Microscopic elucidation of the spatio-temporal control of ALIX during cytokinesis

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Abstract:
During cytokinesis, the final step of cell division, a parental cell is physically separated into two daughter cells. One of the core components of the machinery that mediates the abscission of the intercellular bridge is the scaffolding protein ALIX, which plays an evolutionarily conserved role in recruitment of ESCRT-III that mediates membrane scission. The abscission machinery is sequentially recruited to the intercellular bridge to form the midbody complex, and knockdown or depletion of ALIX leads to delayed abscission. Recently, our lab demonstrated that in Drosophila, ALIX is recruited to the midbody via a mechanism that is analogous to virus budding [1]. On the contrary, in mammalian cells ALIX is recruited to the midbody via direct interaction with CEP55. ALIX itself then promotes the recruitment of the ESCRT-III component CHMP4B to the midbody. Many components of the abscission machinery have been identified and their functions have been described, but the precise regulation and interplay of these components during cytokinesis is not well understood.

In this study we used different microscopic approaches to analyze the spatio-temporal control and functional regulation of ALIX during cytokinesis. We can demonstrate that ALIX is transported along the microtubule network to the intercellular bridge. Consequently disruption of the microtubule network inhibits ALIX transport. FRAP analysis reveals a highly motile ALIX fraction at the midbody, in contrast to ALIX that associates to post-abscission midbody remnants. Furthermore, we analyzed the structural dynamics of ALIX at the midbody by super-resolution microscopy (3D-SIM). Our data show that during cytokinesis ALIX subsequently accumulates at the midbody and eventually forms a spiral-like structure. This structure shows a strong colocalization with the ESCRT-III protein CHMP4B. Moreover, ALIX also localizes to the abscission site.

In summary our data indicates a more complex role of ALIX than it has been suggested before. ALIX seems not only to promote the recruitment of the ESCRT-III machinery to the midbody, but also co-function in the stabilization of the ESCRT-III machinery and the initiation of the abscission site.

[1] A. Lie-Jensen *et al.*, Centralspindlin Recruits ALIX to the Midbody during Cytokinetic Abscission in Drosophila via a Mechanism Analogous to Virus Budding, *Current Biology*, **29 (20)**, 3538-3548.e7 (2019).