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ABSTRACT

Cytokinesis is the process by which a mother cell is physically cleaved into two daughter cells. In animal cells, cytokinesis begins with the contraction of a plasma membrane-associated actomyosin ring that is responsible for the ingression of a cleavage furrow. However, the post-furrowing steps of cytokinesis are less understood. Here, we highlight key recent findings that reveal a profound remodeling of several classes of cytoskeletal elements and cytoplasmic filaments (septins, microtubules, actin and ESCRT) in the late steps of cytokinesis. We review how this remodeling is required first for the stabilization of the intercellular bridge connecting the daughter cells and then for the steps leading up to abscission. New players regulating the abscission (NoCut) checkpoint, which delays abscission via cytoskeleton and ESCRT remodeling in response to various cytokinetic stresses, will also be emphasized. Altogether, the latest discoveries reveal a crucial role for posttranslational modifications of the cytoskeleton (actin oxidation, septin SUMOylation) and an unexpected requirement of ESCRT-III polymer dynamics for successful abscission.

Actin polymerization and turnover are essential for furrow ingression in animal cells [1,2]. After furrow ingression, a microtubule (MT)-filled intercellular bridge connects the daughter cells for several hours before the final cut [3]. Ten years ago, a breakthrough in the field was the finding of a role for the Endosomal Sorting Complex Required for Transport (ESCRT) in cytokinetic abscission, which at that time was known for intraluminal vesicle scission in late endosomes/multivesicular bodies (MVBs) and for retroviral budding [4-6]. Although the detailed mechanism is under debate [7], the current literature indicates that the ESCRT machinery is sufficient for outward membrane budding and fission [8], an event that is topologically equivalent to cytokinesis. In this review, we will focus on 1) how the septin cytoskeleton is regulated to maintain bridge stability, 2) how ESCRT polymers are turned-over to promote abscission and 3) the recent findings regarding MT and F-actin clearance within the bridge prior to abscission to allow for correct ESCRT filament assembly.

Septin filament dynamics and intercellular bridge stability

The stability of the cytokinetic bridge becomes independent of actin filaments soon after furrow ingression but relies on septins [9-11]. Septins are GTP-binding proteins that assemble into non-polar, rod-shaped oligomeric complexes and higher-order structures such as filaments and bundles [12]. Septins localize to the intercellular bridge and their inactivation usually leads to binucleated cells due to either an unstable, and sometimes displaced, cleavage furrow or intercellular bridge relaxation in several animal models [13-16], but not in *C. elegans* [17]. In *Drosophila* S2 cells, the septin Peanut (Septin 7) acts in complex with anillin to maintain connections between the midbody and the plasma membrane, thus maintaining furrow/bridge stability. In addition, septins are required for trimming away excess anillin-positive membranes from the early bridge, which promotes its maturation, both in *Drosophila* [11] and human [16] cells. Among human septins, SEPT9 plays a distinct and later role in cytokinesis, since its inactivation essentially delays abscission but does not generate binucleated cells [14,16,18]. Altogether, recent evidence indicates a role for septins in bridge maturation, a step preceding and required for ESCRT-III recruitment to the abscission site [16,17], but the exact mechanistic roles of septins in this process are not yet fully understood.

In yeast, septins were the first substrates reported to be post-translationally modified by SUMOylation (a moiety resembling Ubiquitin), but this modification is not critical for cytokinesis [19,20]. In contrast, findings in human cells revealed this year that septins are SUMOylated and that this modification is important for cytokinesis [21]. Indeed, non-SUMOylable mutant septins assemble in abnormally long and thick bundles that do not turnover. These bundles likely constitute a physical block within the intercellular bridge, which explains bridge relaxation and observed binucleated cells [21]. Whether SUMO directly controls filament assembly into higher-order structures or whether SUMOylation of septins regulates the interaction of proteins that inhibit the formation of large bundles remains to be investigated. In any case, this highlights a new role for SUMOylation both in cytoskeletal dynamics and cytokinesis (Figure 1A).

ESCRT-III filaments and cytokinetic abscission

As mentioned in the introduction, current models indicate that the ESCRT machinery is responsible for the final abscission, which takes place on the midbody side (also called the "secondary ingression site") [10,16,22-24]. Consistently, depletion of several key ESCRT components or associated proteins such as ALIX, TSG101 (ESCRT-I) and several CHMPs (ESCRT-III) leads to cytokinetic delay, abscission failure and binucleated cells in human cells [4,5,10]. Mechanistically, ESCRT-III can polymerize into filaments *in vitro* [7] and the presence of 17 nm cortical intertwined filamentous helices have been observed at the abscission site in human cells by electron and X-ray microscopy [10,25,26]. In cells depleted for the ESCRT-III component CHMP2A, these helices are no longer present, and concomitantly no cortical constriction is observed [10]. This indicates that either these helices are made of ESCRT, or the ESCRT machinery is required for these filaments to polymerize and constrict the intercellular bridge. This is a crucial point that has to be resolved in our standard model of abscission, perhaps by combining super-resolution and EM tomography. Interestingly, other filamentous structures made of anillin and septins are also observed at the secondary ingression site before CHMP4B recruitment [16]. Thus, the relationship between these different filamentous polymers and the helices observed by EM has to be clarified in future studies. Furthermore, published micrographs show ESCRT-III-

dependent helices in bridges constricted to no less than 150-200 nm [10,26]. Thus, more work is needed to observe helices in further constricted states in order to definitively prove that ESCRT-III-dependent helices drive the final pinch. This might prove tedious if the final constriction step is fast and thus difficult to catch. A provocative, alternative possibility would be that after ESCRT-III constriction to 150-200 nm, there is an additional ESCRT-independent step leading to the final scission of the intercellular bridge at 3-10 nm, perhaps involving lipid modifications [27,28].

Recent findings clarified the mechanisms by which the ESCRT-III machinery is recruited to the intercellular bridge. It was shown that Cep55 plays a pivotal role in directly recruiting TSG101 and ALIX to the midbody in mammalian cells [5,29]. It is now shown that ALIX and TSG101 act in parallel to fully recruit ESCRT-III components [30]: ALIX is activated by phosphorylation [31] and directly interacts with CHMP4B, whereas the ESCRT-I components TSG101 and VPS28 recruit ESCRT-II proteins which in turn recruit the ESCRT-III CHMP4B. Yet, these two parallel pathways are not equivalent, as only ALIX appears to have a specific role in the prevention of binucleation [5,30]. Similarly, CHMP4C, another ESCRT protein involved in the abscission checkpoint (see below), is recruited via ALIX but not TSG101 [30]. A question that remains poorly understood is how ESCRT-III components, initially found at the midbody are later found at the abscission site. Does this involve a separate recruitment from a cytosolic pool? Does ESCRT-III physically translocate from the midbody to the abscission site? Is this related to relaxation of tension within the intercellular bridge [32,33]? Despite these interrogations, it is known that the relocalization of CHMP4B from the midbody to the abscission site depends on anillin/septins [16], as well as F-actin clearance (see below).

Regarding the issue of the spatial and temporal recruitment of the ESCRT machinery at the midbody and the abscission site, important progress has been made this year. Near-endogenous expression levels of tagged proteins combined with fluorescent microscopy revealed that the AAA-ATPase VPS4, which is known to disassemble ESCRT filaments [7], does not arrive to the midbody just before abscission as initially thought [22], but instead is continuously present throughout ESCRT-III subunit recruitment [26]. The presence of VPS4 at the midbody explains why ESCRT-III filaments are highly dynamic (their different subunits display a high turnover, with residence time of 20 sec) [26]. At first counter intuitively, this

process leads to a net increase in the amount of CHMP4B during progress toward abscission. Indeed, depletion of the AAA-ATPase VPS4 abolishes CHMP4B turnover at the midbody, diminishes the recruitment of ESCRT-III, prevents the constriction of the intercellular bridge, and results in a strong delay in abscission [26]. Remarkably, *in vitro* reconstitution assays coupled with high-speed atomic force microscopy (AFM) revealed that ESCRT-III filaments form spirals that undergo both rapid growth and shrinkage in the presence of VPS4 and ATP [26]. Altogether, this suggests that continuous VPS4-dependent turnover of ESCRT-filaments is crucial for filament growth and abscission *in vivo*, revisiting the mechanistic role of VPS4 and ATP hydrolysis in polymer constriction. Interestingly, the spiral helices could not fully contract *in vitro* [26]. Thus, either an additional component is missing for full constriction, or, as mentioned above, the ESCRT machinery is required for successful abscission but does not drive it to completion. Importantly, turnover of ESCRT-III by Vps4 could be a general principle in ESCRT-dependent mechanisms since a continuous and stochastic recruitment of Vps4 throughout the process of intraluminal vesicle budding in yeast MVBs has now been observed [34]. Understanding how polymer remodeling translates into fission is thus a major challenge for the future.

Microtubule and F-actin clearance at the abscission site

Microtubule bundles are locally severed at the secondary ingression site [10,16,22,23] and this is presumably an important step for ESCRT filaments to be able to constrict to completion. Remarkably, the MT-severing AAA-ATPase spastin directly interacts with the ESCRT-III component CHMP1B, and is required for normal abscission [10, 33, 35,36]. This suggests a molecular mechanism coordinating ESCRT recruitment and MT clearance at the abscission site. The same coordination between ESCRT-III and spastin has also been recently observed during nuclear envelope reformation at mitotic exit [37]. However, the importance of spastin for severing MTs at the abscission site is discussed, since spastin-independent, buckling-induced MT severing has also been proposed [38].

In addition to MTs, F-actin must be cleared from the intercellular bridge for successful abscission [23,39,40]. However, whether this has to occur specifically at the secondary site

or all along the bridge has not yet been resolved. Two mechanisms for F-actin clearance have been elucidated. The first mechanism relies on the Rab35 GTPase which recruits an effector, the PtdIns(4,5)P_2 lipid phosphatase OCRL [41], to the intercellular bridge [39]. PtdIns(4,5)P_2 hydrolysis by OCRL limits F-actin oligomerization and is required for normal abscission [28,39]. In addition, Rab11-FIP3-positive endosomes deliver the p50RhoGAP cargo to the bridge to further limit Rho GTPase activation and thus F-actin polymerization in bridges [23]. The second mechanism, also depending on Rab35, was revealed this year and depends on another effector of this GTPase: the oxidoreductase MICAL1 [40] (Figure 1B). MICAL1 is an enzyme that oxidizes methionine residues on F-actin and induces filament depolymerization *in vitro* [40,42] (for a recent review regarding MICAL enzymes see [43]). Rab35 binding to MICAL1 is sufficient to activate the enzymatic activity of MICAL1, which is tightly regulated [40]. In addition, Rab35 recruits a pool of MICAL1 at or close to the abscission site a few minutes before abscission [40]. Altogether, Rab35/MICAL1 actively clear F-actin from bridges, which is a step required for normal abscission and recruitment of ESCRT-III to the abscission site [40]. Interestingly, other proteins that need to be discovered also contribute to MICAL1 recruitment during cytokinesis, and may include other Rab GTPases known to interact with MICAL1 [43]. In summary, two GTPases control parallel pathways that cooperate in F-actin clearance and correct ESCRT-III recruitment at the abscission site. On the one hand, these pathways limit the amount of F-actin polymerization via Rab35/OCRL1 and Rab11/MICAL1 and, on the other hand, actively depolymerize F-actin at the bridge via Rab35/MICAL1. It remains to be established how Rab35 is activated at the midbody and the abscission site, and whether vesicular delivery is involved in enriching this GTPase at specific locations within the intracellular bridge (for a recent review on membrane traffic and cytokinesis, see [44]). Another interesting issue is to determine whether Rab11 and Rab35 act sequentially or in parallel during the process of F-actin clearance.

Conclusion and open questions

Several recent studies have helped to understand the multiple events of cytoskeletal remodeling involved in the late steps of cytokinesis (Figure 2). A number of remaining questions regarding the remodeling of septins, MTs, actin and ESCRT filaments have been

detailed above and the main ones are summarized in the BOX. Besides answering these questions, several areas are expected to yield exciting findings in future studies.

First, the study of cytokinesis in abnormal or pathological situations, such as during aging or when a stress is present. An abscission checkpoint, also called the “abscission checkpoint”, was discovered several years ago in seminal works in yeast (termed the “NoCut checkpoint” in this organism) [45] and in mammalian cells [46]. This evolutionarily-conserved checkpoint delays abscission when lagging chromatin abnormally stays in the intercellular bridge, but also after a variety of other cytokinetic stresses such as defects in nuclear pore reformation or high levels of intercellular tension [33,47-50]. Mechanistically, activated Aurora B mediates the checkpoint by phosphorylating the ESCRT-III subunit CHMP4C, which acts as a negative regulator of ESCRT-III filament assembly [51-53]. In addition, the ATPase VPS4 interacts with the checkpoint regulator ANCHR, which together with CHMP4C retains VPS4 at the midbody and thus delays abscission [54]. The kinase ULK3 also phosphorylates and inhibits IST1, which is an ESCRT-III component required for abscission and a key regulator of Vps4 [55]. Furthermore, recent work identified the CDK-like kinases Clk1, Clk2 and Clk4 as additional activators of Aurora B [56]. Altogether, there is clear evidence that the Aurora B-dependent abscission checkpoint regulates the assembly and constriction of ESCRT-III filaments in response to cytokinetic stresses. Interestingly, activation of the checkpoint is associated with the appearance of F-actin patches at the bridge entrance that presumably play an important role in bridge stability and prevention of tetraploidy while abscission is halted [46]. Despite rapid advancements, many questions remain unanswered: which exact chromatin defects activate the checkpoint [57]? How are diverse cytokinetic stresses recognized and how do they activate checkpoint kinases? How is F-actin maintained at high levels when the checkpoint is activated in mammalian cells? Are double strand breaks observed when the checkpoint is defective, resulting from nuclear envelope rupture and entry of cytoplasmic nucleases [45,55,58,59]? Answering some of these questions should help to understand the multiple changes in F-actin and ESCRT-III remodeling that are observed in response to checkpoint activation.

Second, there is an urgent need to confirm *in vivo* the pathways and mechanisms described within this review in cultured cells. In the few *in vivo* studies, unexpected results have been

numerous. For instance, ESCRTs are required for abscission in female germ cells in *Drosophila*, but not in somatic cells [60,61]. Similarly, SEPTIN7 is required for cytokinesis in mouse fibroblasts but not in hematopoietic stem cells [62]. In addition, the septin Peanut is required for cytokinesis in the first (planar) but not the second (orthogonal) division of sensory precursor stem cells in *Drosophila* [63]. Thus, the filament components described above can be essential or not depending on the context or the organism.

Finally, a recent report exemplified how partial our understanding of late cytokinetic events is. Quite unexpectedly, during the first division of *C. elegans* embryos, no helices could be observed at the abscission site by EM tomography [64]. Instead, ESCRT-III-dependent filamentous helices are unambiguously detected at multiple buds emerging from the midbody. Furthermore, ESCRTs are not essential for abscission in this first division (upon depletion, a slight delay is observed, as opposed to a strong delay found in human cells) [64]. Nevertheless, in both models abscission eventually occurs in most of the cells upon ESCRT-III inactivation. This might be due to residual ESCRT functions or ESCRT component redundancy but might also suggest the existence of an ESCRT-independent mechanism of abscission, which is new territory to be explored in the future.

BOX: Key unanswered questions regarding cytoskeletal remodeling during late cytokinesis

1- Are helices at the abscission site made of ESCRT components?

2- Do ESCRT-dependent helices constrict to completion and trigger abscission?

3- What are the relationships between anillin, septin and ESCRT-dependent helices?

4- How do septins promote ESCRT filament localization?

5- What determines the recruitment of the ESCRT-III pool at the abscission site?

6- How is Rab35 activated and localized to the abscission site?

7- How is F-actin stabilized when the abscission checkpoint is activated?

8- Which pathways are required for abscission *in vivo*?

9- What are the potential ESCRT-independent mechanisms involved in abscission?

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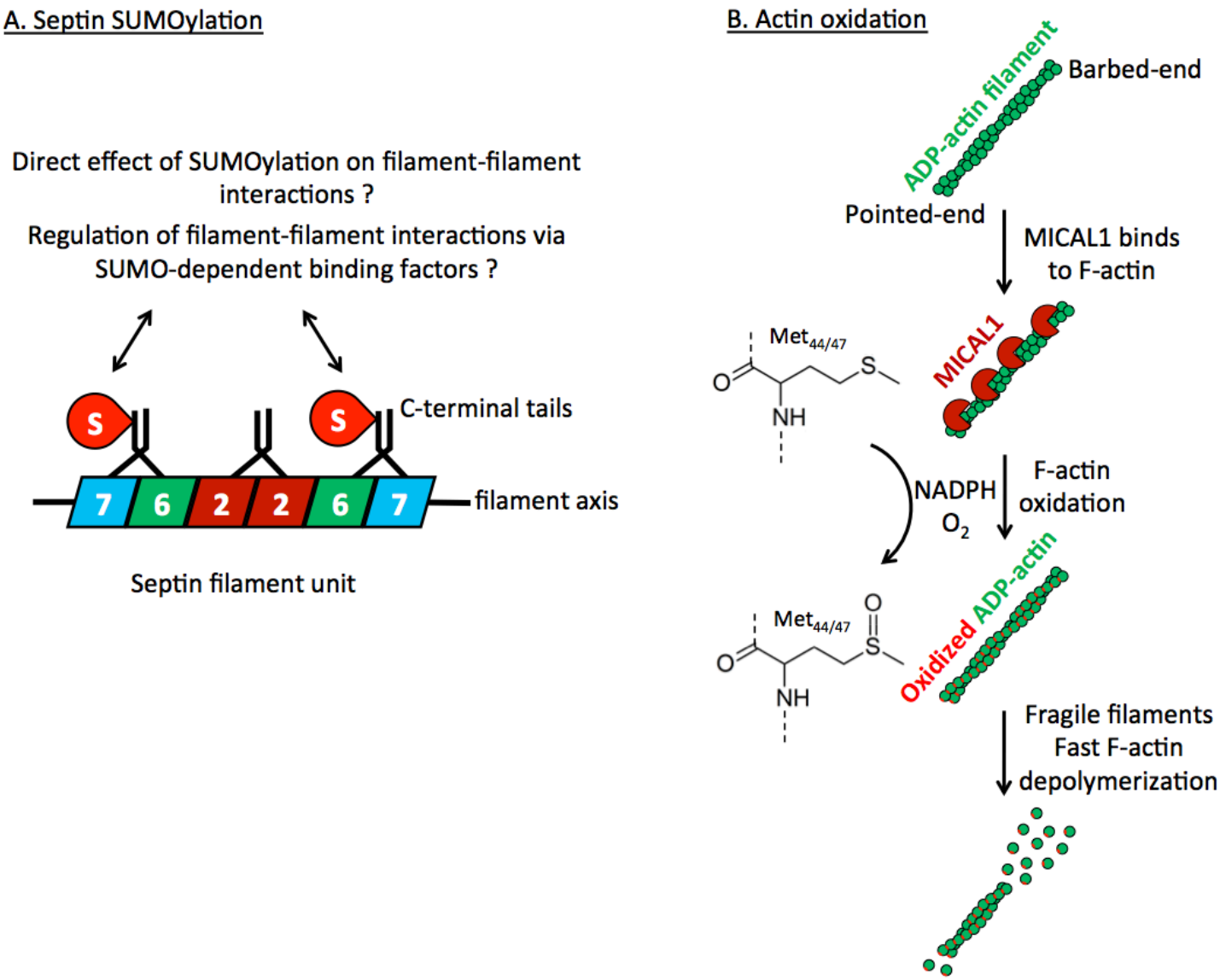

Figure 1: Two post-translational modifications of cytoskeletal elements recently involved in cytokinesis. (A) SUMOylation of Septin 6, 7 and 11 on their C-terminal tails prevents abnormal bundling of Septin filaments and destabilizes cytokinetic bridges in human cells. SUMOylation could either directly affect filament-filament interactions or regulate filament bundling through SUMO-dependent binding factors. **(B)** Actin oxidation of methionine residues (Met44 and M47) into methionine sulfoxide induces fast F-actin depolymerization. The reaction involves the enzyme MICAL1, O_2 and NADPH as a cofactor. MICAL1-dependent actin oxidation is required for correct localization of ESCRT-III at the secondary ingression site and for normal timing of abscission in human cells.

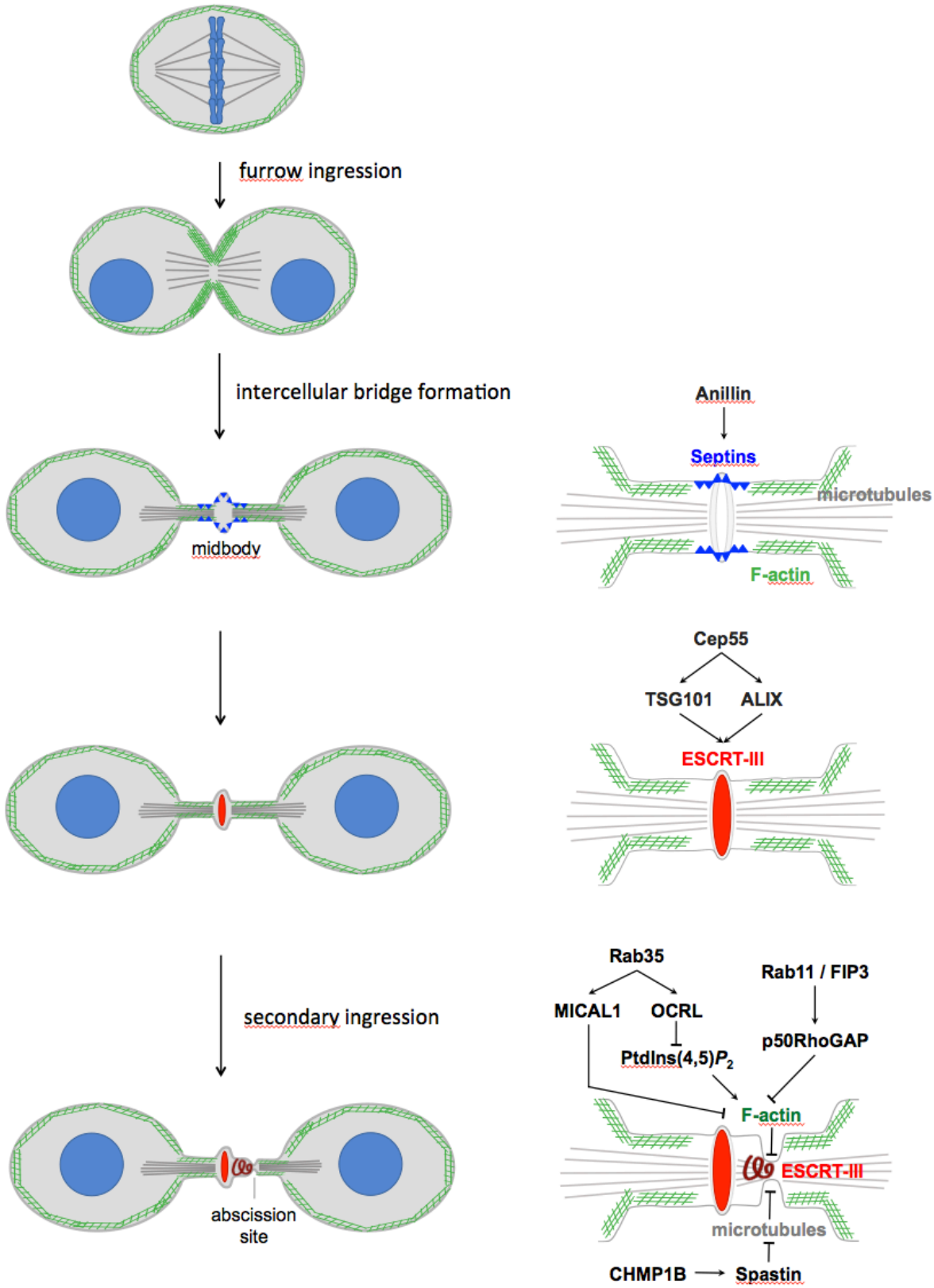

Figure 2: Mechanisms controlling cytoskeletal remodeling in the post-furrowing steps of cytokinesis. Septins, F-actin, microtubules and ESCRT-III filaments are indicated in blue, green, grey and red, respectively. Note that ESCRT-III recruitment and constriction at the abscission site require clearance of both microtubules and F-actin.