadow the chromosomes (3). All the plasmids in a cell are bunched up, and like chromosomes this cluster duplicates during S phase. The clusters then appear to sidle up to a chromosome and go their separate ways as the sister chromatids part and the cell divides. In this way, the plasmid is "like a micro-chromosome or even a chromosome appendage," says Jayaram. Proper positioning of plasmid clusters requires the protein cohesin (4), which fastens sister chromatids together during metaphase. Earlier work also showed that spindle disassembly bumps the plasmid from its nuclear location and halts its partitioning. So Jayaram and his team suspected that motor proteins—which attach to spindle fibers and help haul the chromosomes apart during mitosis—muscle plasmid clusters into place. To test their idea, they analyzed yeast strains lacking one of the motor proteins: Kip1p, Kip3p, Kar3p, or Cin8p.

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The team first tracked a fluorescently labeled reporter plasmid. Mitotic cells missing Kip1p almost always doled out their plasmids unequally between mother and daughter cells (5). But loss of the other three motor proteins did not cause a plasmid imbalance. Using chromatin immunoprecipitation, the researchers then discovered that Kip1p grabs the plasmid at the *STB* locus. To get a grip, though, the motor protein required help from the Rep proteins, which belong to the plasmid's partitioning system that ensures that sister clusters end up in different cells. Kip1p also brought in another crucial component—cohesin—that by holding duplicated plasmids together may ensure their placement on sister chromatids. These findings suggest that Kip1p helps organize the plasmid clusters and ensure their segregation.

Kip1p's absence left plasmids out of place, the team determined. They calculated the distance between the reporter plasmid and a cellular landmark, the spindle pole body. In cells that make Kip1p, most plasmids settle near the spindle pole body, leaving them in position to interact with chromosomes. In cells lacking Kip1p, the distance doubled.

"Kip1p is probably making sure that the plasmid is both attached to the spindle and giving it a ride along the spindle," says Jayaram. The motor protein tugs the plasmid cluster close to one of the chromosomes, the results suggest, but the importance of reaching this location isn't clear. Viral genomes latch onto chromosomes and hitchhike into daughter cells. But researchers aren't sure if the plasmid clusters do the same. The yeast nucleus is so small and the resolution of chromosomes so poor that any tether to the plasmid would be hard to spot, although Jayaram says his group would like to try. "That's a tricky experiment, but potentially do-able." Another possible explanation for the plasmid's proximity to the chromosomes is access to segregation factors that orchestrate chromatid separation during mitosis—and might help parcel out sister plasmid clusters.

That the benign plasmid uses Kip1p isn't surprising, Jayaram says. Kip1p is redundant—Cin8p can take over its job. The parasite's choice of a second-string motor protein accords with its policy of minimal impact on the host. "If you are a parasitic genome, you probably don't want to use a motor that is vital for chromosome segregation," says Jayaram.

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