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An Expanded View of the Eukaryotic Cytoskeleton

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An expanded view of the eukaryotic cytoskeleton

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ABSTRACT A rich and ongoing history of cell biology research has defined the major polymer systems of the eukaryotic cytoskeleton. Recent studies have identified additional proteins that form filamentous structures in cells and can self-assemble into linear polymers when purified. This suggests that the eukaryotic cytoskeleton is an even more complex system than previously considered. In this essay, I examine the case for an expanded definition of the eukaryotic cytoskeleton and present a series of challenges for future work in this area.

INTRODUCTION

Textbooks, lectures, and a vast array of articles collectively define the cytoskeleton as a system of filaments and tubules within cells. The three well-established components of the eukaryotic cytoskeleton are actin filaments, microtubules, and intermediate filaments (Pollard and Earnshaw, 2002). In addition, septin filaments are present in nearly all eukaryotic cells, with the notable exception of plants, and thus represent a potential fourth component of "the cytoskeleton" in eukaryotes (Mostowy and Cossart, 2012). Two common themes linking these cytoskeletal proteins are 1) their ability to self-assemble into linear polymers in vitro and 2) their presence as linear polymers in cells. In many cases, these linear polymers are further organized into higher-order structures by cytoskeletal-associated proteins. These cytoskeletal systems, as both single polymers and high-order structures, have the capacity to generate and/or resist mechanical forces in a cell, leading to roles in an endless list of cellular functions.

Are these properties of the cytoskeleton limited to only four polymer systems? Or are these the first four systems to be uncovered? Recent studies have identified additional proteins that fulfill the criteria of self-assembly into linear polymers in vitro and in cells and have their own associated regulatory proteins. These proteins meet the basic criteria for definition as cytoskeletal polymers, suggesting the possibility that "the cytoskeleton" may be a far more complex system of filaments and tubules than previously realized. It is worth noting that this concept has also been considered for the growing number of filament-forming proteins in bacterial cells (Ingerson-Mahar and Gitai, 2012; Pilhofer and Jensen, 2012). Here I examine the evidence for such new polymer systems as part of an expanded eukaryotic cytoskeleton. This leads to a series of challenges for a comprehensive understanding of the components and functions that underlie the eukaryotic cytoskeleton.

CYTIDINE TRIPHOSPHATE SYNTHASE

Two concurrent studies demonstrated that the conserved metabolic enzyme cytidine triphosphate synthase (CtpS) assembles into filaments in bacteria, yeast, flies, and mammals (Figure 1; Ingerson-Mahar et al., 2010; Noree et al., 2010). This indicates that CtpS filaments may be part of the cytoskeleton in both prokaryotic and eukaryotic cells. Purified bacterial CtpS forms stacked filaments that are roughly 200–400 nm long (Ingerson-Mahar et al., 2010). In bacteria, these filaments function in maintenance of cell shape (Ingerson-Mahar et al., 2010), but a structural role in eukaryotic cells has not been identified. In eukaryotes, CtpS filaments form in neuronal axons but not dendrites, and their assembly depends on growth conditions in yeast (Noree et al., 2010). Thus these filaments may function as classical cytoskeletal components at specific times and places in the cell, perhaps in response to extracellular cues. The role of environmental conditions in filament dynamics will be important in the search for new cytoskeletal polymers. Indeed, CtpS filaments were not observed in initial screens of green fluorescent protein (GFP)-tagged yeast proteins, but only after more detailed observations under multiple growth conditions. Such observations uncovered a number of additional proteins that appear to form filaments (Noree et al., 2010), suggesting a common theme.

Filament assembly by a metabolic enzyme raises the potential for regulatory feedback in pathway signaling. Consistent with this idea, CtpS filament formation is stimulated by cytidine triphosphate (Noree et al., 2010), the end product of its enzymatic activity. Remarkably, a vast number of metabolic enzymes have been shown

In cells   *in vitro*
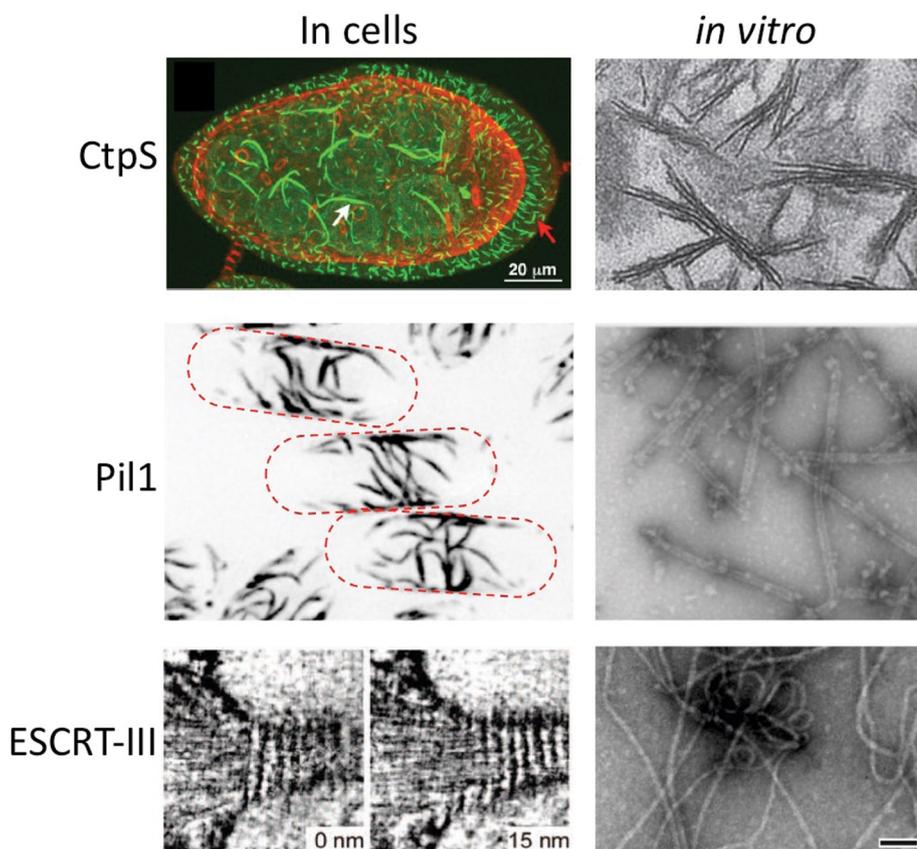
CtpS

Pil1

ESCRT-III

FIGURE 1: Nontraditional cytoskeletal filaments in vitro and in cells. Top, cytidine triphosphate synthase filaments (green) in the *Drosophila* egg chamber and electron microscopy (EM) of purified *Escherichia coli* CtpS filaments. Middle, endogenous Pil1 filaments in outlined *S. pombe* cells and EM of purified recombinant Pil1 tubules. Bottom, electron cryotomography of helical ESCRT-III–dependent filaments at abscission site and EM of purified Snf7 ("activated" R52E mutant). (Images reprinted with permission from Ingerson-Mahar *et al.*, 2010; Noree *et al.*, 2010; Guizetti *et al.*, 2011; Kabeche *et al.*, 2011; Henne *et al.*, 2012.)

to form reversible large-scale structures, suggesting that CtpS filaments may be just the tip of the iceberg (Narayanaswamy *et al.*, 2009). It is unknown whether these structures are cytoskeletal or contain self-assembling properties, but this hints at general mechanisms that might operate in many cell types. Future work might determine how the biophysics of CtpS filaments relates to enzyme regulation in cells. For example, does tension applied to CtpS filaments affect stability and enzyme availability in cells? Do CtpS filaments generate force to support cell structure specifically when enzyme function is not needed? Moreover, how do environmental cues direct regulation of filament dynamics, such as assembly and disassembly? The discovery of CtpS filaments opens intriguing links between cytoskeletal filaments and cell metabolism.

PIL1 EISOSOMES

Eisosomes are linear structures at the plasma membrane of yeast cells (Douglas *et al.*, 2011; Olivera-Couto and Aguilar, 2012), and the core eisosome protein Pil1 self-assembles into 30-nm-wide tubules in vitro (Figure 1; Kabeche *et al.*, 2011; Karotki *et al.*, 2011; Olivera-Couto *et al.*, 2011). Pil1 tubules that line the cell cortex are independent of actin and microtubules and form dramatic invaginations of the plasma membrane that have been evident in freeze-fracture electron microscopy for decades (Moor and Muhlethaler, 1963; Stradalova *et al.*, 2009). These structures are strikingly static in cells (Walther *et al.*, 2006; Kabeche *et al.*, 2011). In many yeast species, a second

Pil1-related protein called Lsp1 also self-assembles into tubules in vitro and participates in the formation of eisosome invaginations in cells (Walther *et al.*, 2006; Karotki *et al.*, 2011). A recent crystal structure identified Pil1 and Lsp1 as members of the BAR-domain family of membrane-remodeling proteins (Ziolkowska *et al.*, 2011). These dimeric proteins are banana shaped, and their inner curvature binds to lipids with the potential to generate lipid tubules (Suetsugu *et al.*, 2010). Indeed, incubation of purified Pil1 with liposomes generates elongated lipid tubules that are encased by Pil1 (Karotki *et al.*, 2011; Olivera-Couto *et al.*, 2011). This indicates that Pil1 self-assembles into cytoskeletal polymers that can "trap" lipids in an elongated tubule. Self-assembly in both the presence and absence of lipids has been observed for at least one other BAR-related protein—the human protein FBP17 (Itoh *et al.*, 2005)—suggesting that mammalian cells may use these proteins as an extended cellular cytoskeleton. The formation of Pil1-lipid tubules in vitro presents an intriguing paradox when compared with the extended half-tubules that Pil1 forms in cells. What prevents the closure of Pil1 tubules in cells? Regulatory proteins might influence the assembly dynamics and structure of Pil1 tubules in cells, but additional regulation from the partitioning of specific lipids might maintain this remarkable geometry in cells. The distinction between Pil1 polymers in vitro and in cells represents a great unknown for this cytoskeletal structure, particularly because they must withstand the immense turgor pressure of the intracellular yeast environment.

The major open question for Pil1 polymers is their cellular function. Deletion of Pil1 does not affect cell growth under a wide range of conditions in most yeast species, including *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. This is quite surprising because Pil1 is one of the most abundant proteins in a yeast cell, and the cytoskeletal structures that it forms are striking and dramatic. Although initially believed to mark sites of endocytosis (Walther *et al.*, 2006), careful follow-up studies demonstrated that Pil1 structures do not directly function or associate with endocytosis (Brach *et al.*, 2011). Instead, the function of these polymers might intersect with signaling pathways, similar to CtpS filaments. Pil1 polymers bind the phospholipid phosphatidylinositol 4,5-biphosphate (PI(4,5)P$_2$) and show genetic interactions with factors that control this lipid (Karotki *et al.*, 2011; Olivera-Couto *et al.*, 2011). In addition, Pil1 polymers were recently proposed to provide spatial control of ceramide synthesis signaling (Berchtold *et al.*, 2012). The position and geometry of Pil1 half-tubules in cells raise the possibility for mechanosensing, analogous to caveolae in animal cells (Sinha *et al.*, 2011). Force-induced breakage of Pil1 half-tubules would release a pool of PI(4,5)P$_2$ and dramatically alter the plasma membrane landscape. As more cellular functions of Pil1 emerge, it will be important to understand how the generation and/or resistance of force by Pil1 polymers can contribute to cellular phenotypes.

ENDOSOMAL SORTING COMPLEX REQUIRED FOR TRANSPORT-III

The conserved endosomal sorting complex required for transport (ESCRT) machinery organizes endosomal trafficking and the formation of multivesicular bodies through several distinct multiprotein complexes (Hurley, 2010; Henne *et al.*, 2011). Within the ESCRT-III complex, one component (Snf7 in yeast; CHMP4A,B,C in humans) displays the properties of a cytoskeletal polymer. Specifically, Snf7 self-assembles into filaments both in vitro and in cells (Figure 1; Ghazi-Tabatabai *et al.*, 2008; Hanson *et al.*, 2008; Guizetti *et al.*, 2011; Henne *et al.*, 2012). A series of elegant studies elucidated key steps in the biochemical regulation of Snf7 polymerization dynamics (Saksena *et al.*, 2009; Teis *et al.*, 2010). Reminiscent of actin and microtubule dynamics, regulatory proteins control the nucleation/assembly (Vps20), stability (Bro1/Alix), capping (Vps24), and disassembly (Vps4) of Snf7 polymers (Henne *et al.*, 2011). The depolymerization of Snf7 filaments by the ATPase Vps4 is particularly noteworthy, as this ATP-dependent step can generate mechanical force for biological functions (Lata *et al.*, 2008). In the "classic" function for ESCRT-III, Snf7 filaments are connected to lipids at the neck of intralumenal vesicle, and their regulated disassembly leads to vesicle scission and/or subunit recycling (Wollert *et al.*, 2009; Henne *et al.*, 2012).

As with other cytoskeletal polymers, ESCRT-III filaments have been functionally repurposed to take on additional force-generating tasks. CHMP4B, a human version of Snf7, is required for cell abscission during the final stages of cytokinesis. Accordingly, filaments that bear a strong resemblance to CHMP4B appear in a spring-like structure during the abscission process (Guizetti *et al.*, 2011). These in vivo filaments have not been formally identified as CHMP4B, but all evidence points to an ESCRT-III spring that pinches apart dividing cells, potentially through ATP-dependent depolymerization. From a cytoskeletal perspective, it is fascinating to consider how this novel polymer structure cooperates with the actomyosin machinery, which drives ingression of the cytokinetic furrow but not complete abscission. This role may reflect an ancient mechanism, as some Archaea use the ESCRT-III machinery to drive cell division (Lindas *et al.*, 2008). ESCRT-III is also a major target of the NoCut pathway, which prevents final abscission in the presence of lagging mitotic chromosomes (Carlton *et al.*, 2012).

The ability of ESCRT-III filaments to generate force has also been harnessed against cells by viruses that hijack the machinery for membrane scission during viral budding (Carlton, 2010). This bears resemblance to cellular pathogens that use other cytoskeletal polymers to drive host invasion. Of interest, these additional functions for Snf7/CHMP4 filaments, which also include authophagy (Hurley, 2010), use some of the same regulators (e.g., Vps4) but not the entire ESCRT machinery (Carlton, 2010). This functional coopting of a self-assembling filament system might relate to how other cytoskeletal systems (e.g., actin, microtubules, etc.) could have evolved a wide range of cellular activities from a single ancestral function.

ONGOING AND FUTURE CHALLENGES

The system of self-assembling filaments and tubes within a eukaryotic cell is not limited to actin, microtubules, intermediate filaments, and septins. Instead, the cytoskeleton appears to represent an extensive collection of diverse polymers with the potential to generate mechanical force and linear spatial information. Two broad challenges exist for understanding the complexity of an expanded eukaryotic cytoskeleton. First, additional filament systems are likely to exist in cells and remain to be identified. Hints of such additions exist, such as the budding yeast protein Fin1, which forms spindle-like structures in cells and assembles into filaments in vitro (van

Hemert *et al.*, 2002, 2003). It is also worth noting that prions and other amyloid-like polymers display many characteristics of cytoskeletal polymers. In many cases, the diffraction limit of light microscopy has precluded the identification of filament-like structures in cells. Increased use of superresolution light microscopy has the potential to uncover such structures, in particular when combined with high-throughput imaging of GFP-tagged protein collections. In addition, electron cryotomography has shown the presence of new filament systems in bacteria (Briegel *et al.*, 2006; Jensen and Briegel, 2007). This technique is limited by specimen depth but has the potential to uncover novel subcellular structures at near-endogenous state. Application of these high-resolution methods to cells will provide new insights into the expanded cytoskeleton.

A second major challenge is to understand the role of polymer biophysics in the cellular function of new cytoskeletal structures. Force generation by polymers can be coupled to both polymerization and depolymerization, as seen by ATP-dependent work generated by ESCRT-III filament disassembly. For ESCRT-III and Pil1, filament assembly might be restricted to membrane sites to facilitate remodeling and/or spatial organization, with similarities to FtsZ cytoskeletal filaments in prokaryotes and septin filaments in eukaryotes. The ability of new polymers to resist force and pressure may also link these systems to signaling pathways, perhaps through the sequestration of key molecules. The rich experimental toolkit used to study polymers such as actin and microtubules—for example, studying single-molecule dynamics by total internal reflection fluorescence microscopy—should be readily applicable to define key parameters in the assembly dynamics of new polymers. Along these lines, fluorescence-based in vitro assays for ESCRT-III have already defined key steps in this regulated filament assembly (Saksena *et al.*, 2009). The formation of linear polymers also has the potential to provide ordered assembly and control of signaling pathways, independent of force generation. This concept might apply to the reversible assembly of large-scale structures by metabolic structures.

CONCLUSIONS

The identification of multiple polymer systems indicates that the cytoskeleton is more elaborate than previously considered. The composition of polymers may vary between different cell types and organisms, but common themes are likely conserved. These new polymer systems suggest roles in membrane remodeling and intracellular signaling reminiscent of traditional cytoskeletal systems. For example, membrane remodeling and scission during endocytosis requires actin filament dynamics in yeast and possibly animal cells. Moreover, the concentration of actin monomers—a byproduct of polymer dynamics—can trigger transcriptional regulatory pathways (Posern and Treisman, 2006), and septin filaments scaffold a wide range of signaling networks in eukaryotic cells (Mostowy and Cossart, 2012). In this sense, functional characterization of new filaments has the potential to unveil new cellular roles for polymer dynamics and may point to new or underappreciated functions for the classic cytoskeletal components. Functional comparison of these novel filament systems in eukaryotes and prokaryotes can additionally point to general mechanisms that operate in diverse organisms. The generation of force and spatial order by new filament systems might also apply to synthetic biological systems. One example might be to engineer yeast-specific Pil1 filaments for expression and function in animal cells, which opens up possibilities to control cell tension and signaling in new and exciting ways. All of these efforts and challenges ultimately require systematic efforts to define and understand the expanded filament systems hiding inside these well-studied eukaryotic cells.

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