JARQ 39 (1), 1 – 4 (2005) http://www.jircas.affrc.go.jp

REVIEW

Cell Cycle Regulation through Ubiquitin/Proteasome-Mediated Proteolysis in Plants

Yuki YANAGAWA[1]* and Seisuke KIMURA[2]

[1] Department of Bioproduction Science, Faculty of Horticulture, Chiba University (Matsudo, Chiba 271–8510, Japan)
[2] Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science (Noda, Chiba 278–8510, Japan)

Abstract

Understanding the mechanism of cell cycle control in plants is important for agricultural technology enabling enhancement of growth rate and then crop yield. Progression of the cell cycle requires the cell cycle-dependent appearance of cell cycle regulatory proteins such as cyclin-dependent kinases (CDKs) and cyclins. These regulatory proteins are controlled at several levels, including expression, phosphorylation, and interaction with other regulatory proteins. Recently, it has become clear that the controlled and timed destruction of the proteins plays an essential role in cell cycle regulation and the 26S proteasome is involved in the destruction. For example, Cyc (cyclin) A, CycB, CycD, and B-type CDK are degraded by 26S proteasome in a cell cycle-dependent manner. In this review, recent advances in our understanding of cell cycle regulation through ubiquitin/proteasome-mediated proteolysis in plants are described.

Discipline: Biotechnology
Additional key words: CDK, cyclin, 26S proteasome

Introduction

The cell cycle is regulated precisely for cell growth and to maintain life. Cell cycle regulatory factors are regulated qualitatively and quantitatively to ensure the cell cycle progresses normally. For qualitative regulation, certain regulatory proteins are activated and/or inactivated during the cell cycle progression. On the other hand, some proteins are regulated quantitatively by their expression and/or degradation. Proteolysis through the ubiquitin/proteasome pathway is one of the most well characterized systems related to the cell cycle. Indeed, there have been several reports about the relationships between cell cycle progression and proteolysis in plants. Here, we focus on recent findings on the regulation of the cell cycle through ubiquitin/proteasome-mediated proteolysis in plants.

Mechanism of cell cycle control in plants

The plant cell cycle is a sequential process involving the replication of the genome, the segregation of chromosomes, and cytokinesis. The sequencing of the complete plant (rice and *Arabidopsis*) genomes and the molecular characterization of plant genes involved in cell cycle control suggested that the basic mechanism of the plant cell cycle is conserved in all other eukaryotes[2]. Progression of the cell cycle requires many proteins (e.g., CDKs, cyclins, CKIs (CDK inhibitors), and retinoblastoma protein homologues) but the most important regulators are heterodimeric serine/threonine protein kinases[2,8,10]. These kinases consist of a catalytic subunit CDK and an activating subunit, cyclin. Yeasts have only one CDK for cell cycle control. In plants, there are 8 CDK and 30 cyclin genes, which have roles at different points in the cell cycle[15]. A classification based on sequence indicates that

Present address:
[1] Plant Physiology Department, Institute of Plant Science, National Institute of Agrobiological Sciences (Tsukuba, Ibaraki 305–8602, Japan)
*Corresponding author: fax +81–29–838–7044; e-mail yana32@yahoo.co.jp
Received 27 September 2004; accepted 5 October 2004.

there are four types of CDKs (A, B, C and E) and five types of cyclins in plants (A, B, C, D, and H)[2,8,15]. The phosphorylation activities of CDKs are controlled by cyclins because the binding of appropriate cyclins to CDK is required for phosphorylation activities. Cyclin was named because of its cyclical appearance. For example, A-type cyclins generally appear at the beginning of S phase, while B-type cyclins appear during G2, and control the G2/M transition. The amounts of cyclins in plant cells are generally determined by a highly regulated transcription as well as by specific protein-turnover mechanisms. CDK activity results in the phosphorylation of substrate proteins at a serine or threonine residue in a specific phase of the cell cycle. One of the most important targets of CDKs is the retinoblastoma (Rb) protein which controls the G1/S transition[9]. Rb functions as a repressor of S phase. Phosphorylation by the CDK/cyclin complex inactivates Rb and promotes DNA replication. Recent studies have revealed that histone H1 in mitotic chromosomes, E2F, components of the cytoskeleton, and other cell cycle control proteins are substrates of CDKs/cyclins. In other cases, CDK phosphorylation results in the destruction of cell-cycle control proteins, because the recognition of targets for ubiquitin-mediated proteolysis usually requires phosphorylation. In these ways, the cell cycle is driven by CDK-controlled phosphorylation.

As mentioned above, the cell cycle-dependent appearance of CDKs, cyclins or other cell cycle regulators is very important for cell cycle control. The cell cycle control genes are regulated at several levels, including expression, phosphorylation, and interaction with regulatory proteins. In the past few years, it has become clear that the controlled and timed destruction of key proteins plays an essential role in cell cycle transitions[6]. The degradation of cell cycle control proteins is not just a way to remove proteins whose roles are finished, but also a way to directly control the cell cycle.

The degradation system and its regulators in the cell cycle

One of the most well-characterized degradation machineries is the 26S proteasome (Fig. 1). The 26S proteasome is highly conserved from yeast to human, and is an ATP-dependent proteinase complex composed of a core 20S proteasome and 19S/22S/PA700 regulatory particles (Fig. 1)[14]. The regulatory particles are also divided into lid and base subcomplexes[5]. The 26S proteasome is thought to degrade several cell cycle regulatory factors enabling the cell cycle to proceed normally to the next stage.

Most substrates degraded by the 26S proteasome are

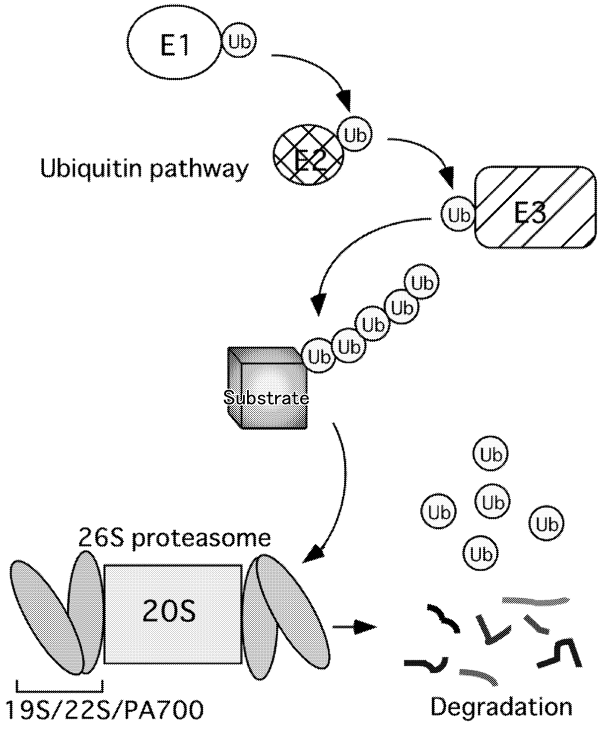

Fig. 1. The ubiquitin/proteasome pathway
The substrate proteins are polyubiquitinated through the ubiquitin pathway following degradation by the 26S proteasome.

polyubiquitinated as target signals. This step involves a cascade named the ubiquitin pathway that includes ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s) and ubiquitin ligases (E3s) (Fig.1)[16]. A database search revealed that the *Arabidopsis* genome encodes 2 E1s and at least 46 E2s and E2-like proteins. Since many (more than 1,200) of E3s are encoded in the *Arabidopsis* genome, the enzymes are thought to show specificity to the substrates mainly. E3s usually comprise multiple components and are classified into 5 groups: APC (anaphase promoting complex), SCF (Skip-Cullin-F-box protein complex), HECT, Ring/U-box, and VBC-Cul2[16]. APC and SCF, help to regulate the cell cycle at metaphase and the M/G1 transition, and at the G1/S and G2/M transitions, respectively. It was shown that a highly conserved motif called the destruction box (D box) at the N terminus of the APC substrate is important for their degradation.

The COP/DET/FUS (constitutive photomorphogenesis/de-etiolated/fusca) proteins were originally identified as negative regulators of photomorphogenesis in *Arabidopsis*, and later found to regulate the ubiquitin/proteasome-related degradation. The COP9 signalosome is composed of 8 distinct subunits of the COP/DET/FUS proteins, and recently was reported to have homol-

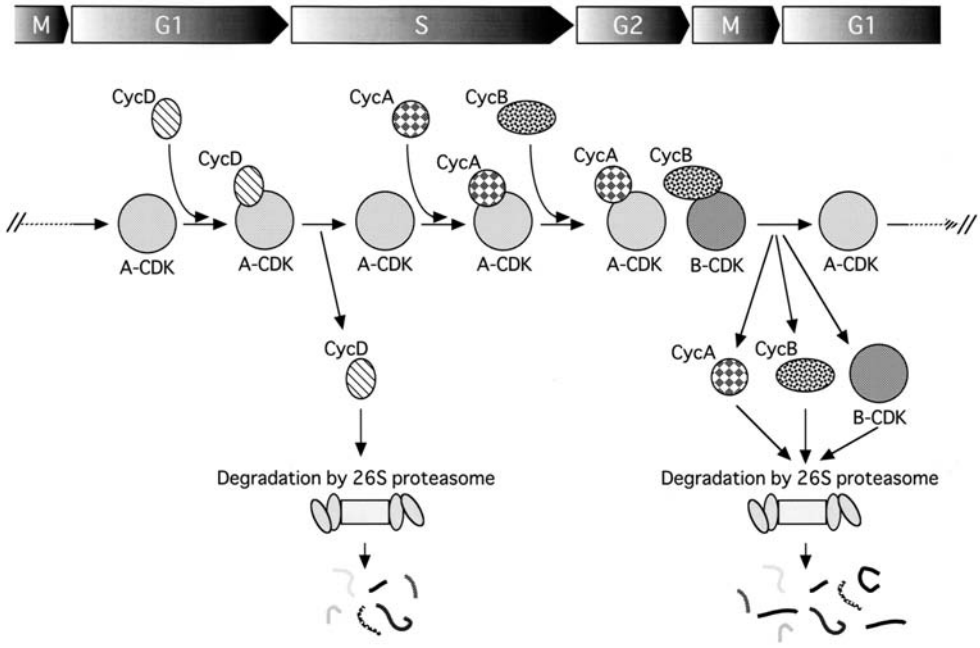

Fig. 2. Degradation of cell-cycle regulatory proteins
Various cell-cycle regulatory proteins are degraded through the ubiquitin/proteasome pathway at specific cell-cycle stages in plant cells.

ogy to a lid particle of the 26S proteasome, and also have the ability to de-neddylate the cullin of SCF-type E3 ligases[13]. In addition, the other COP/DET/FUS proteins, COP1, COP10 and DET1, also regulate the ubiquitin pathway in *Arabidopsis*[16,21]. In mammalian cells, it was shown that the COP9 signalosome regulates the degradation of a cell cycle regulatory protein, p53, through the ubiquitin/proteasome pathway[7]. Recently, COP1 was also reported to be a critical negative regulator of p53[3]. Thus, it is likely that the COP/DET/FUS proteins act to degrade cell cycle regulatory proteins in plants, although there is no evidence of this.

Degradation of cell cycle regulatory proteins

It has been reported that various cell-cycle regulatory proteins are degraded through the ubiquitin/proteasome pathway in specific stages of the cell cycle in plant cells (Fig. 2). Notably, several groups reported the ubiquitin/proteasome-dependent degradation of cyclins. Cyclins A and B from CDK1/cyclin A3 and CDK1/cyclin B1 complexes are degraded by the 26S proteasome so as to complete metaphase[4]. Cyclin B2 is degraded early in M phase[18]. Cyclin D3 is related to the control of the G1/S transition, and is degraded through the ubiquitin/proteasome pathway in *Arabidopsis*[12]. A- and B-type cyclins possess a D box that targets their timely removal by the APC at M phase. D-type cyclins are polyubiquitinated by an SCF complex and subjected to proteasome degradation.

Beside cyclins, proliferating cell nuclear antigen (PCNA) plays a role in DNA replication in S phase, and was suggested to be degraded through the ubiquitin/proteasome pathway in rice[19].

In addition, proteasome inhibitors arrested cells in at least 3 stages; late G2 phase, metaphase and M/G1 transition in tobacco BY-2 cells. Moreover, the 26S proteasome mainly localizes in the nuclear and tubulin configurations such as the preprophase bands, mitotic spindles and phragmoplasts during the cell cycle progression in BY-2 cells[20]. A tobacco kinesin-related protein, TKRP125, is a motor protein needed to form phragmoplasts. Recently, it was suggested it might be a target of ubiquitin/proteasome-mediated proteolysis at the M/G1 transition[11]. The degradation of cyclin B1 is necessary to reorganize the mitotic spindles to the phragmoplasts[17]. Thus, these findings imply that several cell cycle progressing factors are degraded in relation to the configuration of mitotic tubulins.

Concluding Remarks

In this review, the importance of ubiquitin/proteasome-mediated proteolysis during the cell cycle progression was emphasized. Notably, the degradation of cell-cycle regulatory factors such as cyclins and PCNA is necessary for the ubiquitin/proteasome pathway to promote progression through the cell cycle. However, not many factors have been elucidated, and many questions still remain. In addition to the proteins targeted by the 26S proteasome, other regulatory factors such as COP/DET/FUS proteins are implicated in the ubiquitin/proteasome-mediated proteolysis during the cell cycle progression. However, there is no evidence so far in plants, and more experiments are needed. It will also be interesting to know how cell cycle control differs between plants and other organisms.

Previously, Cockcroft et al. showed that a reduction in the length of the G1 phase and faster cell cycling occur with overexpression of cyclin D (CycD2) in transgenic tobacco plants[1]. The plants have normal sized cells and meristems, but elevated overall growth rates and an accelerated development in all stages. This finding suggested that the growth rate of plants could be accelerated by regulating the cell cycle. If the growth rate could be accelerated, the period to harvest could be shortened, enabling an increase in the yield of crops. Further understanding of the cell cycle progression in plants will contribute to agricultural technology.

References

1. Cockcroft, C. E. et al. (2000) Cyclin D control of growth rate in plants. *Nature*, **405**, 575–579.
2. Dewitte, W. & Murray, J. A. (2003) The plant cell cycle. *Annu. Rev. Plant Biol.,* **54**, 235–264.
3. Dornan, D. et al. (2004) The ubiquitin ligase COP1 is a critical negative regulator of p53. *Nature,* **429**, 86–92.
4. Genschik, P. et al. (1998) Cell cycle-dependent proteolysis in plants. Identification of the destruction box pathway and metaphase arrest produced by the proteasome inhibitor mg132. *Plant Cell,* **10**, 2063–2076.
5. Glickman, M. H. et al. (1998) A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3. *Cell,* **94**, 615–623.
6. King, R. W. et al. (1996) How proteolysis drives the cell cycle. *Science,* **274**, 1652–1659.
7. Lyapina, S. et al. (2001) Promotion of NEDD-CUL1 conjugate cleavage by COP9 signalosome. *Science,* **292**, 1382–1385.
8. Mironov, V. V. et al. (1999) Cyclin-dependent kinases and cell division in plants–the nexus. *Plant Cell,* **11**, 509–522.
9. Mittnacht, S. (1998) Control of pRB phosphorylation. *Curr. Opin. Genet. Dev.,* **8**, 21–27.
10. Nigg, E. A. (1995) Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. *Bioessays,* **17**, 471–480.
11. Oka, M. et al. (2004) Inhibition of proteasome by MG-132 treatment causes extra phragmoplast formation and cortical microtubule disorganization during M/G1 transition in synchronized tobacco cells. *Plant Cell Physiol.*, **45**, 1623–1632.
12. Planchais, S., Samland, A. K. & Murray, J. A. (2004) Differential stability of *Arabidopsis* D-type cyclins: CYCD3;1 is a highly unstable protein degraded by a proteasome-dependent mechanism. *Plant J.,* **38**, 616–625.
13. Serino, G. & Deng, X. W. (2003) The COP9 signalosome: regulating plant development through the control of proteolysis. *Annu. Rev. Plant Biol.,* **54**, 165–182.
14. Tanaka, K. & Chiba, T. (1998) The proteasome: a protein-destroying machine. *Genes Cells,* **3**, 499–510.
15. Vandepoele, K. et al. (2002) Genome-wide analysis of core cell cycle genes in *Arabidopsis*. *Plant Cell,* **14**, 903–916.
16. Vierstra, R. D. (2003) The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. *Trends Plant Sci.,* **8**, 135–142.
17. Weingartner, M. et al. (2004) Expression of a nondegradable cyclin B1 affects plant development and leads to endomitosis by inhibiting the formation of a phragmoplast. *Plant Cell,* **16**, 643–657.
18. Weingartner, M. et al. (2003) A plant cyclin B2 is degraded early in mitosis and its ectopic expression shortens G2-phase and alleviates the DNA-damage checkpoint. *J. Cell Sci.,* **116**, 487–498.
19. Yamamoto, T. et al. (2004) Degradation of proliferating cell nuclear antigen by 26S proteasome in rice (*Oryza sativa* L.). *Planta,* **218**, 640–646.
20. Yanagawa, Y. et al. (2002) Cell-cycle dependent dynamic change of 26S proteasome distribution in tobacco BY-2 cells. *Plant Cell Physiol.,* **43**, 604–613.
21. Yanagawa, Y. et al. (2004) *Arabidopsis* COP10 forms a complex with DDB1 and DET1 and enhances the activity of ubiquitin conjugating enzymes. *Genes Dev.,* **18**, 2172–2181.