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Proteasome and cell cycle

Evidence for a regulatory role of the protease on mitotic cyclins in yeast

Birgit Richter-Ruoff, Dieter H. Wolf*

Institut für Biochemie der Universität Stuttgart, Pfaffenwaldring 55, D-70569 Stuttgart, Germany

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The cell cycle of eukaryotic cells is strictly regulated. This regulation is performed by a serine/threonine kinase. The different functions of this kinase in the cell cycle are modulated by different cyclins, which fluctuate in concentration ('cycle') during the different stages of the cell cycle. Using yeast as a model organism we show here that the activity of the multifunctional proteinase, the proteasome, is directly connected to the function of the mitotic cyclin Clb2. Our studies indicate that the proteasome is the proteolytic regulator of this cyclin and thus a central regulator of the cell cycle.

Cell cycle; Proteasome; Cyclin; Yeast; *Saccharomyces cerevisiae*

1. INTRODUCTION

The proteasome is an essential, multisubunit complex highly conserved from yeast to man. It consists of a 20S particle, which is integrated as a core unit into a 26S complex (for reviews, see [1,2]). The proteinase complex harbors at least three different major proteolytic activities and the enzyme has been found to be located in the cytoplasm and the nucleus of cells (for reviews, see [1]). With the aid of yeast mutants defective in proteolytic functions of the proteasome its central role in the proteolysis of ubiquitinated, short-lived and not properly assembled proteins has been discovered [3–9]. The accumulating data on the involvement of the proteasome in the degradation of highly regulated as well as regulatory active proteins ([10,11], Schork, S., Bee, G., Thumm, M. and Wolf, D.H., submitted) leads to the idea that the proteasome is the central cytoplasmic and nuclear protease implicated in the degradation of all short-lived proteins. As expected, severe alterations within a proteinase complex fulfilling such central functions will lead to inviability of the cells. This has been demonstrated for the deletion of several subunits of the yeast proteasome; cells stop growing and die [3,6,7].

The cell cycle is a highly regulated event. All steps of the progression through the cell cycle have to be rigorously controlled (for reviews, see [12–14]). It is gradually emerging that in all cells a serine/threonine kinase, encoded by the *CDC28* gene in yeast, is central to the control of the cell cycle. It is thought that the kinase activity is the key feature which mediates passage of cells through the two predominant control points at the G_1/S (called START) and the G_2/M transition borders (for reviews, see [12–14]). The kinase activity is regulated by a class of proteins called cyclins. Most of the cyclins are very unstable and only present at a particular phase of the cell cycle (for reviews, see [12–14]. In yeast three cyclins (Cln1, Cln2 and Cln3) have been found to be essential for START [15–18], (for review, see [13]). A family of B-type cyclins has been found to act during S-phase or to be necessary for formation and function of the mitotic apparatus [19–22], (for review, see [13]). Of the four B-type cyclins that act in mitosis (Clb1, Clb2, Clb3 and Clb4) Clb2 is essential [13].

In *Xenopus* an abrupt ubiquitin-dependent destruction of the B-type cyclins has been observed which is mediated by a conserved sequence towards their amino termini called destruction box [23]. All four mitotic Clb cyclins of yeast contain sequences similar to the *Xenopus* B-type cyclin destruction box and deletion of this sequence in *CLB2* causes stabilization of the protein [13,24]. The up to now discovered in vivo role of the proteasome in rapidly degrading non-assembled [8] ubiquitinated [3–7], short-lived [4,5] and metabolically regulated ([10,11] Schork, S., Bee, G., Thumm, M. and Wolf, D.H., submitted) proteins led us to the idea, that the enzyme complex might also be involved in cyclin degradation and thus cell cycle regulation. We therefore tested the influence of the proteasome on the mitotic cyclin Clb2.

2. MATERIALS AND METHODS

2.1. *Chemicals*

All enzymes used in DNA manipulation were obtained from Boehringer-Mannheim (Germany). Yeast nitrogen base without amino acids was purchased from Difco (Detroit, USA). All other

*Corresponding author. Fax: (49) (711) 685 4392.

chemicals were obtained from Pharmacia (Freiburg, Germany), Roth (Karlsruhe, Germany), Merck (Darmstadt, Germany) and Serva (Heidelberg, Germany).

2.2. *Media*

E. coli strains were grown in LB [25] medium with or without ampicillin (50 μg/ml). Complete minimal (CM) dropout medium and mineral (MV) medium were prepared according to Ausubel et al. [26]. CM dropout medium contained 0.67% yeast nitrogen base without amino acids and 2% glucose or galactose supplemented with adenine and amino acids. MV medium contained 0.67% yeast nitrogen base without amino acids, 2% glucose and supplements required by auxotrophic strains.

2.3. *Strains*

E. coli strain used was: DH5α {*F⁻ endA1 recA1 gyrA96 thiΔ(argF-lacZYA)U169 (f80ΔlacZdM15)I⁻ hsdR17 supE44 relA1*} [27].

All yeast strains were derivatives of strain WCG4α (*MATα his3-11,15 ura3 leu2-3,112 can GAL*) [7]. The isogenic *pre1-1* mutant strain was constructed by gene replacement of the wild type gene with the mutant allele *pre1-1* [7]. The used yeast strains were: YBR1 [*MATα ura3 his3-11,15 leu2-3,112 can GAL (URA3)*], YBR2 [*MATα ura3 his3-11,15 leu2-3,112 can GAL (GAL1-CLB2 URA3)*], YBR3 [*MATα pre1-1 ura3 his3-11,15 leu2-3,112 can GAL (URA3)*], YBR4 [*MATα pre1-1 ura3 his3-11,15 leu2-3,112 can GAL (GAL1-CLB2 URA3)*].

2.4. *Molecular biological techniques and plasmids*

Purification, restriction, ligation, analysis of DNA and transformation of *E. coli* were carried out according to standard procedures [25]. For transformation of *Saccharomyces cerevisiae* a protocol modified according to [3] was followed. The CLB2 gene was excised from plasmid pRK172 kindly provided by Kim Nasmyth and inserted into plasmid pBM150 (*CEN4 ARS1 URA3* amp^R *GAL1 GAL10*) [28]. Plasmid pBR01 was constructed as follows: The *CLB2* gene was excised from plasmid pRK172 using *Nde*I and *Hin*dIII. The overlapping ends of the resulting 1.4 kb fragment were filled with Klenow Polymerase I to yield a blunt end fragment. pBM150 was cut with *Bam*HI and the overlapping ends were filled with Klenow Polymerase I. The 1.4 kb fragment containing *CLB2* was blunt-end ligated into the filled *Bam*HI site of pBM150.

The yeast strains were transformed with the centromere plasmids pBM150 (*URA3*) and pBR01 (*GAL1-CLB2 URA3*) expressing *CLB2* under control of the galactose inducible *GAL1* promoter.

2.5. *Growth conditions*

Wild-type and mutant strains carrying the CLB2 expressing plasmid were grown to stationary phase in CM dropout medium containing 2% glucose at 30°C. Wild type and mutant strains with and without plasmid pBM150 or plasmid pBR01 were grown in identical fashion. Cells were diluted 1:1000 in CM dropout medium containing 2% galactose and incubated over 72 h at 30°C. Optical density was measured at 578 nm.

3. RESULTS AND DISCUSSION

Monitoring cellular growth is the most sensitive assay to detect functions of essential proteins and their interaction in the cell. The use of yeast mutants defective in the proteolytic activity of the proteasome should make possible the elucidation of any link existing between cyclin Clb2 function and proteasome action. Proteasome mutant cells defective in the *PRE1* gene, carrying an amino acid exchange at position 142 (Ser–Phe) (allele *pre1-1*) show a major defect in the chymotrypsin-like activity of the enzyme complex but exhibit an only rather minor growth phenotype at 30°C [3,7]. This is in contrast to a *pre1* deletion mutant which arrests growth in a budded state [6].

It has been shown that expression from the GAL1 promoter of a single copy of the wild type CLB2 gene is tolerated by cells when grown on galactose, while expression of four copies of the gene is lethal. Also expression of a mutant *CLB2* gene lacking the destruction box leading to a stable Clb2 protein, which cannot be degraded any more is lethal [13,24]. This finding

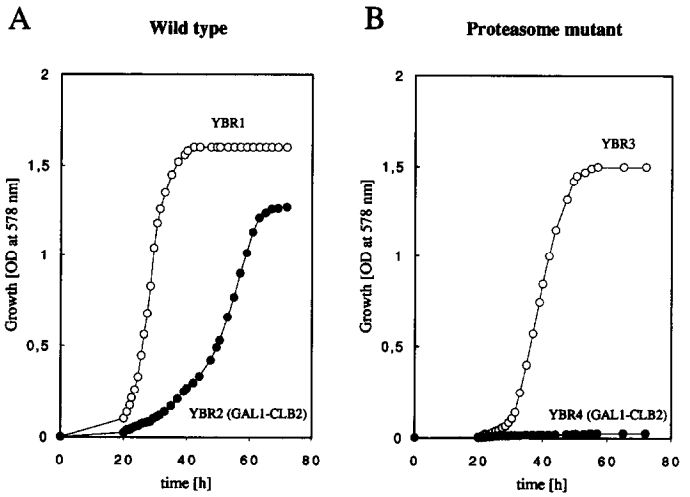

Fig. 1. Growth of wild-type and mutant cells defective in the chymotrypsin-like activity of the proteasome carrying an extra copy of the mitotic cyclin CLB2. Cells were grown as indicated in section 2. (A) Growth of wild type cells YBR1 [*MATα ura3 his3-11,15 leu2-3,112 can GAL (URA3)*] carrying the centromere plasmid pBM150 and YBR2 [*MATα ura3 his3-11,15 leu2-3,112 can GAL (GAL1-CLB2 URA3)*] carrying the centromere plasmid pBR01 into which the CLB2 gene under the control of the *GAL1* promotor is inserted. (B) Growth of proteasome mutant cells YBR3 [*MATα pre1-1ura3 his3-11,15 leu2-3,112 can GAL (URA3)*] carrying the centromere plasmid pBM150 and YBR4 [*MATα pre1-1 ura3 his3-11,15 leu2-3,112 can GAL (GAL1-CLB2 URA3)*] carrying the centromere plasmid pBR01 which contains the *CLB2* gene under the control of the *GAL1* promotor.

implies that growth of cells is very sensitive to the amount of cyclin Clb2 in the cell. It thus can be reasoned that a defect in the proteinase defective in degradation of Clb2 should lead to a growth arrest of cells due to increased accumulation of this cyclin. We therefore transformed isogenic wild type and *pre1-1* mutant cells with a centromere plasmid carrying the *CLB2* gene under the control of the *GAL1* promoter. Wild-type and mutant cells were pre-grown in glucose containing medium and then transferred onto galactose medium to derepress the Clb2 protein. Expression of the extra copy of CLB2 from the plasmid in wild type cells affects growth of cells to some extent, but is still tolerated (Fig. 1A). In contrast, expression of the extra copy of CLB2 in the proteasome mutant cells completely abolishes growth (Fig. 1B). These data clearly demonstrate a link between cyclin Clb2 action in the cell and the defect in the chymotrypsin-like activity of the proteasome. The growth arrest of *pre1-1* mutant cells expressing an extra copy of *CLB2* is due to an enhanced level of Clb2 which is most likely caused by the inability of the defective proteasome to degrade the extra amount of Clb2. The results furthermore imply that there is no inactivation step of Clb2 prior to its proteasome mediated disappearance, indicating that the protease complex is the central regulator of this cyclin and thus of the cell cycle.

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