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MicroCommentary

Cell division without FtsZ – a variety of redundant mechanisms

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Until 1998 it looked like all bacteria and archaea used a universal cytokinetic machine based on FtsZ. A dozen completely sequenced bacterial genomes all had an *ftsZ* gene, as did the several sequenced archaeal genomes. Then in 1998–1999 two species of *Chlamydia* were sequenced and found to have no *ftsZ* (Stephens *et al*., 1998; Kalman *et al*., 1999). Enthusiasts of FtsZ could hold out some hope for its primacy by thinking that these obligate parasites might use some host machinery for division. But the next year the genome of *Aeropyrum pernix*, a free living thermophilic archeon, was found to be without *ftsZ* (Kawarabayasi *et al*., 1999). Additional sequences suggested that the entire kingdom of *Crenarchaea* managed life and cell division without FtsZ. Among the bacteria the following are now known to have no *ftsZ*: the phylum *Planctomycetes* (Pilhofer *et al*., 2008), which is related to *Chlamydiae* but is free-living; *Calyptogena okutanii* (Kuwahara *et al*., 2007) and *Carsonella ruddi* (Nakabachi *et al*., 2006), both intracellular symbionts; *Ureaplasma urealiticum* (Glass *et al*., 2000) and *Mycoplasma mobile* (Jaffe *et al*., 2004). Since all of these prokaryotes divide, there must be mechanisms for cell division that are not based on FtsZ.

ESCRT-III based division

Ten years after the genome sequence of *A. pernix* was published, two groups discovered that the division mechanism of *Crenarchaea* is based on a completely different cytoskeletal system. Both groups studied *Solfolobus acidocaldarius*, and began by screening for genes that were upregulated near the time of cell division. Lindas *et al*. (2008) discovered a three-gene operon that they named *cdv* (for cell division). One of the genes was a homologue of the eukaryotic ESCRT-III, which is involved in vesicle trafficking and membrane scission events (Wollert *et al*., 2009a). In addition to the *ESCRT-III* gene in the *cdv* operon, *S. acidocaldarius* had three additional *ESCRT-III* homologues, a duplication similar to that in eukaryotes (yeast has six). Another gene in the *cdv* operon was a homologue of eukaryotic Vps4, which operates to disassemble ESCRT-III complexes. The Cdv proteins localized to a mid-cell band that appeared to constrict as the cells neared division. In the second study Samson *et al*. (2008) focused from the beginning on the *ESCRT-III* genes as potential replacements for the FtsZ division system. They found that all four *ESCRT-III* homologues were highly expressed near cell division. They identified pairwise interactions between several of the four ESCRT-III homologues. One of the *ESCRT-III* proteins interacted directly with the Vps4 protein, and a crystal structure of the interacting fragments was obtained.

The ESCRT-III proteins form thin filaments that bind to cellular membranes in various curved arrays (Hanson *et al*., 2008). A eukaryotic ESCRT-III system has recently been reconstituted *in vitro* (Wollert *et al*., 2009b). When the ESCRT-III proteins were added to the outside of giant unilamellar vesicles, they caused smaller vesicles to invaginate and pinch off. The mechanism seems to involve curved filaments of ESCRT-III binding to membranes and bending them (Hanson *et al*., 2008; Wollert *et al*., 2009b). This is similar to the mechanism proposed for the constriction force of FtsZ (Osawa *et al*., 2009). However, the three-dimensional structure of the ESCRT-III subunits is completely unrelated to that of FtsZ. This is apparently an example of convergent evolution of mechanical filaments that generate bending forces on membranes.

Traction-mediated cytofission

For almost all bacteria tested, *ftsZ* is an essential gene, as expected given its major role in cytokinesis. However, *ftsZ* is not essential for vegetative growth of *Streptomyces coelicolor* (McCormick *et al*., 1994). This species grows as long hyphae, with only occasional septa. These septa are not formed in the *ftsz* null, and are apparently not essential for vegetative growth. Although *S. coelicolor* normally uses FtsZ for division during vegetative growth, it can

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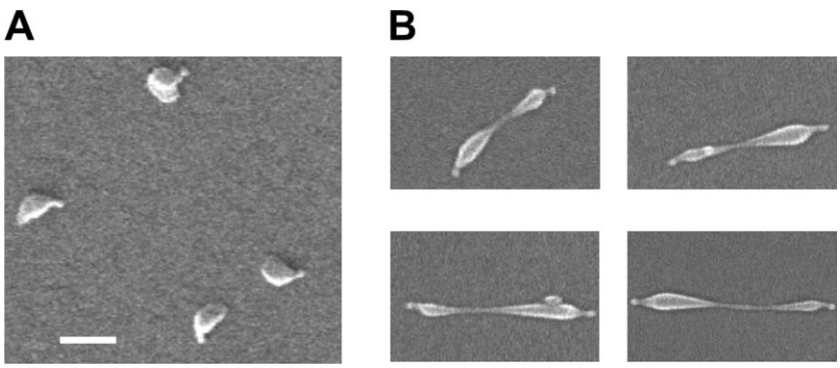

Fig. 1. Scanning electron microscopy images of *Mycoplasma genitalium* G-37 wild-type strain (A) and *ftsZ* null mutant strain (B). The four pictures in (B) show coupled cells linked by a thin filament and exhibiting terminal organelles at both ends. The morphology suggests that these FtsZ-deficient cells are in the late stages of cytokinesis (see Lluch-Senar *et al*., 2007). All images are at the same magnification (scale bar, 500 nm).

apparently live without it by using mechanical breakage of the long hyphae as a crude form of cell division. On the other hand, *ftsZ* is essential for sporulation. When induced to sporulate, *S. coelicolor* assembles multiple Z rings that divide the hyphae into separate spores.

In this issue of *Molecular Microbiology*, Lluch-Senar, Querol and Piñol report their discovery that *Mycoplasma genitalium*, which has an *ftsZ* gene that was previously thought to be essential, can grow and divide quite well without it. This was surprising because an early search using global transposon mutagenesis failed to find any transposon insertion in *ftsZ*, consistent with its being essential (Glass *et al*., 2006). However, in a later survey using a different transposon Lluch-Senar *et al*. (2007) discovered a fully viable strain of *M. genitalium* in which a transposon truncated 60% of FtsZ. This was strong evidence that *ftsZ* is not essential, and that the previous failure to find any insertion was due to that transposon failing to find a favourable DNA sequence. The Piñol group pursued this discovery in their paper in this issue by preparing additional strains in which the *ftsZ* gene was completely deleted. The strain lacking FtsZ had growth kinetics that was identical to the wild-type. They then went on to determine how the cells divide without FtsZ.

The cells apparently divide by using their motile apparatus to pull themselves apart. *M. genitalium,* and some other *Mycoplasma* species, grow as adherent cells, attached to a plastic or glass substrate. While attached they undergo vigorous gliding motility across the substrate. A key to the adhesion and gliding motility is a membrane protrusion called a terminal organelle or attachment organelle. Wild-type cells are mostly rounded, with a single terminal organelle protruding on the leading edge (Fig. 1A). Cells lacking FtsZ are frequently very elongated, with a pair of cells connected by a thin extension (Fig. 1B). The two terminal organelles are pointed in opposite directions, suggesting that gliding motility is pulling the cells apart. Eventually, the thin cytoplasmic bridge ruptures and the membranes heal. Wild-type cells appear to break this bridge much more efficiently, since they are seen paired less frequently. However, the longer time spent in division by the *ΔftsZ* cells does not affect their overall growth rate.

A similar division mechanism was discovered in the eukaryote *Dictyostelium* when its myosin II gene was knocked out (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987). Cytokinesis of eukaryotes is based partly on constriction of an actin-myosin band, and this mechanism was inactivated by the knockout. When grown in suspension the knockout cells became very large and multinucleate, and eventually lysed. However, when these cells were adherent to a substrate they could divide almost as well as wild-type. The division mechanism, called 'illegitimate cell division' or 'traction-mediated cytofission,' appeared to involve two sides of a cell crawling in opposite directions until they mechanically ruptured. Later studies showed that this was only part of the story, since the process of adhesion restored many features of normal furrow formation (Gerisch and Weber, 2000). In *M. genitalium* the mechanism seems much simpler. Traction-mediated cytofission appears to be the complete mechanism for cell division in cells lacking FtsZ.

A *Bacillus subtilis* L-form divides without FtsZ

Leaver *et al.* (2009) created an L-form of *B. subtilis*, a strain that grows without a cell wall. The L-form grew as large and more or less round cells, which occasionally spun off smaller progeny. These pleiomorphic cells might have provided an interesting tool to see how FtsZ functioned inside this new structure. However, when the authors imaged FtsZ–GFP they found a variety of filaments and arcs, but no Z rings. They then made the surprising discovery that the L-form cells could be depleted of FtsZ and this had no effect on their growth and division. Detailed observation led to the proposal that these L-form cells propagate by a novel mechanism that the authors called 'extrusion and resolution.' The cells first increased in size, and then started forming transient blunt protrusions on the surface. These appeared and retracted, and eventually one of them grew into a long, pseudopod-like protrusion. Then, rather abruptly, the pro-

trusion resolved by cleaving into several small, round bodies – the progeny.

This strange division mechanism has two steps, each posing a mechanical question. First, what drives the extrusion of the pseudopod? One possibility is an active chromosome separation system, where the membrane is pushed out by the separating nucleoids. Second, what causes the pseudopod to resolve into small round cells? The authors suggest collapse and resealing of the membrane as a likely mechanism.

We would suggest one additional feature for this mechanism. As a round cell grows larger, its surface area increases as the 2/3 power of its volume. If the cell is programmed to produce membrane at the same rate as its mass increases, as it would for a rod, then the large round cells will accumulate extra membrane. This excess membrane may be the source for the initial blunt protrusions, the pseudopod itself, and finally the extra membrane needed for collapse of the pseudopod and resealing to resolve the progeny.

Excess membrane in *Listeria monocytogenes* L-forms and round *Escherichia coli*

An L-form created from *L. monocytogenes* showed a bizarre form of propagation that may also utilize excess membrane of the large spherical cells (Dell'Era *et al*., 2009). In this case the large spheres did not form protrusions, but invaginated vesicles internally. Somehow these vesicles engulfed the nucleoids but not the cytoplasm, which was labelled with GFP. Eventually, the mother cell membrane disintegrated, releasing the vesicles, which then became active in gene transcription. It is not known if FtsZ or MreB was used in any step.

Bendezú and de Boer (2008) created mutants of *E. coli mre* genes that caused the cells to grow as spheres. They noted that the spheres would have decreased surface area relative to rods, and as the cells grew larger their surface to volume would continually decrease. They found that membrane production was not downregulated in the spheres, meaning that excess membrane accumulated. The excess membrane appeared as invaginations of the cell membrane into the interior, which sometimes pinched off as internal vesicles. The excess membrane accumulated aberrant FtsZ structures, which may have depleted the supply, since cells needed excess FtsZ to divide in most conditions.

Redundancy and evolution

Redundant systems are frequent in biology. It is sometimes suggested that this provides a backup in case of failure of one system. However, we think that in most or all cases the redundant systems are used together to make the overall process more efficient. In the case of *M. genitalium*, the cells can divide quite efficiently using traction alone, but they spend a lot more time resting stationary in the division stage. FtsZ greatly speeds this part of the cell cycle. Also, Lluch-Senar *et al*. (2010) in this issue suggest that as the *Mycoplasma* cells are engulfed in the host vesicles they may pass through a phase where there is no substrate for adhesion. Then the FtsZ-mediated division may be essential.

When two redundant mechanisms can each complete the function alone, even if less efficiently, we should expect to find cases where one evolves to be so efficient that the other is discarded. *M. mobile* has apparently discarded FtsZ, and may rely on traction-mediated cytofission for cell division. *Chlamydiae* and *Planctomycetes* also probably had FtsZ originally and discarded it, since the genomes of some species have *murC, ddl* and *FtsQ,* genes that typically cluster with *ftsZ* (Pilhofer *et al*., 2008). What replaced the FtsZ-based mechanism is still not known.

The ESCRT-III system also likely originated as redundant with FtsZ. Some low-temperature *Crenarchaea*, which share some characteristics of *Euryarcheae,* have both *ftsZ* and *cdv* genes (Lindas *et al*., 2008). We propose that FtsZ was the primordial system because the lack of tryptophan in most FtsZ's suggests that it is an ancient protein (Davis, 2002; Erickson, 2007). In this scenario all bacteria and archaea would have used FtsZ for division in early evolution. A precursor of the *Crenarchaea* then acquired an *ESCRT-III* gene, and this gene evolved into the multiple proteins of the modern filamentous complex. Its early functions were probably not in cell division, but when it evolved the ability to make filaments that could bend the membrane, it could assist FtsZ in cell division. Eventually, the ESCRT-III system became so efficient that FtsZ was discarded by most *Crenarchaea*.

Looking back even farther in evolution (Leaver *et al*., 2009) suggested that extrusion–resolution may have been the earliest mechanism of cell division. This is a very attractive idea, and we would add the speculation that this mechanism may require only that the cell be able to synthesize extra membrane over what is needed for the surface area of the sphere. That this mechanism can still function in the *B. subtilis* L-form probably attests to the simplicity of a system that may depend only on the synthesis and mechanics of membranes.

In this scenario FtsZ would have evolved later, and when it did it provided such an efficient division machine that it now dominates the bacterial and archaeal domains of life. But FtsZ systems later acquired redundant partners. One of these was the ESCRT-III system; another was traction-mediated cytofission. No doubt other cell division systems are still awaiting discovery in various bacteria without FtsZ.

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