Roles of proteasomes in cell growth

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Abstract

Proteasomes are large, unique protein complexes catalyzing energy- and ubiquitin-dependent proteolysis. Recent studies have revealed that these complexes are involved in two important cellular functions. One is to make antigen fragments for major histo-compatibility complex (MHC) class I-restricted antigen presentation and the other is to regulate the cell cycle by proteolysis. Here we review only the latter function of proteasomes. Proteasomes are widely distributed in eukaryotic cells, but their levels have been shown to be particularly high in various immature cells, such as cancerous, fetal and lymphoblastic cells, and agents inducing cell differentiation were found to suppress their expression. These conditions also regulate the expression of ubiquitin genes in a similar way, suggesting that proteasomes act ubiquitin-dependently in their 26S form in immature cells. High levels of proteasomes were found immunochemically in the nuclei of rapidly growing cells, indicating that proteasomes are important for eukaryotic cell growth. Indeed, gene disruptions of most subunits of proteasomes in yeast resulted in total suppression of cell growth and cell death. Short-lived regulatory factors of the cell cycle, such as Fos, p53, Mos, and cyclins are degraded by the proteasome-ubiquitin pathway under phosphorylated or dephosphorylated conditions. Ornithine decarboxylase, which is also a short-lived enzyme and is involved in the early phase of cell growth, is quickly degraded by proteasomes with antizyme, but without ubiquitination. Recently, we found that one of the regulatory factors of 26S proteasomes, p31, is a homologue of Nin1p, whose mutation caused inhibition of the cell cycle in yeast. These results indicate that proteasomes play important roles in regulation of the cell cycle in eukaryotes.

Introduction

Proteasomes are widely distributed in eukaryotic cells and are protease complexes of a unique shape consisting of over 30 different proteins. Their central part is cylindrical and is seen as 4 rings, each of which contains 7 subunits. The molecular weight and sedimentation coefficient of this central part are about 750 kDa and 20S, respectively [1, 2]. This cylindrical part has multicatalytic proteolytic activities, but its physiological role is not known. Both ends of this cylindrical part have additional proteins which include over 15 regulatory factors, giving the proteasome a symmetrical caterpillar like shape, with a molecular weight and sedimentation coefficient of over 2,000 kDa and 26S, respectively [3]. This 26S proteasome is supposed to play an energy-and ubiquitin-dependent role in proteolysis in vivo [4, 5].

From these unique characters, proteasomes are supposed to have specific functions in cells, rather than simply in degradation of excess proteins. In fact, recent studies revealed at least two essential roles of the proteasome in cellular activities; one is an immunological function in catalyzing antigen processing, and the other is a function in regulating the cell cycle by specific proteolysis. Here we review only functions of proteasomes involved in regulation of the cell cycle. For information on the function of proteasomes in MHC antigen presentation, reference should be made to other articles [6, 7].
Growth of immature cells and expression of proteasomes

Proteasomes have been found in all eukaryotic cells examined, but their levels of expression are particularly high in rapidly growing embryonic tissues of rats, chicks, *Xenopus* and *Drosophila* [8-11]. Cells during normal growth, such as those in the liver of neonatal rats and in regenerating liver of adult rats after partial hepatectomy, did not show any increase of proteasomal expression [8]. Blastocysts induced from normal lymphocytes by treatment with phytohemagglutinin also expressed high levels of proteasomes [12]. These results indicate that rapidly growing immature cells require an increased level of proteasomes to regulate the active cell cycle, but that neonatal or regenerating cells contain sufficient basal levels of proteasomes to regulate normal growth. This idea is supported by the fact that proteasomal expression is high in all cancerous cells examined, such as human and rat hepatomas [8], human leukemic cells and their cell lines [12, 13], and human primary kidney cancers and their cell lines [14]. It is interesting that in these cells, the expressions of various subunits of the 20S proteasome increase in parallel, but no marked increase of any particular subunit, suggesting that there is a concerted mechanism of gene expression to increase the expressions of the various subunits to form the proteasome complex. We, therefore, searched for some consensus sequence in the upstream regions of some subunit genes, but failed to find any particular element controlling their parallel expressions [15].

Similarly in leukemic cells, the gene expressions of various subunits of proteasomes and various ubiquitin species are increased in parallel and treatments of these cells with various compounds, such as phorbol esters, dimethylsulfoxide, retinoic acid or butyric acid, caused differentiation into monocytes, granulocytes and erythrocytes depending on the cell type together with down-regulations of the expressions of proteasomal subunits and ubiquitins in parallel [14, 16]. These findings also suggest that active proteasomes in these cells are the 26S form, not the 20S form, because ubiquitin/ATP is required for activity of the 26S proteasome, but not the 20S proteasome. This possibility is also supported by the fact that in rapidly growing cells, the expressions of over 10 regulatory factors that combine with the 20S proteasome to form the 26S proteasome also increase in parallel with the expressions of various subunits of the 20S proteasome (Tanaka et al., to be published). Therefore, subunits of the 20S proteasome, regulatory factors of the 26S form and ubiquitins all increase in harmony to control this specific proteolysis in active cell growth. However, the mechanism involved in the parallel expressions of various genes related to proteasomal activities is still unknown. There are some indications that during development of some tissues [10] and in interferon-γ-treated cells [7], the composition of subunits of the 20S proteasome changes, and that these altered proteasomes may have specific proteolytic functions, but details of the exchanges of subunits and their physiological significance are unknown. It should be mentioned that increases of the levels of mRNAs and protein syntheses of various subunits are evident in these immature cells, but immunochemical studies revealed no increase in the total amount of proteasomal proteins in these cells [13, 14]. This puzzling phenomenon can be explained by supposing the intracellular coexistence of two pools of mRNA and protein of proteasomal subunits, respectively, one a large pool with a slow turnover, and the other a small pool with a rapid turnover [13]. This latter pool may be responsible for regulation of active cell growth.

We also showed increase of proteasomes in the nucleus of rapidly growing cells [8, 14, 17] and found that some subunits contain nuclear translocation signals [18]. A recent immunochemical study also demonstrated that a high concentration of proteasomes is associated with spindle microtubules in dividing cells [19]. Therefore, it is likely that proteasomes with rapid turnover are synthesized in the cytosol during rapid cell growth and then transferred to the nucleus to regulate the cell cycle. Other evidence that proteasomes are involved in cell growth is that disruptions of the genes of yeast proteasome subunits caused complete suppression of cell growth followed by cell death [20, 21]. Although a defect of one subunit, Y13, did not have a lethal effect [22], the results indicated that most proteasomal subunits are essential for cell survival. Perhaps defects of these subunits result in failure to form the proteasome complex.

Involvement of proteasomes in cell cycle regulation

There are several reports indicating a close relation between ubiquitination and cell growth. Differences have been found in the ubiquitinated protein patterns in tumor cells and normal cells [14], some ubiquitin genes are fused with ribosomal protein genes [23],