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Article

APC/C^{Cdh1} Targets Aurora Kinase to Control Reorganization of the Mitotic Spindle at Anaphase

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Summary

Background: Control of mitotic cell cycles by the anaphase-promoting complex or cyclosome (APC/C) ubiquitin ligase depends on its coactivators Cdc20 and Cdh1. APC/C^{Cdc20} is active during mitosis and promotes anaphase onset by targeting mitotic cyclins and securin. APC/C^{Cdh1} becomes active during mitotic exit and has essential targets in G1 phase. It is not known whether targeting of substrates by APC/C^{Cdh1} plays any role in the final stages of mitosis. Here, we have investigated the role of APC/C^{Cdh1} at this time in the cell cycle by using siRNA-mediated depletion of Cdh1 in human cells.

Results: In contrast to the current view that Cdh1 takes over from Cdc20 at anaphase, we show that reduced Cdh1 levels have no effect on destruction of many APC/C substrates during mitotic exit but strongly and specifically stabilize Aurora kinases. We find that APC/C^{Cdh1} is required for assembly of a robust spindle midzone at anaphase and for normal timings of spindle elongation and cytokinesis. The effect of Cdh1 siRNA on anaphase spindle dynamics requires Aurora A, and its effect can be mimicked by nondegradable Aurora kinase.

Conclusions: Targeting of Aurora kinases at anaphase by APC/C^{Cdh1} participates in the control of mitotic exit and cytokinesis.

Introduction

Mitotic cell division requires segregation of duplicated chromosomes and coordinated partitioning of the cell by cytokinesis into two daughter cells with identical complements of chromosomes. Key to this process in all eukaryotic cells is the anaphase-promoting complex or cyclosome (APC/C), a multisubunit ubiquitin ligase that targets substrates for ubiquitination and subsequent destruction by the 26S proteasome [1, 2]. The accurate segregation of chromosomes at anaphase requires the timed, APC/C-dependent destruction of anaphase inhibitors securin and mitotic cyclins. Subsequent mitotic exit and cytokinesis are accompanied by destruction of many additional substrates of the APC/C [3], but the significance of these destruction events is mostly unknown.

The activity of APC/C in somatic cell cycles is dependent on two WD40-repeat-containing coactivator proteins, named Cdc20 and Cdh1 after their budding yeast homologs. These associate with the APC/C at different times in the cell cycle. Cdc20 interacts with the APC/C at mitotic entry and is regulated by the spindle checkpoint for control of anaphase onset. Cdh1 activates the APC/C during interphase and is required in G1 for maintaining low levels of mitotic cyclins and preventing unscheduled accumulation of S phase-promoting activities [4–8]. Because the interaction of Cdh1 with the APC/C is negatively regulated by mitotic cyclin-dependent kinase (cdk) phosphorylation of Cdh1 on multiple sites, APC/C^{Cdh1} is thought to become active at anaphase onset, once cyclin B1 has been destroyed. Targeting of Cdc20 by APC/C^{Cdh1} is thought to complete the switch from APC/C^{Cdc20} to APC/C^{Cdh1} during mitotic exit.

Entry into anaphase is accompanied by a broadening of APC/C-substrate specificity that is usually attributed to the activation of APC/C^{Cdh1}, in line with the idea that Cdc20 and Cdh1 are involved in substrate recognition. Whereas APC/C^{Cdc20} targets substrates containing a D-box destruction motif, APC/C^{Cdh1} can additionally target substrates containing alternative motifs, such as the KEN box, and shows less substrate specificity in vitro [9]. Current models propose that Cdc20 and Cdh1 participate in the formation of stable ternary complexes with substrate and APC/C to influence the processivity of multiubiquitination on substrates (reviewed in [2]). This in turn should determine the efficiency with which substrates are processed by the 26S proteasome for destruction. Indeed, it has been proposed that the ordering of substrate destruction seen at anaphase [10] could be generated by differences in the processivity of ubiquitination reactions carried out on different substrates by APC/C^{Cdh1} [11].

In mammalian cells, the mitotic regulators Polo-like kinase-1 (Plk1) and members of the Aurora kinase family are among APC/C substrates destroyed during anaphase [10, 12]. All play multiple essential roles in mitosis. Aurora A associates strongly with the mitotic spindle poles throughout mitosis, whereas Aurora B, the enzymatic component of the chromosome passenger complex (CPC) [13], shows dynamic localization through mitosis. At anaphase, both Plk1 and the CPC are recruited to the spindle midzone, an antiparallel microtubule array (also known as the central spindle) that assembles between the segregated chromosomes, where they play essential roles in the organization of cytokinesis signals. These signals are delivered to the overlying cortex at the cell equator, leading to an accumulation of active RhoA that triggers actomyosin contraction in the cleavage furrow. Delivery of cytokinesis signals to the equatorial cortex critically requires microtubules of the anaphase spindle [3, 14].

There is no evidence that destruction of anaphase-specific APC/C substrates is essential for mitotic exit. However, because the APC/C is critical for the proper execution of events earlier in mitosis, it has proved difficult to investigate the importance of the mitotic activity of the APC/C after anaphase entry.

Here, we have used siRNA-mediated knockdown of Cdh1 in cells synchronized at mitosis to investigate the role of APC/C activity after anaphase onset in human cells. We have found that the activation of APC/C^{Cdh1} is unlikely to account for the targeting of many APC/C substrates at anaphase. Instead,

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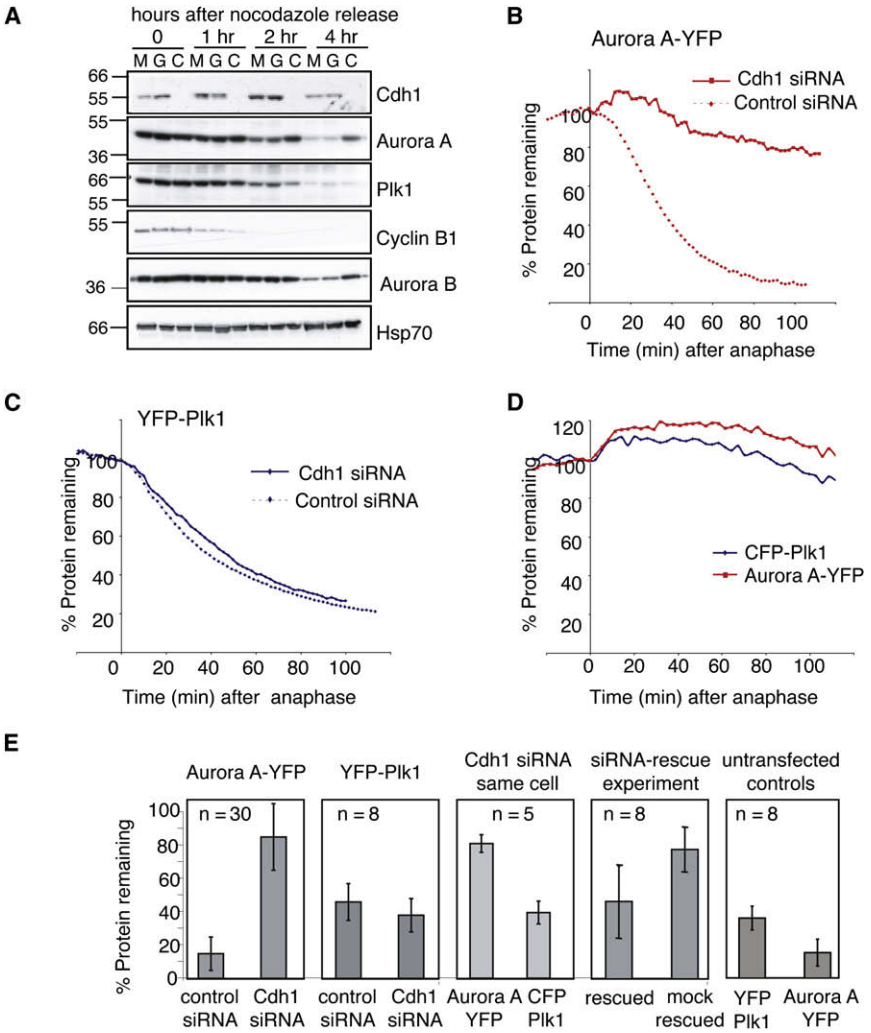

Figure 1. Targeting of Aurora Kinases by APC/C^{Cdh1} during Mitotic Exit

(A) Synchronized HeLa cells, mock treated ("M") or treated with siRNA against control (GAPDH, "G") or Cdh1 ("C") sequences, were released from nocodazole at t = 0. Samples were taken at the time points indicated for immunoblot analysis. The blots shown are typical of at least three separate experiments.

(B and C) Synchronized HeLa cells treated with siRNA against control (GAPDH) or Cdh1 sequences were injected in G2 phase with plasmids encoding Aurora A-YFP (B) or YFP-Plk1 (C) and imaged through mitosis. Fluorescence levels in individual cells were measured and plotted against time. Graphs shown are typical of the experiments summarized in (E).

(D) Synchronized HeLa cells treated with siRNA against APC3 sequences were coinjected in G2 phase with plasmids encoding Aurora A-YFP and CFP-Plk1. The graph shown is typical of at least five cells from two separate experiments.

(E) Histograms summarizing substrate degradation in different experiments (level of fluorescent protein remaining 90 min after anaphase, expressed as percentage of the level before anaphase onset). For the "Cdh1 siRNA same cell" experiment, Aurora A-YFP and CFP-Plk1 were coexpressed in single cells (see Movie S1). For the siRNA-rescue experiment, cells were prepared as in (B), except that Aurora A-YFP plasmid was coinjected with a plasmid encoding an siRNA-resistant version of Cdh1, Cdh1-SR ("rescue"), or with empty vector ("mock rescue"). The minimum number of cells analyzed in each experiment is indicated, and data were collected from at least two separate experiments. Error bars indicate standard deviations.

we identify a clear role for APC/C^{Cdh1} in targeting Aurora kinases during mitotic exit to regulate different aspects of mitotic exit. We show for the first time that APC/C^{Cdh1} is involved in control of mitotic exit in animal cells.

Results

Cdh1 siRNA Specifically Stabilizes Aurora Kinases during Mitotic Exit

We have shown previously that there is ordered proteolysis of mitotic regulators during exit from mitosis in human cells [10]. GFP-tagged Plk1 and Aurora A are both degraded at anaphase, and the timing of their degradation suggests that they are substrates for the Cdh1-associated form of the APC/C in vivo; moreover, both Plk1 and Aurora A have been shown to be substrates for APC/C^{Cdh1} in vitro [11, 15]. To test directly whether Cdh1 targets these regulators for destruction at anaphase, we examined levels of different APC/C substrates during mitotic exit in cells depleted of Cdh1 by siRNA treatment. Using double-stranded RNA oligo previously shown to give efficient knockdown of expression [16], we routinely obtained more than 95% depletion of Cdh1 (Figure S1 available online). siRNA directed against a different sequence in Cdh1 was less efficient but gave similar results where tested (Figure S1 and data not shown). To analyze substrate degradation during mitotic exit, we synchronized HeLa cells at G1/S and treated them with siRNA before harvesting mitotic cells arrested with the microtubule poison nocodazole. After we washed out nocodazole, cells exited mitosis, and we collected samples at different times for analysis of substrate levels. Mitotic-index measurements of parallel samples (Figure S2) and flow-cytometry profiles (data not shown) showed that exit from mitosis was complete 2 hr after release from nocodazole. Surprisingly, our immunoblot analyses showed that whereas Aurora A was clearly stabilized by Cdh1 knockdown during the first 4 hr after nocodazole release, Plk1 appeared to be degraded as normal (Figure 1A). This result indicated that Cdh1 might not be involved in targeting Plk1 for destruction by the APC/C. However, we also considered the possibility that Plk1 could be a much better substrate for the APC/C than Aurora A (as suggested by Rape and colleagues [11]) and that incomplete depletion of Cdh1 would allow degradation of Plk1, but not Aurora A. To look at this question more closely, we turned to live-cell degradation assays. We injected cDNAs encoding YFP-tagged versions of Aurora A and Plk1 into G2 phase HeLa cells transfected with siRNA oligos. Quantification of fluorescence levels in mitotic cells showed that Aurora A-YFP degradation was always blocked by Cdh1 siRNA treatment (Figures 1B and 1E). Coinjection of a plasmid encoding siRNA-resistant Cdh1 gave partial but significant rescue of Aurora A-YFP degradation in Cdh1 siRNA-treated cells (Figure 1E), consistent with the conclusion that Aurora A stabilization resulted from