Drosophila suzukii contains a peptide homologous to the *Drosophila melanogaster* Sex-peptide and functional in both species. Insect Biochem. Molec. Biol. *23*, 571–579.
17. Cirera, S., and Aguadé, M. (1998). Molecular evolution of a duplication: the SP (Acp 70A) gene region of *Drosophila subobscura* and *Drosophila madeirensis*. Mol. Biol. Evol. *210*, 247–254.
18. Nagalakshmi, V.K., Applebaum, S.W., Azrielli, A., and Rafaeli, A. (2007). Female sex pheromone suppression and the fate of Sex-peptide-like peptides in mated moths of *Helicoverpa armigera*. Arch. Ins. Biochem. Physiol. *64*, 142–155.

Zoological Institute, University of Zurich-Irchel, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
E-mail: ekubli@zool.uzh.ch

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Plasmid Segregation: Is a Total Understanding within Reach?

Recent *in vitro* and *in vivo* studies of the proteins responsible for the active partitioning of bacterial plasmids suggest that it will be possible to develop a quantitative, molecular understanding of this form of DNA segregation.

Daniel J. Needleman

The continual propagation of genetic material from one generation to the next is one of the most basic characteristics of all organisms. In eukaryotes, DNA is segregated into the two daughter cells by a highly dynamic, self-organizing structure called the spindle. While spindle formation and chromosome segregation have been intensely studied for over one hundred years, the ultimate goal of quantitatively explaining how these phenomena arise from the collective interactions of molecules seems far out of reach. Indeed, biologists are still debating basic questions, such as the existence of an organizing mechanical scaffold [1], and whether diffusible signals provide a global blueprint that determines spindle morphology [2]. In the last few years tremendous progress has been made in understanding another form of DNA segregation: the partitioning of plasmids in bacteria. Plasmids are non-essential circular pieces of DNA, some of which are actively segregated by cytoskeletal polymers that form dynamic structures analogous to the eukaryotic spindle [3]. A recent live imaging study by Campbell and Mullins [4] indicates that the structure and dynamics of these bacterial spindles can be understood in terms of the *in vitro* behavior of their constituents. This paper, combined with previous work, suggests that it will be feasible to develop a quantitative, biophysically based molecular model of a form of DNA segregation.

The most thoroughly studied plasmid partitioning system is the one responsible for segregating the 100 kilobase multidrug resistant plasmid R1. The active segregation of plasmid R1 uses no host factors and requires just three components: two proteins which the plasmid encodes, ParM and ParR, and a centromere-like DNA sequence called *parC* [3]. About five years ago, immunofluorescence of fixed cells revealed that ParM, an actin homolog, forms filaments [5] with plasmids positioned at the ends [6], suggesting that segregation is caused by ParM polymerization pushing apart plasmids. This view has been further refined through *in vitro* studies which show that while ParM filaments readily nucleate, they are highly unstable, and grow and shrink bidirectionally in an active, fluctuating manner reminiscent of microtubule dynamic instability [7]. Furthermore, ParR binds cooperatively to *parC in vitro* and the resulting complex promotes ParM assembly *in vitro* [5].

These results led to a model of segregation in which ParM filaments are continually nucleating and disassembling, searching for ParR–*parC* complexes, and when a ParM filament bridges two plasmids it becomes selectively stabilized and grows, forcing the plasmids apart [7] (Figure 1). Aspects of this model were strikingly confirmed by another *in vitro* study which demonstrated that ParM can push apart ParR–*parC* coated beads in precisely the predicted manner [8]. Thus it seems that the molecules required for the active partitioning of plasmid R1 are known [3], their structures have been determined [9–11], and their *in vitro* interactions can mimic DNA segregation [8]. But is this all really sufficient to explain what happens *in vivo*?

In the new work, Campbell and Mullins [4] directly studied the behaviors of plasmids and ParM in living *Escherichia coli* cells by using time-lapse fluorescence microscopy. They observed that short, dynamic filaments of ParM seem to grow from the sides of isolated plasmids, implying that ParM filaments are partially stabilized by their interactions with the ParR–*parC* complex *in vivo*, as had been suggested. These structures are reminiscent of the ParM asters formed around isolated ParR–*parC* coated beads *in vitro* [8]. When two plasmids come into close proximity, a ParM bundle polymerizes between them, pushing them apart. The initial encounter between plasmids occurs throughout the cytoplasm and the spindles begin growing at random orientations. The plasmids eventually find their way to opposing poles only because the growing spindle pushes against the cell sides, forcing it to align with the long axis of the cell. Precisely the same process causes *in vitro* spindles — made from two ParR–*parC* coated beads bridged by growing ParM filaments — to orient along the long axis of microchannels [8].

The authors [4] used photobleaching to show that *in vivo* spindles grow symmetrically from both ends, as occurs in the reconstituted system. After elongating for a short while, the ParM filaments suddenly undergo a catastrophic switch to shrinking, indicating that they grow by dynamic instability *in vivo* as they do *in vitro* [7]. These dynamics cause the spindles to continually fall apart and reform independently of the cell cycle, further arguing against any regulation of plasmid segregation by other factors. Amazingly, even the rates of ParM polymerization and depolymerization are similar *in vivo* and *in vitro*, but this may just be

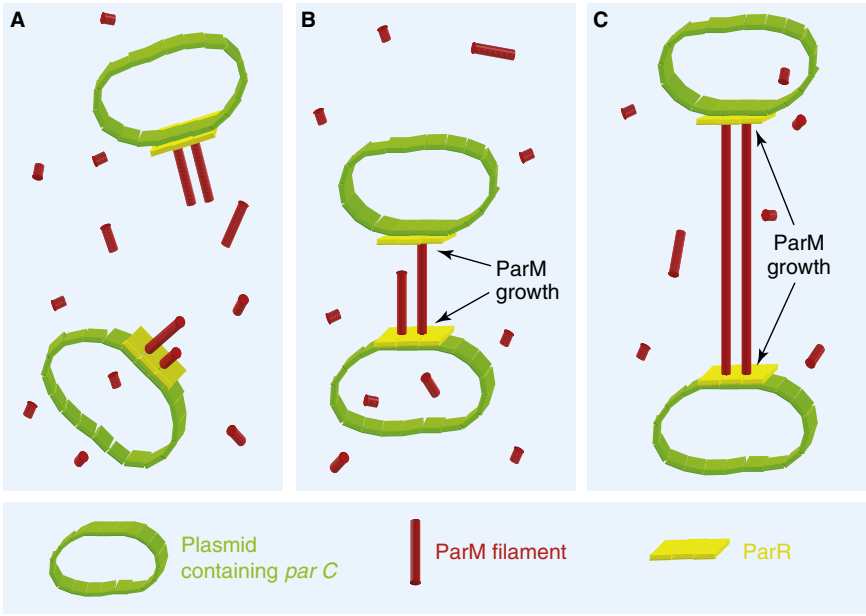

Figure 1. The search-and-capture model of plasmid R1 segregation.

(A) ParM filaments are nucleated throughout the cytoplasm and become stabilized at one end when bound to the ParR–*parC* complex. The free end of the ParM filament searches for another plasmid, but rapidly depolymerizes by dynamic instability if no successful contact is made. (B) When two plasmids come into close proximity the ParM filaments can be captured by another ParR–*parC* complex and become stabilized at both ends. (C) The growing ParM bundle pushes the plasmids apart, segregating them to opposite sides of the cell. The ParM filaments eventually depolymerize by dynamic instability, freeing the plasmids to diffuse independently, and the process repeats.

a coincidence because these values will depend on various details such as the exact ionic conditions and the concentration of inert macromolecules.

Taken together these results further support the previously developed model of R1 plasmid segregation [7] (Figure 1), and suggest that the entirety of the partitioning process can be understood in-terms of the *in vitro* properties of ParM, ParR and *parC*. Of course there are still many outstanding questions. On a biophysical level, how does ATP hydrolysis give rise to ParM dynamic instability? And how does the interaction with ParR-*parC* stabilize ParM filaments? How can one spindle consist of multiple ParM filaments, as Campbell and Mullins [4] demonstrated, and are the observed 'plasmids' actually clusters of multiple plasmids [12]? Moving up in complexity, it is not obvious that the proposed search-and-capture mechanism can account for all the observed interactions between plasmids. Naively one might expect that the probability of a successful search event would be quite low, particularly if it requires both ParR–*parC* complexes to be in the correct orientation. Furthermore, when separate plasmid foci move in close proximity they transiently diffuse together before forming a spindle, suggesting that plasmids can have some intermediate state of association between being independent and being connected by a growing ParM bundle. Finally, the ultimate question is: can knowledge of the biophysical properties of the R1 plasmid, ParM, and ParR be used to explain the statistics of plasmid partitioning, both the degree to which the *par* locus promotes plasmid stability and the incompatibility of two plasmids which carry the same centromere [13]?

In 1982, Pickett-Heaps, Tippit, and Porter began a review article [14] on eukaryotic spindles by approvingly quoting the classic manuscript, *Mitosis* [15], written 40 years earlier:

"Since about 1870 there has been a succession of periods in which triumph seemed to stand on the threshold as, first, observers of the living cell, then students of the morphology of the fixed cell, and lastly the physiologists, marshaled the evidence furnished by their different attacks…each of these periods had a corresponding aftermath of disillusion, always accompanied by a new appreciation of the difficulties of the problem."

Now, 25 years later, tools from the molecular revolution have allowed researchers to discover hundreds of proteins involved in chromosome segregation, but while a great deal has been learned, we seem barely closer to understanding the eukaryotic spindle. In contrast, the active segregation of R1 plasmids requires just three components, all of which are well studied. This simplicity — combined with the ingenuity and hard work of many investigators — is allowing researchers to begin to understand the *in vivo* behavior of segregating plasmids in terms of the *in vitro* properties of the relevant molecules. While much work remains, it seems we may finally be at the threshold of developing a quantitative, molecular understanding of some form of DNA segregation.

References

1. Travis, J. (2007). Return of the matrix. Science *318*, 1400–1401.
2. Bastiaens, P., Caudron, M., Niethammer, P., and Karsenti, E. (2006). Gradients in the self-organization of the mitotic spindle. Trends Cell Biol. *16*, 125–134.
3. Ebersbach, G., and Gerdes, K. (2005). Plasmid segregation mechanisms. Annu. Rev. Genet. *39*, 453–479.
4. Campbell, C.S., and Mullins, R.D. (2007). In vivo visualization of type II plasmid segregation: bacterial actin filaments pushing plasmids. J. Cell Biol. *179*, 1059–1066.
5. Moller-Jensen, J., Jensen, R.B., Lowe, J., and Gerdes, K. (2002). Prokaryotic DNA segregation by an actin-like filament. EMBO J. *21*, 3119–3127.
6. Moller-Jensen, J., Borch, J., Dam, M., Jensens, R.B., Roepstorff, P., and Gerdes, K. (2003). Bacterial mitosis: ParM of plasmid R1 moves plasmid DNA by an actin-like insertional polymerization mechanism. Mol. Cell *12*, 1477–1487.
7. Garner, E.C., Campbell, C.S., and Mullins, R.D. (2004). Dynamic instability in a DNA-segregating prokaryotic actin homolg. Science *306*, 1021–1025.
8. Garner, E.C., Campbell, C.S., Weibel, D.B., and Mullins, R.D. (2007). Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog. Science *315*, 1270–1274.
9. van den Ent, F., Moller-Jense, J., Amos, L.A., Gerdes, K., and Lowe, J. (2002). F-actin-like filaments formed by plasmid segregation protein ParM. EMBO J. *21*, 6935–6943.
10. Moller-Jensen, J., Ringgaard, S., Mercogliano, C.P., Gerdes, K., and Lowe, J. (2007). Structural analysis of the ParR/*parC* plasmid partition complex. EMBO J. *26*, 4413–4422.
11. Orlova, A., Garner, E.C., Galkin, V.E., Heuser, J., Mullins, R.D., and Egelman, E.H. (2007). The

structure of bacterial ParM filaments. Nat. Struct. Mol. Biol. *14*, 921–926.
12. Weitao, T., Dasgupta, S., and Nordstrom, K. (2000). Plasmid R1 is present as clusters in the cells of *Escherichia coli*. Plasmid *43*, 200–204.
13. Bouet, J.-Y., Nordstrom, K., and Lane, D. (2007). Plasmid partition and incompatibility – the focus shifts. Mol. Micro. *65*, 1405–1414.
14. Pickett-Heaps, J.D., Tippit, D.H., and Porter, K.R. (1982). Rethinking mitosis. Cell *29*, 729–744.
15. Schrader, F. (1944). Mitosis: The Movements of Chromosomes in Cell Division (New York: Columbia University Press).

Department of Systems Biology, Harvard Medical School, Boston, Massachusetts 02115, USA.
E-mail: Daniel_Needleman@hms.harvard.edu

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Animal Navigation: The Longitude Problem

Determining longitude is incredibly difficult — for humans. Are animals fooling us into thinking that they have bicoordinate maps? New experiments show that at least some creatures effortlessly solve the seemingly insoluble problem of longitude.

James L. Gould

A variety of animals regularly return to relatively precise locations after migration or displacement. This behavior seems to imply a 'map sense' from which the creatures read either absolute or relative location from at least two coordinates, though previous calibration in the target area appears crucial. Perhaps the best known homers are pigeons, which are routinely taken 100 kilometers or more from their home loft to unfamiliar locations, often in apparent sensory deprivation [1]. Upon release these experimentally convenient creatures circle and then depart in roughly the correct direction, and return home along fairly efficient routes.

As judged by tests involving flight with frosted goggles (which eliminate form vision beyond a few centimeters), the accuracy of pigeons is on the order of one or two kilometers. The amazing — to some, literally incredible — precision of this homing suggests to many that the behavior cannot plausibly depend on a bicoordinate map. Because longitude in particular is so difficult to measure, there is an eerie attraction to the idea that pigeons are fooling us; perhaps their purported maps are, after all, a romantic illusion in the minds of scientists. And if pigeons do not need maps, quite possibly no animals have them. But two new papers [2,3] show that migrating birds do in fact somehow measure longitude, as also apparently (over shorter distances) do sea turtles [4], spiny lobsters and newts [5].

Essentially all the evidence on map use comes from displacement tests, in which the animals are captured and transported to a 'release' site. The creatures may then be tracked, or recoveries near the goal logged, or the early visual or radio vanishing bearings recorded, or the attempts to escape from a cage monitored and averaged. Regardless of what is measured, recovery from displacement is different from migrational navigation in two fundamental ways: displacements generally require the animal to return from shorter distances; and they do so after a shorter interval of only a few hours or perhaps days. The extent to which experiments involving reduced temporal and geographic scales call upon the same mechanisms at work in migration is an important question. Unlike pigeons, displaced migrants are rarely tracked, and even when they are, the accuracy of any map sense can be hard to judge (there being no equivalent to goggles to eliminate local cues, or even in some cases any very clear idea of the exact target — at least in the mind of the experimenters). Moreover, judging longitude for humans depends critically on accurately measuring time differences between the release site and the goal; time intervals are infinitely easier to estimate for a creature displaced only the previous day compared to one migrating home after several months away from the goal.

For human navigators, map position is specified by latitude, the angular distance from the equator, and longitude, the angular distance from an arbitrary reference point — the Greenwich meridian for most of the world. Animals, of course, might use another grid pair, or (being notorious for redundancy and back-up systems) employ more than two parameters.

Latitude is fairly simple to judge, and there is good evidence that animals have this variable well under control [6]. The elevation of the pole point at night gives the latitude directly; memorizing the constellations allows at least some species to infer the pole point through broken clouds. Human navigators of the past determined latitude from the elevation of the sun at solar noon. They then compared the reading to a table of values for each date, which is necessary to correct for seasonal variations in the apparent path of the sun though the sky. (For animals lacking innate tables, comparing ever-changing solar elevations in two locations to judge displacement would be useful over an interval of only a few days.) Polarization patterns allow at least some species to infer the sun's position when it is hidden from direct view, and in theory the pole point is evident in daylight as the spot in the sky around which the vectors representing the polarization of the sunlight by the sky rotate. Latitude can also be read from magnetic inclination, though there is some inaccuracy because the North magnetic pole lies about 800 kilometers from the geographic pole (near Ellesmere Island in Canada); moreover, the magnetic pole drifts 10–40 kilometers annually. As a cue for use at least hundreds of kilometers away from the poles (95% of the earth), or in a familiar area, magnetic latitude is generally reliable.

Longitude, by contrast, is very much harder to determine. Early navigators were often reduced to the inefficient expedient of sailing north or south to the desired latitude, and then east or west ('westing') to the target. In the early 1600s the British instituted a competition for a practical method of determining the longitude; it generated much interest, but little real progress for about a century. The essential problem (at least as our species conceives it) is in determining the local time and comparing it to the time at the