Entangled but Finicky Ingression Protein Complexes for Successful Cytokinesis

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Abstract
Cytokinesis is the final stage of mitosis that leads to the physical separation of two daughter cells and comprises a sequence of events such as actomyosin ring contraction, ingression and remodeling of the extracellular matrix. All these processes are tightly regulated in space and time through a network of proteins. Defects in cytokinesis may increase the risk of tumor formation. Using a combination of cell biology and molecular techniques, along with biochemical experiments, Foltman et al. [27] have dissected how the "ingression protein complexes" (IPCs) localize and coordinate to ensure proper cytokinesis. Interestingly, a particular glycosyltransferase, named Chs2, is the hub protein that assures a successful cytokinesis in budding yeast.

Keywords: actomyosin ring, cytokinesis, glycosyltransferase, membrane ingression, yeast

Introduction

The cell cycle comprises an ordered and precise set of events such as faithful duplication of chromosomes (in S phase [synthesis]) and their segregation (in M phase [mitosis]). Gap phases (G1 and G2) intervene between each M and S phase and each S and M phase, respectively. Cytokinesis, which leads to the physical separation of the two daughter cells, is the final step of cell division and takes place at the end of the M phase.

In eukaryotic cells, cytokinesis is a complex process that must be spatially and temporally coordinated with sister chromatid partition. Successful cytokinesis is achieved by the coordination of the actomyosin ring (CAR) at the division site and the ingression of the plasma membrane and remodeling of the extracellular matrix (ECM) [1-6]. A deep understanding of cytokinesis is of crucial importance, because cytokinesis failure and the subsequent generation of polyploid cells have been often associated with tumorigenesis [7-8].

The budding yeast *Saccharomyces cerevisiae* is a powerful system to investigate the molecular mechanisms underlying cytokinesis because of its tractable genetics, powerful biochemistry, proteomics, cellular and molecular biology. In addition, the core machinery and cell division mechanisms have been proved to be largely conserved from budding yeast to humans.
In the last three decades, a large group of proteins have been involved in CAR contraction, such as F-actin, type II myosin heavy chain, the Iqg1 protein (IQGAP), Inn1 (required for INgressioN), the F-BAR protein Hof1 (Homolog of cdc Fifteen), Cyk3 (CYtoKinesis), and the glycosyltransferase Chs2 (also named Chitin Synthase 2).

In budding yeast, CAR assembly takes place in different steps that start in late G1 [6,9]. First, the single myosin type II heavy chain Myo1 is recruited to the presumptive bud site [10,11]. Then, in late mitosis successive events listed below lead to CAR assembly. For instance, the IQGAP protein Iqg1 joins the bud neck and recruits a second wave of Myo1 [12] as well as F-actin that crosslinks actin through its amino-terminal calponin homology domain. In parallel, Iqg1 also binds to Hof1 protein, a key regulator of CAR assembly and stability [6]. It is worth mentioning that Hof1 interacts directly with type II myosin Myo1 through its F-BAR N-terminal domain. Moreover, Hof1 localization at the cleavage site is Myo1-dependent [13,14]. Hof1 also contains an SH3 domain in its C-terminus

from which it interacts with other proteins involved in CAR. For example, Hof1-SH3 domain binds to proline-rich motifs located at the C-terminus of Inn1 [15,16], also found at the N-terminus of Cyk3 [16], a protein that in turn binds to Iqg1. Beside, Hof1 has a redundant function with the BAR amphiphysin yeast Rvs167 to promote F-actin assembly at the CAR [17].

The fully assembled CAR contracts with primary septum formation, a special layer of the ECM synthetized by the bud-neck-localized Chs2 protein [18-20]. Chs2 is synthesized in G2/M and accumulates in the endoplasmic reticulum (ER) until the end of mitosis [18-21]. Chs2 is shifted from the ER to the bud neck along actin cables in a Myo2-dependent manner [22]. However, its delivery from the ER to the bud neck is triggered upon activation of the Cdc14 (Cell Division Cycle 14) phosphatase [20,23,24]. Cdc14 is a key component of the signal transduction cascade called Mitotic Exit Network (MEN) [25,26]. Cdc14 promotes two sets of events converging on CDK inactivation, a prerequisite for mitotic exit and also cytokinesis.

Despite all efforts, the detailed picture about how all these proteins interact together and coordinate CAR contraction during cytokinesis was missing. Recently, Alberto Sanchez-Diaz's laboratory has successfully dissected how CAR contraction is achieved using budding yeast as a model organism [27].

Authors' Results

It was reported that Inn1 interacts with Chs2 [28]. However, the biological function of these proteins was not fully characterized. Now, Alberto Sanchez-Diaz's laboratory further investigated how these proteins work in cells. By using different approaches such as yeast genetics, cell biology, *in vitro* binding assays and mass spectrometry, they have demonstrated that the C2 domain of Inn1 directly binds to and controls the catalytic activity and localization of Chs2. In addition, they have dissected how these two proteins coordinate with a large complex of proteins that they named "ingression protein complexes" (IPCs: Iqg1, Hof1, Myo1 and Cyk3 proteins) to ensure the entangled cytokinetic events, like contraction of the actomyosin ring, ingression of the plasma membrane and ECM remodeling.

Since most IPC proteins are essential in budding yeast, simple yeast genetics cannot be used to understand their function in cells. To override this problem, the authors have used sophisticated genetics. They have combined two powerful yeast degron systems: the "heat-inducible degron" [29-31] and the "auxin degron" [32]. These systems allowed them to smartly inactivate one by one these proteins and dissect their physiological function in yeast cells.
Foltman et al. [27] proposed that Myo1 and Iqg1 serve as scaffold for the rest of IPC components (Hof1, Inn1, Cyk3) [33-36], since specific inactivation of Myo1 or Iqg1 prevents IPC localization. The authors have shown that Inn1 and Hof1 interact with the Myo1-Iqg1 scaffold. In addition, Inn1 and Hof1 at the bud neck are required for Chs2 localization at the cleavage site. For instance, Chs2 was unable to localize at the bud neck upon deletion of Hof1 and Inn1.
As previously mentioned, Chs2 travels through the ER in G2/M until the end of mitosis [18-21] when it is translocated upon Cdc14 phosphatase activation.

Foltman et al [27] have also clarified this point. They have discovered that, in spite Chs2 is delivered to the plasma membrane in a Hof1-Inn1 dependent manner, it is kept in an inactive form through the C-terminus of Inn1. Afterward, Chs2 is activated *in situ* by a mechanism that depends on the interaction of Chs2-Inn1 with Cyk3, the latest protein that binds to the IPCs. Moreover, Foltman et al. [27] corroborated that these events are highly regulated by the Cdc14 phosphatase. For instance, the Chs2-Inn1-Cyk3 ternary complex is solely formed when Chs2 and Inn1 are dephosphorylated by the Cdc14 phosphatase [37,38]. Of note, Cdc14 is activated only after dropping cyclin B/cyclin dependent

kinase complexes (cyclin B-CDK) activity. When the three proteins (Chs2-Inn1-Cyk3) are bound, Chs2 is activated, because Cyk3 counteracts Inn1 inhibitory function on Chs2 activity at the site of division.

Interestingly, Chs2 is the only component of the IPCs with a transmembrane domain embedded in the plasma membrane. In other species such as *S. pombe,* the glycosyltransferase Bgs1 (beta (1,3)-glucan synthase) lays down the primary septum during cytokinesis [39]. In spite of producing a different polysaccharide (a D-glucose instead of an N-acetylglucosamine), Bgs1 could be analogous to Chs2, because it is also an integral membrane protein with its catalytic domain located at the cytoplasmic side of the membrane, besides possessing similar function. In animal cells, glycosyltransferases are essential to the synthesis of polysaccharides of the ECM during cytokinesis. Taking into account the conservation of the cytokinetic mechanism, further studies would help to determine whether a glycosyltransferase similar to Chs2 plays a key role during cytokinesis in human cells.

Acknowledgements

I would like to thank the reviewers for their constructive comments.

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