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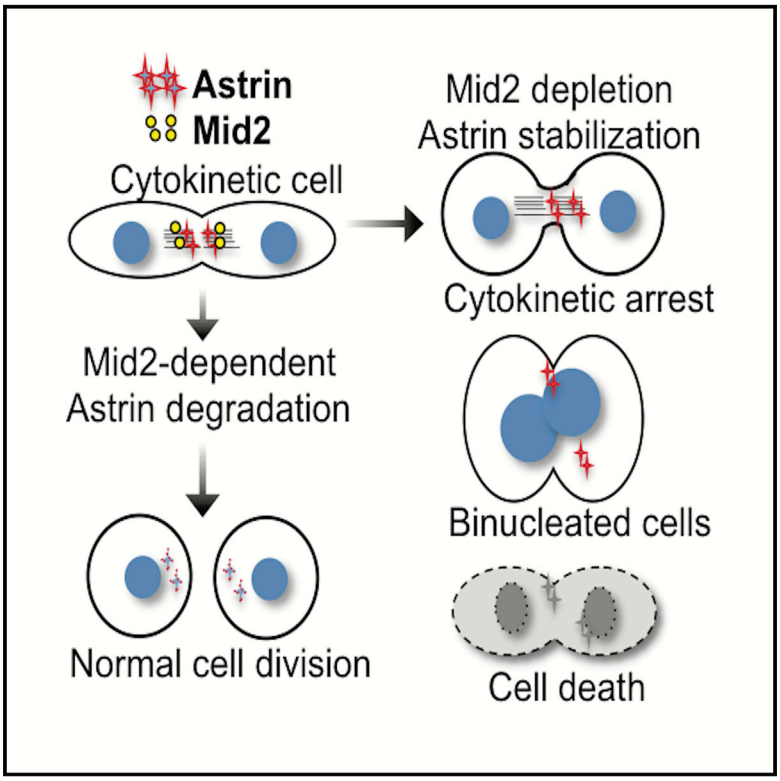
The X-Linked-Intellectual-Disability-Associated Ubiquitin Ligase Mid2 Interacts with Astrin and Regulates Astrin Levels to Promote Cell Division

Graphical Abstract



Authors

Ankur A. Gholkar, Silvia Senese, Yu-Chen Lo, ..., Joseph Capri, Julian P. Whitelegge, Jorge Z. Torres

Correspondence

torres@chem.ucla.edu

In Brief

Gholkar et al. demonstrate that the Mid2 ubiquitin ligase that is found mutated in some individuals with X-linked intellectual disability is important for human cell cytokinesis. Mid2 binds astrin, ubiquitinates astrin on lysine 409, and targets a pool of astrin for destruction during mitotic exit, which is required for proper cytokinesis.

Highlights

- Astrin co-purifies and localizes with Mid1 and Mid2 to cytokinetic microtubules
- Mid2 ubiquitinates astrin on K409 and targets it for degradation during cytokinesis
- Mid2 depletion leads to astrin stabilization and increased cytokinetic defects
- K409A astrin accumulates at cytokinetic microtubules and leads to cytokinetic defects

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The X-Linked-Intellectual-Disability-Associated Ubiquitin Ligase Mid2 Interacts with Astrin and Regulates Astrin Levels to Promote Cell Division

Ankur A. Gholkar,[1] Silvia Senese,[1] Yu-Chen Lo,[1,2] Edmundo Vides,[1] Ely Contreras,[1] Emmanuelle Hodara,[1] Joseph Capri,[3] Julian P. Whitelegge,[3,4,5] and Jorge Z. Torres[1,4,5,*]

[1]Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA 90095, USA
[2]Program in Bioengineering, University of California, Los Angeles, Los Angeles, CA 90095, USA
[3]Pasarow Mass Spectrometry Laboratory, The Jane and Terry Semel Institute for Neuroscience and Human Behavior, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA
[4]Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA 90095, USA
[5]Jonsson Comprehensive Cancer Center, University of California, Los Angeles, Los Angeles, CA 90095, USA
*Correspondence: torres@chem.ucla.edu
http://dx.doi.org/10.1016/j.celrep.2015.12.035

SUMMARY

Mid1 and Mid2 are ubiquitin ligases that regulate microtubule dynamics and whose mutation is associated with X-linked developmental disorders. We show that astrin, a microtubule-organizing protein, co-purifies with Mid1 and Mid2, has an overlapping localization with Mid1 and Mid2 at intercellular bridge microtubules, is ubiquitinated by Mid2 on lysine 409, and is degraded during cytokinesis. Mid2 depletion led to astrin stabilization during cytokinesis, cytokinetic defects, multinucleated cells, and cell death. Similarly, expression of a K409A mutant astrin in astrin-depleted cells led to the accumulation of K409A on intercellular bridge microtubules and an increase in cytokinetic defects, multinucleated cells, and cell death. These results indicate that Mid2 regulates cell division through the ubiquitination of astrin on K409, which is critical for its degradation and proper cytokinesis. These results could help explain how mutation of *MID2* leads to misregulation of microtubule organization and the downstream disease pathology associated with X-linked intellectual disabilities.

INTRODUCTION

The TRIM/RBCC (tripartite motif composed of a RING finger, zinc binding motif/s called B-box/s, and a coiled coil region) superfamily of E3 ubiquitin ligases has variable roles in cellular homeostasis, including signaling, regulation of cytoskeletal structures, and cell-cycle progression (Meroni and Diez-Roux, 2005). The Mid1 (midline 1, or Trim18) and Mid2 (midline 2, or Trim1) ubiquitin ligases belong to the C-I TRIM subfamily and share a similar domain architecture, with an N-terminal tripartite motif and FN3 (fibronectin type III) and B30.2-like/RFP (Ret finger protein) domains at their C terminus (Meroni and Diez-Roux, 2005; Figure 1A). Mid1 and Mid2 also contain a COS (C-terminal subgroup one signature) box between the coiled coil and FN3 motif that mediates the binding of these proteins to microtubules (Short and Cox, 2006; Figure 1A).

Mid1 and Mid2 have critical roles during development, and mutation of *MID1* has been linked to Opitz syndrome (MIM: 300000), an X-linked disease characterized by congenital anomalies that is manifested by the abnormal closure of midline structures (Cainarca et al., 1999), whereas mutation of *MID2* has been linked to X-linked intellectual disabilities (MIM: 300204; Geetha et al., 2014). Mid1 and Mid2 homo- and heterodimerize (Cainarca et al., 1999; Short et al., 2002) and have been implicated in anchoring proteins to microtubules, in the bundling and stabilizing of microtubules, and in organizing microtubules during neural tube closure (Berti et al., 2004; Short et al., 2002; Suzuki et al., 2010). Therefore, Mid1 and Mid2 have roles in microtubule stability and organization; however, whether their ubiquitin ligase activities are required for regulating these processes remains to be determined. Although Mid1 ubiquitination substrates include alpha4, the catalytic subunit of protein phosphatase 2A, and Fu, whether ubiquitination of these proteins leads to changes in microtubule organization has not been explored (Du et al., 2013; Schweiger et al., 2014; Trockenbacher et al., 2001). Additionally, to our knowledge, there are no known substrates of Mid2 that can explain its role in regulating microtubule organization.

To better understand the molecular basis of X-linked developmental diseases associated with mutation of *MID1* and *MID2*, we analyzed the Mid1 and Mid2 interactomes and identified SPAG5 (sperm-associated antigen 5, aka hMAP126 and astrin). Astrin is a microtubule-bundling/organizing protein critical for regulating microtubules during cell division (Gruber et al., 2002; Mack and Compton, 2001; Thein et al., 2007). Mid1 and Mid2 showed an overlapping localization with astrin to intercellular bridge microtubules (ICBMTs). Depletion of Mid1 or Mid2 arrested cells during cytokinesis and generated binucleated cells. Importantly, Mid2 ubiquitinated astrin on lysine 409 during mitotic exit and