Seminars in Cell & Developmental Biology 37 (2015) 3–10

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

An intrinsically disordered linker plays a critical role in bacterial cell division

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ARTICLE INFO

Article history:
Available online 13 October 2014

Keywords:
FtsZ
Cell division
Cytokinetic ring
Cooperative assembly
Intrinsically disordered peptide
Bacteria

ABSTRACT

In bacteria, animals, fungi, and many single celled eukaryotes, division is initiated by the formation of a ring of cytoskeletal protein at the nascent division site. In bacteria, the tubulin-like GTPase FtsZ serves as the foundation for the cytokinetic ring. A conserved feature of FtsZ is an intrinsically disordered peptide known as the C-terminal linker. Chimeric experiments suggest the linker acts as a flexible boom allowing FtsZ to associate with the membrane through a conserved C-terminal domain and also modulates interactions both between FtsZ subunits and between FtsZ and modulatory proteins in the cytoplasm.

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Mechanistically, cytokinesis exhibits an extraordinary degree of conservation across all domains of life. In bacteria, animals, fungi, and many single celled eukaryotes, division is initiated by the formation of a ring of cytoskeletal protein at the nascent division site. This ring serves as a framework for assembly of the division machinery and constricts at the leading edge of the closing septum during cytokinesis. In animals and fungi this ring is composed of the ATPases actin and myosin.

In bacteria, the essential GTPase FtsZ serves as the foundation for the cytokinetic ring. A distant relative of eukaryotic tubulin, FtsZ forms single stranded polymers *in vitro* that resemble the 13 protofilaments that run the length of microtubules. Like tubulin, FtsZ binds GTP as a monomer but the active site for GTP hydrolysis is formed at the interface between the two subunits. *In vitro*, GTP-binding promotes FtsZ polymerization, which further promotes GTP hydrolysis. Although it appears static in conventional micrographs, fluorescence recovery after photo bleaching (FRAP) indicates that the FtsZ ring is highly dynamic with subunit turnover on the order of seconds [1].

In contrast to tubulin, FtsZ does not form structures as highly ordered as a microtubule under standard *in vitro* assembly conditions. Single stranded polymers, however, do interact laterally *in vitro*, forming parallel bundles and sheets [2,3]. Super-resolution microscopy and electron cryotomography (ECT) support a model in which single-stranded FtsZ polymers also interact laterally *in vivo* to form a loosely associated wreath-like structure that varies in thickness around its circumference [4–8].

The single-stranded nature of FtsZ filaments and the apparently cooperative nature of its assembly *in vitro*, has for years presented a perplexing paradox. Multi-stranded filaments, such as actin and microtubules, undergo cooperative assembly [9–11]. Cooperative filaments assemble in two stages: an energetically

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http://dx.doi.org/10.1016/j.semcdb.2014.09.017
1084-9521/

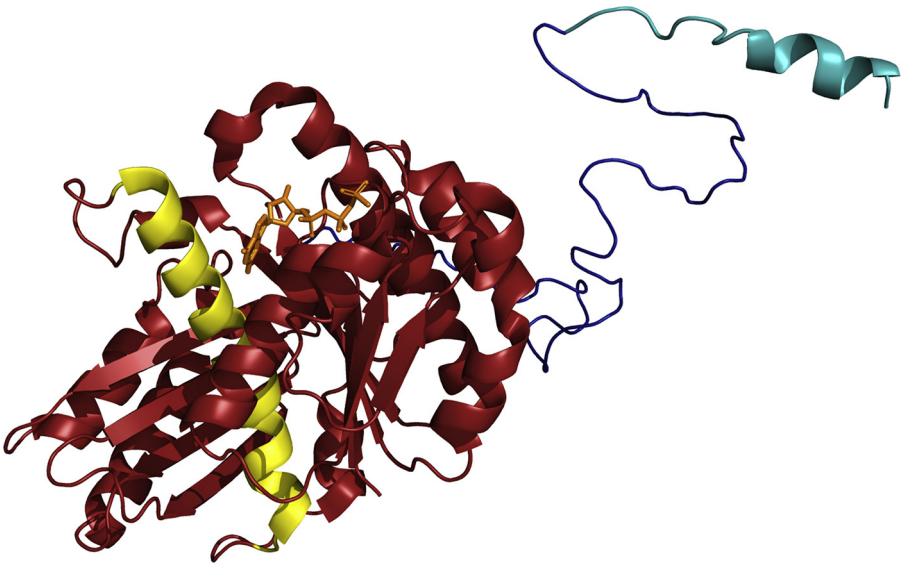

Fig. 1. Structure of *B. subtilis* FtsZ. The N-terminal core (maroon) is depicted as bound to GDP (from PDB entry 2RHO [61]). The flexible C-terminal linker (blue) is depicted here as an unstructured peptide. The conserved C-terminal tail (CTT), in cyan, is depicted as a short α helix based on the structure of the *E. coli* CTT in complex with ZipA [62].

unfavorable nucleation phase and an ensuing elongation phase. The affinity of subunits for the filament end is higher than the affinity of a subunit for itself due to the interaction between an adding subunit and multiple other subunit interfaces already at the filament end. Critical concentration and a concentration dependent lag phase are defining features of cooperative assembly. Cooperative filaments display a critical concentration (C_c), a minimum concentration of subunits below which polymerization will not occur. When the subunit concentration rises above the C_c, all additional protein assembles into filaments. At steady state, the equilibrium concentration of monomers will equal the critical concentration. Additionally, cooperative assembly features polymers undergoing a concentration-dependent lag phase during which the nucleus, or smallest species for which elongation is preferred over disassembly, is formed.

Quantitative calorimetric and spectroscopic methods indicate that FtsZ assembly is cooperative [12–14]. FtsZ polymers display a critical concentration for assembly and exhibit the characteristic lag phase seen in many other cytoskeletal filaments. At the same time, the observation that FtsZ forms stable single-stranded filaments *in vitro*, is ad odds with standard models for cooperative assembly in which the adding subunit interacts with multiple subunits on the growing polymer. Single stranded polymer formation is more consistent with an isodesmic assembly model in which the affinity for dimer formation is the same as polymer elongation. This discrepancy between *in vitro* experimental data and FtsZ polymer structure have rendered the existing kinetic models of actin or tubulin assembly insufficient for modeling FtsZ assembly kinetics [15]. To overcome these limitations, numerous models have been employed to describe how FtsZ assembles cooperatively yet primarily appears as single-stranded filaments [15,16]. These models predict that subunits undergo conformational changes that facilitate a high affinity active state favoring polymerization. However, detailed molecular and structural evidence for such a high affinity state remains elusive.

1. The FtsZ C-terminal linker: a model intrinsically disordered region (IDR)

Structurally FtsZ consists of four functional domains: a globular tubulin-like core, an intrinsically disordered C-terminal "linker" (CTL), a highly conserved ~11 residue region known as the C-terminal tail or grappling hook peptide (CTT or GHP), and short, variable set of residues termed the C-terminal variable region or CTV (Fig. 1). The negatively charged core encompasses FtsZ's GTP binding site and the coordinating T7 "loop." The interface between the nucleotide binding site and the T7 loop in dimers and higher order polymers forms the active site for GTP hydrolysis. The CTT or GHP is the site of interaction between FtsZ and a host of modulatory proteins including the conserved membrane associated protein FtsA that serves to anchor FtsZ to the plasma membrane. Finally, depending on its charge, the CTV can play a significant role in mediating lateral interactions between FtsZ polymers *in vitro* and in the integrity of the FtsZ ring *in vivo*.

The CTL, the focus of this review, has received scant attention until recently. The sequence of the CTL is highly variable and like most archetypal IDPs a singular structure cannot be resolved from crystals of FtsZ that contain the CTL. Experimental data support a model in which the CTL serves as a flexible tether between FtsZ and the plasma membrane [17,18]. The importance of the intrinsically disordered CTL to the function of FtsZ has been established unequivocally in recent investigations.

Consistent with its intrinsically disordered nature being important for function, the primary sequences of CTLs are largely fungible as determinants of FtsZ activity. Not only is the linker poorly conserved at the sequence level across species, the CTL of both *Escherichia coli* and *Bacillus subtilis* FtsZ can be replaced by non-homologous linkers from other FtsZs and intrinsically disordered regions (IDR) from unrelated proteins without obvious deleterious effects on FtsZ assembly or function [17,18]. CTLs of varying lengths are also tolerated, up to a point. Replacing the ~50 residue native linker with one that is approximately twice as long (~100 residues) has only a modest deleterious impact on FtsZ ring formation in *B. subtilis* and *E. coli*. However, increasing linker length further (over 95 residues in *E. coli* and 100 residues in *B. subtilis*) completely disrupts FtsZ function *in vivo*, leading to a lethal block in division [17,18]. Reducing linker length by 50% (from ~50 to ~25 residues for *B. subtilis* FtsZ) has little effect on FtsZ assembly, whereas replacing *E. coli*'s linker with a significantly shorter (≤24 residues) IDP from alpha adducin results in a non-functional FtsZ that is unable to complement the heat sensitivity of a conditional *ftsZ* allele, *ftsZ84* [17,18].