The presence of too many or too few centrosomes at mitosis can disrupt the timely formation of a bipolar spindle and may lead to aneuploidy and cancer. Strict control of centrosome duplication is therefore crucial. Centrosome duplication must occur once per cell cycle and the number of new centrioles made must be tightly controlled. The importance of protein degradation for the orderly progression of the cell cycle has long been recognized, but until recently the role of proteolysis in the regulation of centrosome duplication had not been appreciated. Recent evidence suggests that restricting protein levels so that a single new centriole is built next to each pre-existing centriole is one way in which centrosome duplication is controlled. Here we discuss our recent finding that the SCF ubiquitin ligase complex regulates centrosome duplication in *C. elegans* in the larger context of the proteolytic regulation of centrosome duplication.

**Promoting Centrosome Duplication**

The centrosome is composed of two centrioles and a surrounding cloud of pericentriolar material. Control of centrosome number is achieved by strict regulation of centrosome duplication so that it occurs once per cell cycle. Centrosome duplication is a semi-conservative process where a new (daughter) centriole is built next to each existing (mother) centriole, regulated by the sequential recruitment of the five key proteins, SPD-2, ZYG-1/Plk4, SAS-6, SAS-5/ANA2/STIL and SAS-4/CPAP. These key regulators of centrosome duplication were first discovered in *C. elegans* and this core pathway is conserved in Drosophila and humans, although additional complexity and specialization is apparent in higher organisms.

Loss of any of the core centrosome duplication proteins leads to a failure in centrosome duplication. Conversely, overexpression of some of these factors, including PLK4 and SAS-6, leads to the formation of extra centrosomes. Overexpression data suggest that a delicate balance of PLK4 and SAS-6 activity must be maintained in the cell: the presence of either too little, or too much of either protein leads to the formation of too few, or too many centrosomes. Ensuring the appropriate level of each centrosome duplication factor to allow the formation of only a single daughter centriole next to each mother is therefore key to maintaining appropriate centrosome numbers. Understanding how these proteins are degraded, what molecules are involved and how the process is regulated, is central to determining the role of proteolysis in the control of centrosome duplication.

**Protein Degradation: Controlling the Masses**

It has long been recognized that cell cycle progression is regulated by the periodic accumulation and degradation of important regulatory proteins. For example, accumulation of the cdk1 activator, cyclin B, promotes mitotic entry and, once the important events of mitosis are completed, the destruction of cyclin B leads to mitotic exit. It is the irreversibility of protein degradation that ensures an orderly stepwise...
progression through the cell cycle. Recent evidence suggests that centrosome duplication is also regulated by the timely destruction of its positive regulators. Moreover, the involvement of the same molecules in both cell cycle and centrosome regulation leads to co-regulation of the two processes.

Regulated ubiquitination is the key step in the control of protein degradation because tagging a protein with a polyubiquitin chain targets it for destruction by the 26S proteasome. Ubiquitin conjugation is catalyzed by one of two E3 ubiquitin ligation enzymes, the SKP-CUL1-F-box (SCF) complex or the anaphase promoting complex/cyclosome (APC/C). Substrate specificity of the SCF complex is achieved by its selective association with one of many F-box proteins, which are involved in substrate recognition. The APC/C associates with the co-activator subunits CDC20 or CDH1 in a temporally restricted manner to achieve specificity. Both the SCF and APC/C participate in the regulation of centrosome duplication.

Moderating Plk4/ZYG-1 Levels

Regulation of Plk4 activity is achieved through regulated protein degradation mediated by the SCF E3 ubiquitin ligation complex. The importance of proteolysis for the regulation of appropriate centrosome numbers was initially recognized in Drosophila, where supernumerary centrosomes are evident when SCF activity is impaired in SkpA and Slimb mutant cells. More recently the molecular target of the SCF was found to be Plk4. In Drosophila and human cells, SCF recognition and thus degradation of Plk4 is mediated by the homologous F-box proteins Slimb and βTrCP. In C. elegans, ZYG-1 is the functional homolog of Plk4 and our work in the worm has uncovered a similar role for the SCF complex in regulating ZYG-1 levels. A notable difference in our findings, however, is that recognition of ZYG-1 by the SCF employs two F-box proteins: the slimb/βTrCP homolog LIN-23 and a second F-box protein SEL-10 (Fig. 1). The use of these two F-box proteins for ZYG-1 degradation in C. elegans raises interesting questions about conservation of this regulatory pathway.

Does degradation of Drosophila or mammalian plk4 also use additional F-box proteins? A systematic screen of Drosophila F-box proteins found Slimb to be the only F-box protein with a role in Plk4 degradation. If this is truly the case, it represents an evolutionary divergence between C. elegans and Drosophila. There remains, however, the possibility that a minor role in Plk4 degradation is played by a second F-box protein in Drosophila, or that a second F-box protein plays a cell-type-specific. Differentiation of these possibilities will require further work.

In contrast there is already evidence to suggest that mammalian Plk4 levels, like those of ZYG-1, are regulated by the combined action of multiple ubiquitin ligases. Deletion of the βTrCP binding site in Plk4 did not prevent its ubiquitination in human cells, nor did it prevent protein degradation. Based on our results it is tempting to speculate that the human homolog of SEL-10, FBW7, may play a role in Plk4 degradation. Indeed existing evidence makes this an attractive hypothesis. First, inactivation of FBW7 has previously been associated with centrosome amplification. Second, in human cells Plk2 promotes centrosome duplication by phosphorylation and destabilization of FBW7. FBW7 has a well-established role in cyclin E degradation, and increased cyclin E activity following FBW7 down-regulation may contribute to centrosome amplification/duplication. Nevertheless it is an intriguing possibility that FBW7 also regulates Plk4 and that when FBW7 is downregulated increased Plk4 levels contribute to centrosome duplication, perhaps in combination with cyclin E. In the future it will be important to clarify if FBW7 plays a role in the regulation of Plk4 to better understand how its inactivation leads to centrosome amplification. This in turn may give important insights into how FBW7 inactivation contributes to cancer.
in the levels of FBW7 substrates.\(^7\) It is currently unknown whether SEL-10 or LIN-23 levels/activity are cell cycle-regulated and thus whether this contributes to changes in ZYG-1 levels.

Our finding that degradation of ZYG-1 is regulated by the SCF complex is the first evidence that centrosome duplication in \textit{C. elegans} is regulated by proteolysis. We find regulation of ZYG-1 and Plk4 to be similar, suggesting evolutionary conservation of these pathways. In addition to regulating Plk4 levels, proteolysis also regulates other aspects of centrosome duplication in human cells, notably SAS-6 levels.

**Regulating SAS-6: Multiple Pathways**

SAS-6 is a structural component of the centriole and in human cells it begins to accumulate in G1, reaches maximal levels at G2/M and sharply decreases in mitosis.\(^2\) Regulation of this cyclical pattern of protein accumulation and destruction is achieved through proteolysis regulated by two different E3 ubiquitin ligase activities (Fig. 1). First, APC/C-CDH1 targets hSAS-6 for degradation at the end of mitosis, through binding to a KEN box in the C-terminal portion of SAS-6.\(^2\) Temporal regulation is achieved by the availability of the CDH1 targeting subunit, which peaks in M/G1. Second, the SCF\(^{FBXW5}\) complex targets SAS-6 for degradation in S phase.\(^2\) Degradation of SAS-6 by SCF\(^{FBXW5}\) is regulated in a complex fashion. FBXW5 levels are cell cycle regulated through degradation of FBXW5 by the APC/C complex during M/G1. Notably, the APC/C downregulates both SAS-6 and FBXW5 at the M/G1 transition, which suggests that it is the APC/C rather than SCF\(^{FBXW5}\) that is responsible for the large-scale destruction of SAS-6 seen at this time. FBXW5 levels subsequently increase reaching a maximum in S-phase. However, phosphorylation of FBXW5 by PLK4 prevents the ubiquitination of SAS-6 during the G1/S transition. SCF\(^{FBXW5}\) downregulates SAS-6 activity in late S phase, when PLK4 activity abates. Because global SAS-6 levels do not appear to decrease in S phase\(^2\) the importance of SCF\(^{FBXW5}\) activity for SAS-6 degradation at the time is unclear and perhaps represents a requirement for spatially restricted degradation.

The involvement of Plk4 in regulating the timing of SAS-6 degradation is intriguing and shows that proteolytic control of centrosome duplication involves a network of regulatory inputs and connections. This highlights that understanding the integration of these controls requires us to focus on the global picture.

**Proteolysis: Converging Controls of Centrosome Duplication**

Both Plk4/ZYG-1 and SAS-6 levels are determined by cell cycle-regulated degradation. How do the pathways fit together to ensure a coordinated system of proteolysis that ensures appropriate regulation of centrosome duplication? I have combined data from humans and \textit{C. elegans} to summarize what is currently known about the network of interactions relevant to Plk4/ZYG-1 and SAS-6 regulation (Fig. 1).

In human cells Plk2 regulates FBW7 levels, but whether a similar regulation of the FBW7 homolog, SEL-10, exists in worms is unknown. In \textit{C. elegans} SEL-10, in combination with βTrCP, downregulates ZYG-1 to control centrosome duplication timing. This pathway is at least partially conserved. In humans, PLK4 phosphorolysates FBXW5 to prevent SAS-6 degradation. In \textit{C. elegans} there is no clear FBXW5 homolog, but ZYG-1 phosphorolysates SAS-6 to promote centrosome duplication.\(^2\) The phosphorylated residue is not conserved in hSAS-6, so it is unclear whether this interaction exists in humans. Nevertheless in both worms and humans SAS-6 activity is regulated by Plk4/ZYG-1. Lastly, an additional level of regulation is provided by the APC/C\(^{CDH1}\)-mediated degradation of human SAS-6 at the G1/M transition. The \textit{C. elegans} SAS-6 protein lacks the KEN box recognition sequence for CDH1 binding therefore it seems unlikely that SAS-6 is degraded by a conserved mechanism in the worm. The requirement for APC/C\(^{CDH1}\) activity in the larger context of centrosome duplication has not been tested in \textit{C. elegans} and it remains possible that the APC/C recognizes alternative substrates involved in centrosome duplication.

**Perspectives**

While our recent work has provided the first insights into the regulation of ZYG-1, many interesting questions remain. Of particular importance are questions relating to whether the pathway is conserved between humans and worms, how this degradation pathway is cell cycle-regulated and how it is integrated with other proteolytic controls of centrosome duplication. One particularly interesting question in the future will be to understand the spatial importance of protein degradation for the regulation of centrosome duplication.

Recent years have brought many crucial advances in understanding the contribution that regulated protein degradation makes to controlling centrosome duplication. In the future it will be interesting to discover how these systems fit together to integrate control of the cell cycle and centrosome duplication.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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