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## DISPATCH

### Cell Biology: Alix ESCRTs Pavarotti During Cell Division

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**Cytokinesis leads to the physical separation of the daughter cells and requires the constriction of ESCRT filaments. How the ESCRT machinery is recruited in non-vertebrate organisms was puzzling, and is now shown to rely on a direct interaction between the ESCRT-associated protein ALIX and the kinesin motor Pavarotti in *Drosophila*.**

Cytokinesis is the last step of cell division, and physically cleaves the mother cell into two independent daughter cells once the genetic material has been segregated. In animal cells, cytokinesis initiates with the contraction of a furrow, which relies on the acto-myosin cytoskeleton at the cell cortex [1–3]. The daughter cells are then left connected by an intercellular bridge filled with microtubules, which is eventually cut during a final step called abscission [1–3]. Abscission occurs on the side of the midbody, which stands at the centre of the intercellular bridge, and depends on the recruitment of the ESCRT (endosomal sorting complexes required for transport) machinery [4,5]. The ESCRT machinery was first discovered for its role in cargo sorting and intraluminal vesicle budding within the endosomal pathway at the level of late endosomes/multivesicular bodies (MVBs) [6]. This evolutionarily conserved machinery has then been implicated in similar topologically related membrane scission events, such as viral budding and cytokinesis [6]. Strikingly, its role in cytokinesis likely represents the ancestral function since the ESCRTs are also implicated in cytokinesis in some Archea [7].

The ESCRT machinery is composed of more than 20 proteins assembled in complexes (ESCRT-0, -I, -II and -III) and its activity culminates with the assembly of constricting filaments made of ESCRT-III proteins [6]. As during many ESCRT-dependent processes, the ESCRT-associated protein ALIX and the ESCRT-I protein TSG101 play a key role during cytokinesis by recruiting ESCRT-III to where the scission event must occur. In human cells, ALIX and TSG101 indeed cooperate and recruit ESCRT-III at the midbody via two parallel pathways [8] (Figure 1A). Importantly, the midbody protein CEP55 directly interacts with ALIX and TSG101 and is essential for their recruitment at the midbody and thus for abscission in mammalian cells [4,5,9,10]. CEP55 is therefore believed to be the main upstream factor that recruits ALIX, TSG101 and the ESCRT-III machinery at the midbody. Importantly, CEP55 directly interacts with the kinesin MKLP1 [9], which is part of the Centralspindlin complex. Centralspindlin is an evolutionarily conserved heterotetrameric complex made of MKLP1 (called Pavarotti in *Drosophila*, ZEN-4 in *C. elegans*) and MgcRacGAP (called RacGAP50C/Tumbleweed in *Drosophila*, CYK4 in *C. elegans*) [11]. Centralspindlin plays an essential role in initial steps of cytokinesis by activating the RhoA GTPase as well as in the structural integrity of the midbody [11–13]. Since the midbody contains overlapping microtubule plus ends, it concentrates the kinesin MKLP1. Altogether, the MKLP1–CEP55 interaction provides a complete molecular mechanism explaining why the ESCRT machinery is recruited at the midbody during the terminal steps of cytokinesis.

However, a long-standing and puzzling observation indicated that this model could not be universal. Indeed, there is no recognizable CEP55 homologue in non-vertebrate organisms, including *Drosophila*, which nevertheless relies on ESCRTs for cytokinetic abscission [14–16]. The study by Lie-Jensen *et al.*, published in this issue of *Current Biology*, now provides an alternative molecular explanation of how the ESCRT machinery is recruited at the midbody in the absence of CEP55 [17].

Lie-Jensen *et al.* addressed this question using *Drosophila* cultured cells, and validated their main results in the *Drosophila* female germline *in vivo*. The authors first determined which domain of *Drosophila* Alix is necessary and sufficient for localizing the protein at the midbody. Human ALIX and *Drosophila* Alix are composed of three distinct domains: an N-terminal Bro1 domain that can directly interact with ESCRT-III proteins, a central V-domain that binds endosomal cargos and viral proteins, and a C-terminal proline-rich region (PRR). In human cells, it was found that the PRR domain is sufficient

for the localization of ALIX to the midbody, consistent with the fact that the GPPX<sub>3</sub>Y motif present in the PRR is essential for its interactions with CEP55. In contrast, this motif is not necessary for Alix localization at the midbody or for cytokinesis in *Drosophila*. Instead, the V-domain is both necessary and sufficient for Alix localization at the midbody in cultured cells and in flies.

To understand how Alix could be localized to the midbody, the authors searched for partners interacting with the V-domain using immunoprecipitation and mass spectrometry. They found that the components of centralspindlin — Pavarotti and RacGAP50C — are two putative partners of the Alix V-domain, and demonstrated that endogenous Pavarotti indeed coimmunoprecipitates with overexpressed Alix. *In vitro* experiments further revealed that the Alix–Pavarotti interaction is direct. On the Alix side, it involves a hydrophobic patch within the V-domain, which is involved for the direct interaction with (L)YPX<sub>n</sub>L/LxxLF motifs in viral proteins. On the Pavarotti side, it requires an LxxLF motif located in the motor domain. Thus Alix V-domain directly interacts with the motor domain of Pavarotti in *Drosophila* (Figure 1B).

Functionally, the direct interaction between Pavarotti and Alix is crucial for Alix recruitment at the midbody and for cytokinetic abscission. Indeed, a point mutation in the LxxLF motif of Pavarotti that disrupts the interaction with Alix does not perturb Pavarotti localization at the midbody or the formation of the Centralspindlin complex, but prevents Alix localization at the midbody. Reciprocally, mutations in the Alix V-domain that disrupt the interaction with Pavarotti prevent the recruitment of Alix (but not Pavarotti) at the midbody. Importantly, mutations in the V-domain of Alix are unable to rescue the cytokinetic defects associated with Alix inactivation *in vivo*. Consistent with Pavarotti acting upstream, Alix is not recruited at the midbody in Pavarotti-depleted cells, whereas Pavarotti is correctly localized in Alix-depleted cells. Importantly, depletion of either Pavarotti or Alix impairs the localization of the ESCRT-III component Shrub at the midbody, explaining the requirement of Pavarotti–Alix interactions for proper cytokinesis. Altogether, the direct interaction between Pavarotti and Alix bypasses the need for CEP55 for coupling Alix–ESCRT to centralspindlin in *Drosophila* (Figure 1B).

This work raises several interesting questions. First, how is Tsg101 recruited in *Drosophila*? This is particularly important, since the authors found that Alix and Tsg101 are individually required for Shrub localization at the midbody (Figure 1B), whereas in

mammalian cells the depletion of both ALIX and TSG101 is required for losing ESCRT-III at the midbody (Figure 1A). In mammalian cells, the situation is simple since CEP55 interacts with both ALIX and TSG101 [10]. In the absence of CEP55, ALIX as well as TSG101 are thus lost from the midbody [4,5,10]. In *Drosophila* cells, Lie-Jensen *et al.* reported that Tsg101 recruitment at the midbody depends on Pavarotti but not on Alix. This thus raises the possibility that, as for Alix, Centralspindlin, recruits Tsg101 to the midbody either directly or indirectly.

A second unanswered question deals with the regulation of the interaction between Alix and Pavarotti. In mammalian cells, CEP55 phosphorylation by PLK1 prevents its interaction with MKLP1 and ensures that CEP55 (and thus the ESCRT) are not recruited too early to the anaphase spindle, which would be detrimental for cytokinesis [18]. In *Drosophila* cells, which lack CEP55, the interaction between Alix–Tsg101 and Pavarotti must be regulated by other means, perhaps by direct phosphorylation of Alix, Tsg101 and/or Pavarotti by Plk1 or by other mitotic kinases. Another interesting question is to determine the significance, if any, of the shift from a simple Pavarotti–Alix–Tsg101–ESCRT to a more complex MKLP1–CEP55–ALIX–TSG101–ESCRT for ESCRT recruitment in cytokinesis. CEP55 has additional properties other than interacting with ALIX and TSG101: it can self-assemble and has a microtubule-bundling activity [9], which might be important for successful abscission in vertebrates.

Intriguingly, the LxxLF motif in Pavarotti is found as a related LxxIF motif in the kinesin-6 family protein Subito in *Drosophila*, as well as in *Caenorhabditis elegans* ZEN-4 and in *Homo sapiens* MKLP1 and MKLP2. It should thus be tested whether these other kinesins can also bind directly to ALIX and might help ALIX recruitment to the midbody. This would raise new questions in mammals, since in cultured cell lines ALIX is lost from the midbody after CEP55 depletion whereas MKLP1/MKLP2 are still correctly localized at the midbody. Perhaps this alternative mechanism of recruiting ALIX is required only in specific cells, since some cell types express low levels of CEP55 [19]. Actually, MKLP1 directly interacts with another ESCRT component, the ESCRT-III protein CHMP4C [20].

Finally, this work suggests that the direct binding between the Centralspindlin and Alix, without the need for CEP55, might represent the ancestral way of recruiting the ESCRT machinery during cytokinesis. Testing this hypothesis in other invertebrates, such as *C. elegans*, would help to confirm this hypothesis.

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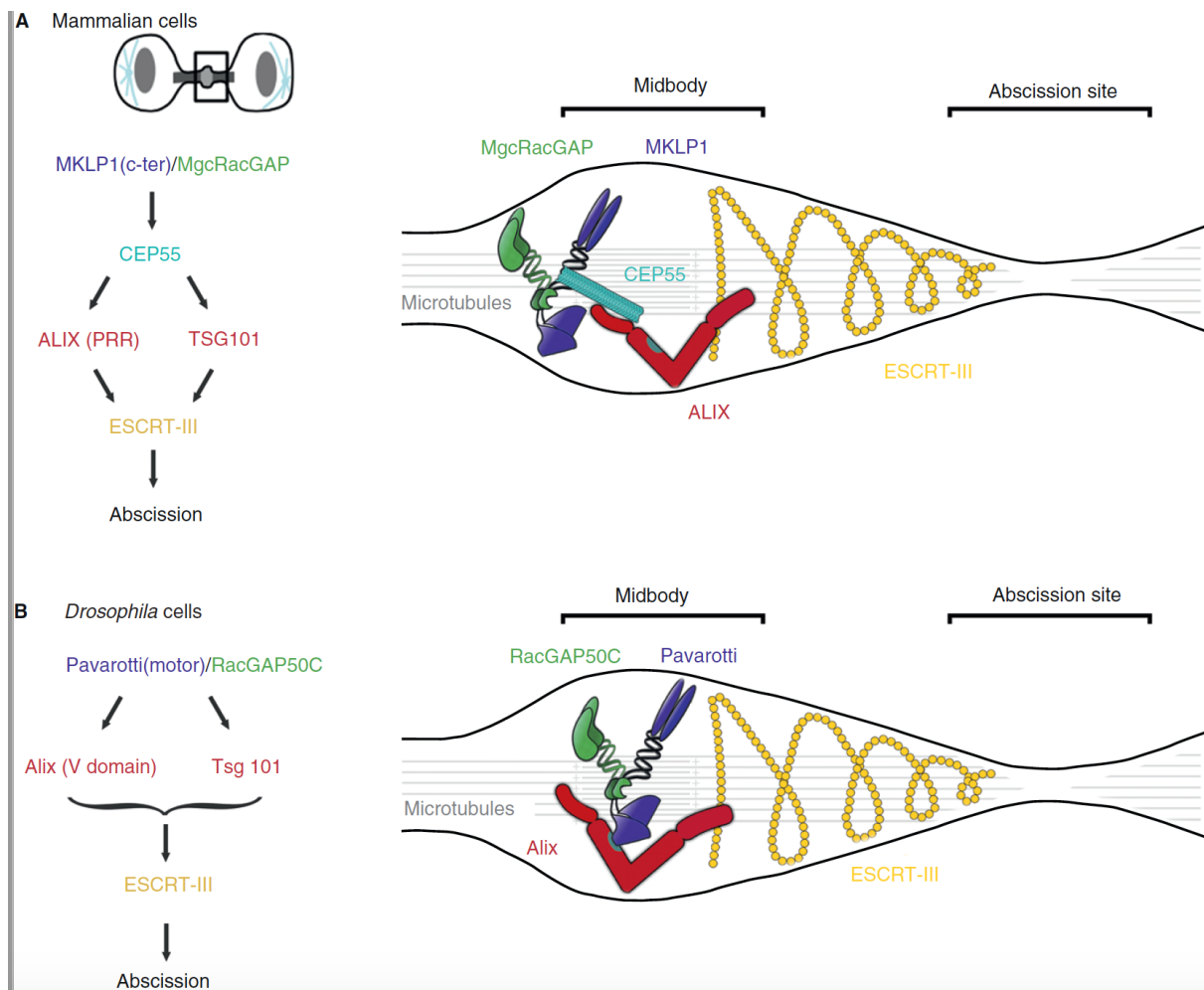


Figure 1: Alternative mechanisms for the recruitment of ESCRT-III at the midbody in mammalian cells and in *Drosophila* cells. In mammalian cells (A), the C-terminal part of the kinesin MKLP1 directly interacts with CEP55 and recruits this protein at the midbody, the central part of the intercellular bridge. There, CEP55 recruits both ALIX (via its C-terminal proline rich region or PRR) and TSG101 through direct interactions. ALIX and TSG101 directly and indirectly recruit the ESCRT-III machinery, respectively. In *Drosophila* cells (B), MKLP1 homologue Pavarotti interacts directly via its motor domain with the central, V-domain of Alix. The N-terminal part of Alix directly interacts with ESCRT-III and promotes ESCRT recruitment to the midbody. Tsg101 also participates to ESCRT localization at the midbody and is recruited to the intercellular bridge in a Pavarotti-dependent manner. In both mammalian and *Drosophila* cells, ESCRT-III recruitment at the midbody is required for cytokinetic abscission.

In Brief

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