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Review

Non-mitotic functions of the Anaphase-Promoting Complex

Manuel Eguren, Eusebio Manchado, Marcos Malumbres*

Cell Division and Cancer Group, Spanish National Cancer Research Center (CNIO), Madrid, Spain

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ABSTRACT

The Anaphase-Promoting Complex or Cyclosome (APC/C) is an E3 ubiquitin ligase whose activation requires the binding of a cofactor, either Cdc20 or Cdh1. While APC/C-Cdc20 is a major player during mitotic exit, APC/C-Cdh1 plays a central role in maintaining quiescence and controlling the onset of DNA replication. In addition, APC/C-Cdh1 is essential for endoreduplication, a process in which several rounds of DNA synthesis occur without mitosis. Recent data suggest that the APC/C is also involved in differentiation and metabolism, and plays important roles in postmitotic cells such as neurons. Thus, the APC/C is not only critical for anaphase onset but also regulates many other cellular processes during G1/S or in quiescent cells.

Contents

1. Introduction

The Anaphase-Promoting Complex/Cyclosome (APC/C) was initially identified more than 15 years ago because of its role in cyclin degradation and the metaphase to anaphase transition [1]. This protein complex functions as a E3 ubiquitin ligase by entailing the assembly of polyubiquitin chains on substrate proteins, thus targeting them for degradation by the 26S proteasome. The APC/C requires the binding of a cofactor, Cdc20 or Cdh1, in order to select substrates and perform its activity. These co-activators participate in substrate recognition by interacting with specific elements such as the KEN-box, A-box or D-Box. This interaction is mediated by the C-Terminal WD40 domain present in both APC/C co-activators [2–5]. Substrates are recruited to the APC/C by binding to a bipartite substrate receptor composed of one of the cofactors and Doc1 (also known as Apc10), an APC/C subunit also implicated in processive substrate ubiquitination [6].

APC/C activity is tightly regulated through the cell cycle. Since Cdc20 and Cdh1 associate transiently with the APC/C, the regulation of this interaction is a key event that defines the timing of APC/C activation. Cdc20 is expressed during DNA synthesis (S-phase), G2 and mitosis; however, it can only bind to the APC/C when several subunits of this complex have been phosphorylated by mitotic kinases. APC/C-Cdc20 drives mitotic exit by initiating cyclin degradation thus finally resulting in decreased Cdk activity [2]. On the contrary, Cdh1 phosphorylation by Cyclin-dependent kinases (Cdks) during S-phase, G2 and mitosis impairs its binding to the APC/C. During mitotic exit, the inactivation of Cdks and subsequent activation of mitotic exit phosphatases allows Cdh1 dephosphorylation and binding to the APC/C. APC/C-Cdh1 complexes also target mitotic cyclins for destruction completing Cdk1 inactivation. In addition, this com-

* Corresponding author at: Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernández Almagro 3, E-28029 Madrid, Spain. Tel.: +34 91 7328000; fax: +34 91 7328033.
E-mail address: malumbres@cnio.es (M. Malumbres).

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plex participates in mitotic exit through the ubiquitination of many other cell cycle regulators such as mitotic kinases or some of their regulators (Plk1, Aurora A, B and Tpx2). Finally, APC/C-Cdh1 also targets Cdc20 for degradation favoring the complete switch from APC/C-Cdc20 to APC/C-Cdh1 during the exit from mitosis [2–5].

In mammals, Cdc20 is an essential protein since it initiates the degradation of mitotic cyclins, the subsequent inactivation of Cdk1 and the activation of mitotic phosphatases [7,8]. Cdh1, on the other hand, is dispensable for the cell cycle [9–11]. However, this protein seems to play relevant roles in maintaining quiescence, preventing replication and perhaps in regulating differentiation in specific cell types. In this review, we will briefly discuss the non-mitotic functions of the APC/C. Although most of these functions have been assigned to Cdh1, Cdc20 may also play a specific role in the control of dendrite growth in neurons (see below).

2. Exiting from the cell cycle

In multicellular organisms such as mammals, most adult cells do not divide and are maintained in a state known as quiescence. When these cells re-enter into the cell cycle, they need to synthesize most of the proteins required for cell cycle progression; i.e. the factories involved in DNA replication and the structures and regulators involved in chromosome segregation. Most of the control of the cell cycle during these early phases therefore relies on the regulation of transcription. Entry into the cell cycle depends on the activation of Cdks and the subsequent inactivation of the retinoblastoma protein (pRb), a general repressor of the transcription of many cell cycle genes [12]. To be able to decide whether to continue cycling or to exit to quiescence, cells need to eliminate the cell cycle machinery synthesized during G1/S/G2. APC/C-Cdc20 first drives the exit from mitosis by targeting securin and mitotic cyclins for degradation, thus resulting in the necessary inactivation of mitotic Cdk [2]. Cdh1 also contributes to mitotic exit by targeting mitotic cyclins, although this cofactor is not essential at this stage [10,11]. In addition, Cdh1 targets many other cell cycle regulators for degradation. First, several components of the mitotic machinery such as Plk1, Aurora kinases, Tpx2, Bub1, Cdc20 or Sgo1 among others, are eliminated during mitotic exit in an APC/C-Cdh1 dependent manner [3]. Many of these proteins have important roles in chromosomal segregation and cytokinesis, and its degradation may be critical to maintain chromosomal integrity [13–17]. Indeed, in Cdh1-deficient cells these mitotic substrates accumulate and cells re-enter into a new cell cycle with increased levels of these mitotic regulators. Whether this leads to a defective cell cycle exit and cytokinesis is not clear since mitotic exit occurs normally in the presence of APC/C-Cdc20 despite the lack of degradation of these mitotic regulators [10,11]. Yet, since Cdh1-null cells accumulate genomic aberrations, it is possible that the excess of these proteins may result in abnormal chromosome segregation in the following mitotic cycles.

3. Preventing unscheduled DNA replication and genomic instability

In normal cells, the mitogen-dependent accumulation of G1 cyclins and Cdk activity results in the inactivation of the pRb pathway and the transcription of genes required for DNA synthesis [12]. In order to properly regulate DNA replication, cells need to alternate between periods of low Cdk activity and low geminin levels, in which the pre-replicative complexes (preRCs) are assembled (licensing); periods of high Cdk activity and high geminin levels, in which origin firing and DNA replication occurs (Fig. 1A) [18,19]. APC/C-Cdh1 is crucial to properly regulate the switch between these two states.

During G1, the active APC/C-Cdh1 complexes keep low Cdk activity by two different mechanisms. On one hand, APC/C-Cdh1 directly targets mitotic cyclins (A/B types) for degradation and maintains this function during G1 [20–22]. On the other hand, APC/C-Cdh1 also eliminates the Cdk activator Cdc25A as well as Skp2 and Cks1, two cofactors of the SCF E3 ubiquitin ligase. In the presence of Cdh1, SCF activity is low allowing the accumulation of Cdk inhibitors of the Cip/Kip family ($p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$) [23,24]. These alterations result in Cdk inhibition, which is required for the formation of the preRC and the subsequent loading of MCM complexes onto chromatin by ORC, Cdc6 and Cdt1 (Fig. 1A) [18,19]. APC/C-Cdh1 also ubiquitinates the inhibitor of Cdt1, Geminin. The oscillation in geminin levels is necessary for the proper regulation of the loading of MCM complexes and to avoid re-duplication events [25,26].

The accumulation of mitogenic signaling during G1 leads to the activation of G1 Cdks such as Cdk4 or Cdk2 [12]. At the G1-S transition, Cdh1 is inactivated by inhibitory phosphorylation by active Cdks and binding to Emi1, a protein that inhibits the APC/C acting as a pseudosubstrate (Fig. 1A) [27–29]. In addition, APC/C-dependent ubiquitination is also prevented at this stage by degradation of its E2 enzymes UbcH10 and Ube2S [30,31]. The increase in Cdk activity and geminin levels then allows the firing of DNA replication origins (Fig. 1A). The temporal separation between these processes, DNA licensing and firing, guarantees that the origins are only fired once per cycle and, therefore, re-replication is prevented.

The relevance of APC/C-Cdh1 during G1 and the G1/S transition has been explored by loss-of-function studies in mammals. Loss of the APC/C core subunit Apc2 leads to unscheduled proliferation in hepatocytes, even in the absence of proliferative stimuli, suggesting a critical role for the APC/C in maintaining quiescence in these cells [32]. Absence of Cdh1 results in a shorter G1 phase and early entry into S phase due to a premature increase in the levels of cyclins that lead to Cdk activation (Fig. 1B) [9–11,33]. In Cdh1-depleted cells, the shortening of G1 phase goes along with a prolonged and defective S phase [9,11]. Loss of Cdh1 function impairs the loading of MCM complexes onto chromatin and the formation of preRCs at the replication origins. In addition, the increase in geminin levels and untimely Cdk activation is likely to favor DNA synthesis (Fig. 1B). The precocious initiation of DNA synthesis without the proper machinery leads to a slower S phase progression, which could result in stalled replication forks and under-replicated DNA. These replicative defects, as well as the upregulation of mitotic kinases, can finally lead to genetic damage [11]. Thereby, this early and unscheduled entry into S phase generates some levels of genomic instability and a DNA damage response, with the activation of p53-$p21^{Cip1}$ pathway [9,33]. Yet, these deficiencies in replication together with the alterations during mitotic exit are not sufficient to stop cell cycle progression, and Cdh1-deficient cells proliferate and accumulate a variety of genomic aberrations [11,33].

Another important role of the APC/C during these stages of the cell cycle is the destruction of two key enzymes in dTTP formation: thymidine kinase 1 (TK1) and thymidylate kinase (TMPK) during G1 [34,35]. The inactivation of APC/C-Cdh1 at the G1-S transition, together with the transcriptional activation of these kinase genes during G1 [36,37], allows the expansion of the dTTP pool necessary for DNA replication. Disruption of the normal degradation of TK1 and TMPK in Cdh1-deficient cells results in a severe imbalance in the dNTP pool, and an increased rate in gene mutation since fidelity of DNA synthesis is compromised [35]. To sum up, APC/C-Cdh1 plays critical roles in maintaining G1 phase and controlling the onset of DNA replication, thus protecting chromosomal integrity.