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Extra Views

The Cdc48/p97-Ufd1-Npl4 Complex

Its Potential Role in Coordinating Cellular Morphogenesis During the M-G_1 Transition

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ABSTRACT

The AAA ATPase Cdc48/p97 together with its adaptors, Ufd1-Npl4, regulate membrane-related functions and mitotic spindle disassembly by directly binding to membrane-associated proteins or spindle assembly factors, modulating their interactions with membranes or spindles, respectively. Here, we discuss the possibility that the Cdc48/ p97-Ufd1-Npl4 complex has a more general role in mediating morphological transitions as the cell exits mitosis and enters G_1.

The AAA ATPase *CDC48* was first identified in *Saccharomyces cerevisiae* as a cell cycle division gene.[1] Cdc48, as well as its homolog p97 in vertebrates, forms a homohexameric ring that functions as an active ATPase.[2] Together with its adaptor proteins, the Ufd1-Npl4 heterodimer,[3] the Cdc48/p97-Ufd1-Npl4 complex participates in a variety of membrane-related functions. First, this complex is required to release polypeptides from the ER membrane into the cytosol for subsequent degradation by the 26 S proteasome.[4-7] In addition, it acts as a chaperone to separate a transcription factor from its membrane-anchored precursor—a function that does not lead to degradation by the proteasome.[8,9] Finally, the p97-Ufd1-Npl4 complex is needed for an early stage of nuclear envelope assembly in interphase *Xenopus* egg extracts, although the mechanism of action in this case is unknown.[10]

During each cell cycle, the microtubule cytoskeleton undergoes drastic transformations, especially in the processes of spindle formation and disassembly. While the mechanism of bipolar spindle assembly has been the focus of many studies, how a highly dynamic mitotic spindle reorganizes into a relatively stable interphase microtubule array is poorly understood. It is commonly accepted that the downregulation of Cdc2 kinase activity after mitotic exit results in the inactivation of spindle assembly factors, thereby leading to spindle disassembly. However, considering that spindle disassembly has to be coordinated with many other morphological transformations including chromosome decondensation, cytokinesis, and nuclear envelope assembly, additional regulatory control(s) is likely to exist. Since *cdc48* mutants arrest in late mitosis with aberrant medium length spindles,[11] we reasoned that the Cdc48/p97-Ufd1-Npl4 complex might regulate spindle disassembly at the end of mitosis. Using *Xenopus* egg extracts and yeast genetics, we found that Cdc48/p97-Ufd1-Npl4 regulates spindle disassembly at the end of mitosis in a separate pathway from the mitotic exit network (MEN), and this complex is able to bind to certain spindle assembly factors and regulate their interactions with microtubules at mitotic exit. Our studies demonstrate that in order to successfully reestablish interphase microtubule arrays, both Cdc2 inactivation and the activity of Cdc48/p97-Ufd1-Npl4 are required.[12]

CDC48/P97-UFD1-NPL4, A GENERAL REGULATOR OF M-G_1 TRANSITION?

Cdc48/p97-Ufd1-Npl4 acts as a chaperone to regulate membrane-related functions and mitotic spindle disassembly. The key similarity is in that this complex recognizes proteins of large cellular structures such as ER and the mitotic spindle and regulates the interaction of these proteins with the cellular structures. It is tempting to speculate that Cdc48/ p97-Ufd1-Npl4 might use a similar mechanism to assist other morphological transformations in cell division, particularly in establishing a proper G_1 phase after mitosis. Consistent with this idea, Cdc48/p97-Ufd1-Npl4 is required for post-mitotic nuclear envelope reassembly,[10] which is one of the major morphological transformations occurring at the end of cell division. Interestingly, we observed that either a dominant-negative allele of p97 (p97QQ) or the depletion of Ufd1-Npl4 blocked chromosome decondensation and the formation of interphase nuclei after the cell cycle returned to the interphase state in

Xenopus extracts. This suggests that Cdc48/p97-Ufd1-Npl4 could play a role in post-mitotic chromosome decondensation. Studies have suggested that post-mitotic chromosome decondensation and nuclear envelope assembly occur in a coordinated manner. The docking of nuclear envelope and nuclear pore proteins to late anaphase and early telophase chromosomes represents some of the earliest steps of post-mitotic nuclear envelope assembly.[10,13-15] Successful nuclear envelope assembly may require the reorganization or decondensation of mitotic chromatin at the end of mitosis to create sites for nuclear membrane docking and fusion. Cdc48/p97-Ufd1-Npl4 could assist this chromatin change by removing proteins involved in chromosome condensation from the highly condensed mitotic chromatin. In addition, this complex may also directly regulate nuclear envelope assembly by assisting proper assembly of nuclear envelope proteins on the chromatin. Our preliminary studies show that both Ufd1 and Npl4 are localized on condensed chromosomes throughout mitosis in mammalian and *Xenopus* tissue culture cells (our unpublished observation), which puts the proteins at the right place to coordinate post-mitotic chromosome decondensation with nuclear envelope assembly.

Cytokinesis is another major event occurring at the end of mitosis. Considering the complex changes of microtubules, F-actins, and membranes during cytokinesis, it is of great interest to examine whether Cdc48/p97-Ufd1-Npl4 is involved in coordinating some of these changes. We have shown that Cdc48/p97-Ufd1-Npl4 interacts with both the vertebrate polo kinase and its budding yeast homolog Cdc5. In fact, Cdc48/p97-Ufd1-Npl4 is required for Cdc5 degradation at the end of mitosis.[12] This may link Cdc48/p97-Ufd1-Npl4 to cytokinesis since accumulating evidence has shown that temporal and special regulation of polo kinase plays critical roles in cytokinesis.[16-18]

In addition to its role in cytokinesis, Cdc5 also turns on MEN to inactivate Cdc2/Cyclin B.[19,20] After successful mitotic exit, it is necessary to turn off the MEN to allow the cell to reenter G_1 properly. Although not much is known about how MEN is turned off, in budding yeast, one mechanism may involve the destruction of Cdc5 via APC^{Cdh1}-directed ubiquitination.[21] Our observation that Cdc48-Ufd1-Npl4 mediates Cdc5 proteolysis at the end of mitosis suggests an additional role for Cdc48/p97-Ufd1-Npl4 as a novel regulator in turning off MEN. In addition, a recent study reports a role of Cdc48 in the execution of Start in G_1 by mediating the proteolysis of the G_1-CDK inhibitor Far1.[22] This finding is consistent with the idea that Cdc48/p97-Ufd1-Npl4 has a general role in regulating proper M-G_1 transition.

Although the ideas discussed above are highly speculative, they offer some new angles to study the events occurring at the M to G_1 transition. Thus far, much effort in studying cell division has been focused on how Cdc2 kinase activity is turned on and off in a cell cycle. Cdc48/p97-Ufd1-Npl4 should provide a means to study how the cell cycle machinery is coupled with cellular morphogenesis, especially at the M-G_1 transition.

THE REGULATION OF CDC48/P97-UFD1-NPL4 AT THE END OF MITOSIS

As discussed above, Cdc48/p97-Ufd1-Npl4 regulates membrane-bound proteins, spindle assembly factors, and the G_1-CDK inhibitor Far1. One obvious question is how the specificity of Cdc48/p97-Ufd1-Npl4 is controlled so that it recognizes its substrates at the appropriate time and place during the cell cycle. It is possible that Cdc48/p97-Ufd1-Npl4 itself is cell cycle-regulated. Supporting this idea, Cdc48 in budding yeast is tyrosine-phosphorylated in vivo in a cell cycle-dependent manner.[23] It will be important to understand whether this phosphorylation regulates Cdc48 activity. It is not clear whether Ufd1 or Npl4 is also phosphorylated in a cell cycle-dependent manner. However, p47, another well-characterized adaptor for p97, is phosphorylated on Ser-140 by Cdc2 during mitosis.[24] The phosphorylation is important for Golgi disassembly in mitosis.[25] This suggests that Ufd1-Npl4 might also be regulated by phosphorylation. It is possible that activation of MEN could lead to phosphorylation of Ufd1-Npl4, which might in turn allow Cdc48/p97-Ufd1-Npl4 to recognize spindle assembly factors at mitotic exit.

In addition to regulation of the Cdc48/p97-Ufd1-Npl4 complex itself, another attractive way to control the specificity of this complex is to regulate its substrates. Cdc48/p97-Ufd1-Npl4 can bind to ubiquitin directly through Cdc48/p97, or to a much greater extent via Ufd1-Npl4.[26] Therefore, regulated ubiquitination of substrates could confer specificity for Cdc48/p97-Ufd1-Npl4. Although this idea is attractive, it is important to note that whether ubiquitination of substrates is required for recognition by Cdc48/p97-Ufd1-Npl4 has been controversial. While Cdc48/p97-Ufd1-Npl4 clearly recognizes certain substrates in a ubiquitination-dependent manner, it can also bind to substrates that are not ubiquitinated. For example, by immunoprecipitation, Cdc48/p97-Ufd1-Npl4 can precipitate an ERAD (ER associated protein degradation)-substrate and several spindle assembly factors that are not ubiquitinated.[7,12] However, since this interaction was shown by immunoprecipitation, the possibility exists that Cdc48/p97-Ufd1-Npl4 may indirectly recognize its substrate by binding to an unidentified ubiquitinated protein that forms a complex with the substrates. Clearly, we are still at a very early stage of exploring the functions of the Cdc48/p97-Ufd1-Npl4 complex. How this complex regulates cellular morphogenesis in the context of cell cycle regulation and how it achieves specificity in substrate recognition represent some of the most interesting questions to study in the future.

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