Seminars in Cell & Developmental Biology 22 (2011) 544–550

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Processive ubiquitin chain formation by the anaphase-promoting complex

Hermann-Josef Meyer, Michael Rape*

University of California at Berkeley, Department of Molecular and Cell Biology, 522 Barker Hall, Berkeley, CA 94720-3202, United States

ARTICLE INFO

Article history:
Available online 6 April 2011

Keywords:
Ubiquitin
Anapahase-promoting complex
APC/C
Ube2C
Ube2S
Processivity

ABSTRACT

Progression through mitosis requires the sequential ubiquitination of cell cycle regulators by the anaphase-promoting complex, resulting in their proteasomal degradation. Although several mechanisms contribute to APC/C regulation during mitosis, the APC/C is able to discriminate between its many substrates by exploiting differences in the processivity of ubiquitin chain assembly. Here, we discuss how the APC/C achieves processive ubiquitin chain formation to trigger the sequential degradation of cell cycle regulators during mitosis.

Contents

1. Introduction

During mitosis, eukaryotic cells undergo dramatic structural reorganizations that allow them to evenly distribute the genetic material between their daughter cells. To accomplish this with high precision, the dividing cells need to adhere to a carefully scripted and highly conserved program that starts with nuclear envelope breakdown and chromosome condensation and proceeds through spindle formation, sister chromatid separation and cytokinesis. How the sequence of mitotic reactions is established is an area of intense research.

Among the many pathways controlling mitosis, protein degradation has emerged as a key player in orchestrating the series of events that leads to successful cell division. In eukaryotes, most proteins are degraded by the 26S proteasome [1]. This tightly regulated, compartmentalized protease recognizes its substrates after these have been covalently modified with ubiquitin chains connected through Lys11 or Lys48 of one ubiquitin molecule and the C-terminus of the next ubiquitin [2–4]. Underscoring the importance of protein degradation for mitosis, the inhibition of the 26S proteasome or loss of K11- or K48-linked ubiquitin chain formation interfere with cell division in multiple organisms [4,5].

The attachment of proteolytic ubiquitin chains to cell cycle regulators requires the collaborative effort of at least three enzymatic activities, referred to as E1, E2, and E3. An E1 activates ubiquitin in an ATP-dependent reaction by forming a thioester bond between a Cys residue at its active site and the C-terminus of ubiquitin [6]. Following activation, ubiquitin is transferred to a Cys residue in an E2 active site [7]. Ubiquitin-charged E2 enzymes are then recognized by E3s, the majority of which contain a signature RING-domain [8,9]. These RING-E3s bind a charged E2 and a specific substrate at the same time, thereby promoting the transfer of ubiquitin from the E2 active site to a substrate lysine. When these reactions are repeated, but Lys residues in substrate-attached ubiquitin molecules are modified, polymeric ubiquitin chains are being generated.

* Corresponding author. Tel.: +1 510 289 4413.
E-mail address: mrape@berkeley.edu (M. Rape).

1084-9521/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2011.03.009

Although several of the ~600 human E3s have been ascribed critical roles during mitosis [10], it is one RING-E3, the anaphase-promoting complex (APC/C), that is essential for establishing the correct temporal order of mitotic events [11]. Rather than degrading all its substrates at the same time, the APC/C ubiquitinates its targets only after they have fulfilled their mitotic functions. This creates a sequence of APC/C-dependent degradation reactions: inhibitors of sister chromatid separation are degraded prior to proteins required for spindle elongation, and those are ubiquitinated before components of the cytokinetic machinery (Fig. 1; [12–14]). At the end of this proteolytic sequence, the APC/C promotes the degradation of its own E2 enzymes to shut off its mitotic activity [15,16]. By degrading key regulators at specific times during cell division, the APC/C orchestrates progression of cells through mitosis.

Given its many substrates, determining the proper time of substrate degradation is no easy task for the APC/C. Based on the current literature and unpublished screens performed in this laboratory, the human APC/C can be estimated to have more than 100 substrates (Table S1), and indeed, activation of this global mitotic player leads to a dramatic increase in the abundance of K11-linked ubiquitin chains, the product of human APC/C-activity [17]. Due to its importance for accurate cell division, the ability of the APC/C to discriminate between its substrates is controlled by multiple mechanisms. Early in mitosis, the spindle assembly checkpoint inhibits the APC/C to prevent chromosome missegregation [18]. However, few APC/C-substrates, such as cyclin A or Nek2A, are degraded under these conditions, thereby moving these proteins to the top of the proteolytic line [19,20]. Once the spindle checkpoint has been satisfied, the APC/C ubiquitinates a small number of proteins, including cyclin B1, that are delivered by a WD40-repeat protein, Cdc20 [21,22]. This burst in APC/C-activity results in a drop in cyclin B1-levels and leads to the activation of a different APC/C-adaptor with much broader substrate spectrum, Cdh1 [23–25]. Thus, the switch from Cdc20 to Cdh1 provides another layer of ordering mitotic degradation events. Finally, fully active

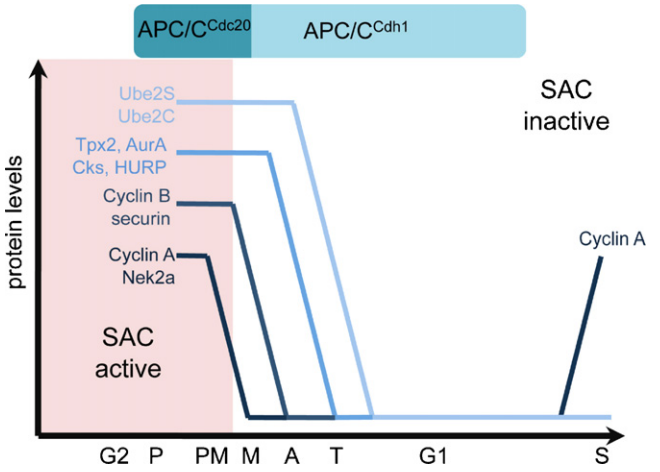

Fig. 1. Sequential degradation of APC/C substrates during mitosis. The APC/C degrades its many substrates only after they have fulfilled their mitotic functions, thereby creating a sequence of degradation events. Processive APC/C-substrates are degraded earlier in mitosis than distributive substrates, and distributive substrates can re-accumulate more easily during late G1. In early mitosis, APC/C^{Cdc20} is inhibited by the spindle assembly checkpoint, but few substrates, such as cyclin A and Nek2 can be degraded under these conditions. Later in mitosis, the APC/C switches to a substrate adaptor with broader specificity, Cdh1. During G1, APC/C^{Cdh1} activity decreases through the degradation of the E2s Ube2C and Ube2S. P: prophase, PM: prometaphase, M: metaphase, A: anaphase, T: telophase.

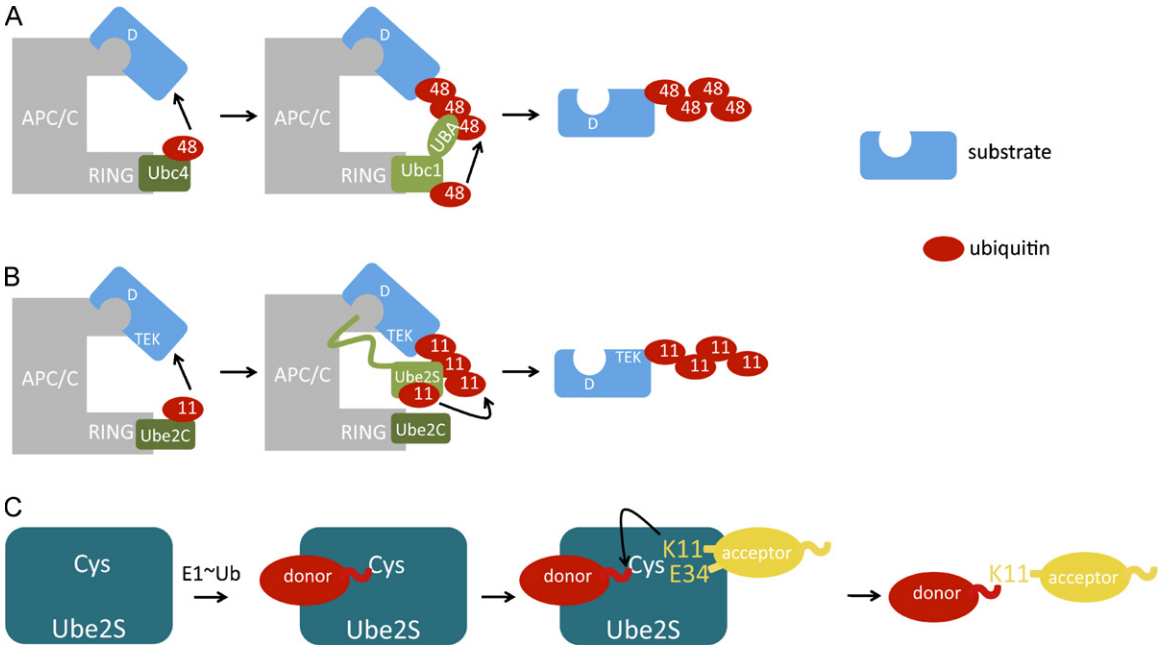

Fig. 2. Mechanism of processive ubiquitin chain formation by the APC/C. (A) Yeast APC/C catalyzes the attachment of K48-linked ubiquitin chains to its substrates. The E2 Ubc4 first modifies substrates with ubiquitin molecules to initiate chain formation. The E2 Ubc1 then extends K48-linked ubiquitin chains with high processivity. Ubc1 requires a UBA-domain for processive chain formation. As both Ubc4 and Ubc1 have to interact with the RING domain in APC11 for activation, they need to act sequentially. (B) Metazoan APC/C forms K11-linked ubiquitin chains on its substrates. Chain initiation is catalyzed by the E2 Ube2C, which interacts with the APC/C through the RING-domain in APC11. A second E2, Ube2S then elongates K11-linked ubiquitin chains with high processivity. Binding of Ube2S to the APC/C is most likely independently of the RING finger, and requires a positively charged C-terminal appendix. (C) Mechanism of linkage-specific chain elongation by Ube2S. Activated ubiquitin (the "donor") is transferred from the E1 to the catalytic site Cys residue of Ube2S. The donor ubiquitin engages in a non-covalent interaction with Ube2S, which is required for processive chain elongation. The "acceptor" ubiquitin, whose Lys11 will be modified, is then recognized by a different surface on Ube2S. Binding of the correct acceptor ubiquitin surface leads to formation of a catalytically competent active site, which is composed of residue of both Ube2S and the acceptor ubiquitin (E34). Thus, K11-linkage specific chain formation by Ube2S depends on substrate-assisted catalysis.