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The 26S proteasome of the fission yeast *Schizosaccharomyces pombe*

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The 26S proteasome is the multiprotein complex that degrades proteins that have been marked for destruction by the ubiquitin pathway. It is made up of two multisubunit complexes, the 20S catalytic core and the 19S regulatory complex. We describe the isolation and characterization of conditional mutants in the regulatory complex and their use to investigate interactions between different subunits. In addition we have investigated the localization of the 26S proteasome in fission yeast, by immunofluorescence in fixed cells and live cells with the use of a GFP-tagged subunit. Surprisingly, we find that in mitotic cells the 26S proteasome occupies a discrete intracellular compartment, the nuclear periphery. Electron microscopic analysis demonstrates that the complex resides inside the nuclear envelope. During meiosis the localization showed a more dynamic distribution. In meiosis I the proteasome remained around the nuclear periphery. However, during meiosis II there was a dramatic relocalization: initially, the signal occupied the area between the dividing nuclei, but at the end of mitosis the signal dispersed, returning to the nuclear periphery on ascospore formation. This observation implies that the nuclear periphery is a major site of proteolysis in yeast during mitotic growth and raises important questions about the function of the 26S proteasome in protein degradation.

Keywords: 26S proteasome; meiosis; mitosis; *Schizosaccharomyces pombe*

1. INTRODUCTION

The main non-lysosomal intracellular proteolytic pathway is the ubiquitin pathway. Ubiquitin is a protein of 76 amino acids which, when covalently attached as a polyubiquitin chain to a lysine residue of a protein, targets that protein for destruction. The ubiquitin pathway has been implicated in the turnover of a number of important proteins, such as p53, c-Myc, c-mos and the mitotic cyclins (Hershko & Ciechanover 1992).

The means by which ubiquitin is attached to the target protein has been characterized by cell fractionation studies. First, the ubiquitin is activated by the E1 or ubiquitin-activating enzyme. It is then transferred to an E2 or ubiquitin-conjugating enzyme, which, either by itself or with the help of a coenzyme, ubiquitin ligase (E3), attaches the ubiquitin to the target protein. Subsequent addition of further ubiquitin molecules to form a polyubiquitin chain on the target protein allows it to be recognized by the 26S proteasome, which degrades the substrate to peptides and recycles the ubiquitin. The different components of this pathway are highly conserved from yeast to humans (Hershko & Ciechanover 1992; Hochstrasser 1995).

The 26S proteasome is made up of two multiprotein complexes, called the 20S catalytic particle and the 19S regulatory particle. The 20S particle is made up of 14 different subunits (Coux *et al*. 1996). These can be classified further into seven α- and seven β-particles, based on their homology to the simpler version of the proteasome found in the archaebacterium *Thermoplasma acidophilum*. The α- and β-subunits form seven-membered rings. The mature 20S complex has a cylindrical structure made up of seven α seven β seven β seven α rings with a channel running through the centre of the structure. The peptidase catalytic sites reside within the β-subunits and are located inside the cylinder. Proteins enter the structure from either end of the cylinder through a narrow pore. The crystal structure of the 20S particle from *T. acidophilum* and budding yeast has been solved (Lowe *et al*. 1995; Groll *et al*. 1997). In the *T. acidophilum* 20S particle there is a narrow pore at each end whereas in the budding yeast particle the openings are closed. Presumably, in yeast, the pore opens after binding of the regulatory complex to each end of the 20S complex. In both cases, however, as the pore is narrow, proteins have to be unfolded before entry into the 20S chamber.

The 19S complex is made up of 17 different subunits. These are classified into two different groups, the ATPases, of which there are six, and non-ATPase subunits, of which there are 11 (Glickman *et al*. 1998*a*). It is generally believed that the six ATPases form a ring and bind to the top of the 20S particle, opening the channel in the a ring of the eukaryotic 20S complex. In addition, they are thought to participate in the anti-chaperone activity of the 19S complex, unfolding the protein substrate and translocating it to the 20S catalytic particle for degradation (Rubin *et al*. 1998). The role of the non-ATPases is less clear. Possible functions include participation in anti-chaperone activity, recognition of substrates and an isopeptidase activity to remove the polyubiquitin

Table1. *Schizosaccharomyces pombe* genes isolated in methylbenzylcarbamylate (MBC^R) screen and the corresponding homologues in *Saccharomyces cerevisiae* and humans

S. pombe	*S. cerevisiae*	human
mts1$^+$	RPN9	—
mts2$^+$	RPT2(YTA5)	S4
mts3$^+$	RPN12 (NIN1)	S14
mts4$^+$	RPN1 (NAS1)	S2 (Trap-2)
mts5$^+$ (*pad1*$^+$)	RPN11(MPR1)	Poh1

tag from the substrate to recycle the ubiquitin that is not degraded by the proteasome. An isopeptidase activity has been identified in purified 19S complexes from mammalian cells although the subunit(s) responsible for the activity has not yet been identified (Lam *et al.* 1997). To date, only one subunit has been implicated in the recognition of ubiquitin conjugates. When the purified 19S complex from human erythrocytes was run on an SDS–PAGE gel and transferred to nitrocellulose membrane one subunit, S5a, was able to recognize polyubiquinated chains by a filter assay (Deveraux *et al.* 1994). Surprisingly, deletion of the *RPN10*(*MCB1*) gene, which encodes S5a in budding yeast, was not a lethal event (Van Nocker *et al.* 1996*b*). This is in marked contrast to other 19S subunits, most of which have been found to be essential for cell viability (Coux *et al.* 1996). This finding suggests that there are other proteins involved in the recognition of polyubiquitinated substrates.

We have investigated the 26S proteasome in the fission yeast *Schizosaccharomyces pombe*. Fission yeast is an attractive model organism for the study of cell biology (Moreno *et al.* 1991). It is amenable to genetic manipulation and also provides suitable material for biochemical experiments, allowing a combined approach to solving biological problems. Furthermore, a wide range of cytological methods can be used routinely in this organism. In this review we describe the isolation of mutants in the 19S regulatory complex and their use to study the structure and function of the 26S proteasome. We also describe the localization of the complex during the mitotic cycle and show that, surprisingly, it localizes to a discrete cellular compartment, implying that in fission yeast most proteolysis occurs at a fixed position within the cell.

2. THE ISOLATION AND CHARACTERIZATION OF TEMPERATURE-SENSITIVE MUTANTS IN THE FISSION YEAST 19S COMPLEX

We have isolated a number of different conditional lethal temperature-sensitive mutants of the regulatory complex of the 26S proteasome in fission yeast. We call them *mts* mutants because they were isolated in a screen to isolate mutants that were resistant to the mitotic poison methylbenzylcarbamylate that were also temperature-sensitive for growth. As can be seen in table 1, this screen turned out to be remarkably efficient in isolating conditional mutants in different subunits of the 26S proteasome. Out of six complementation groups, five, *mts1*$^+$ to *mts5*$^+$ (Gordon *et al.* 1993, 1996; Wilkinson *et al.* 1997; Penney *et al.* 1998; C. Gordon, unpublished results), encoded subunits of the 19S complex. One, *mts5*$^+$,

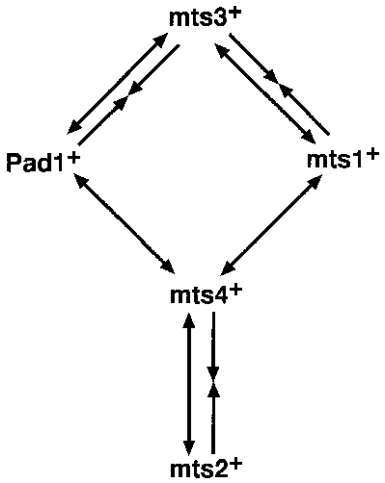

Figure 1. Genetic interactions obtained with the *mts* genes. The arrows represent partial rescue of the temperature sensitivity of different *mts* mutants by overexpression of a *mts*$^+$ cDNA. The arrowheads point to the mutants which are partially rescued. The inverted arrows represent synthetic lethality.

encoded the previously known *S. pombe* gene *pad1*$^+$. The *pad1*$^+$ gene was thought to encode a positive activator of the Papl transcription factor (Shimanuki *et al.* 1995). However, recent work from our laboratory and others has demonstrated that it encodes a subunit of the 19S regulator, subunit 13 (Spatoro *et al.* 1997; Glickman *et al.* 1998*a*; Penney *et al.* 1998). The deletion of any of the above *mts* genes was a lethal event; this result demonstrated that in each case the gene was essential for mitotic growth. Furthermore, in each case the deletion phenotype was essentially the same as that found for the corresponding temperature-sensitive mutant when incubated at the restrictive temperature. This indicated that the conditional lethal mutants were all lack-of-function mutants at the restrictive temperature.

The phenotype observed for each mutant is shown in figure 1. Each mutant was found to enter mitosis, as shown by the presence of a mitotic spindle, but then arrested with a short spindle typical of the metaphase stage of mitosis in fission yeast. Consistent with this finding, the DNA was found not to separate. The mutants were therefore defective in the metaphase–anaphase transition (see figure 1). Unlike budding yeast, where 26S proteasome mutants arrest at two points in the cell cycle, G1/S phase as well as the metaphase–anaphase transition, the fission yeast mutants only arrest at the metaphase–anaphase transition. This is due to the fact that in budding yeast the destruction of the cdk inhibitor Sic1 is required to enter S phase. This protein

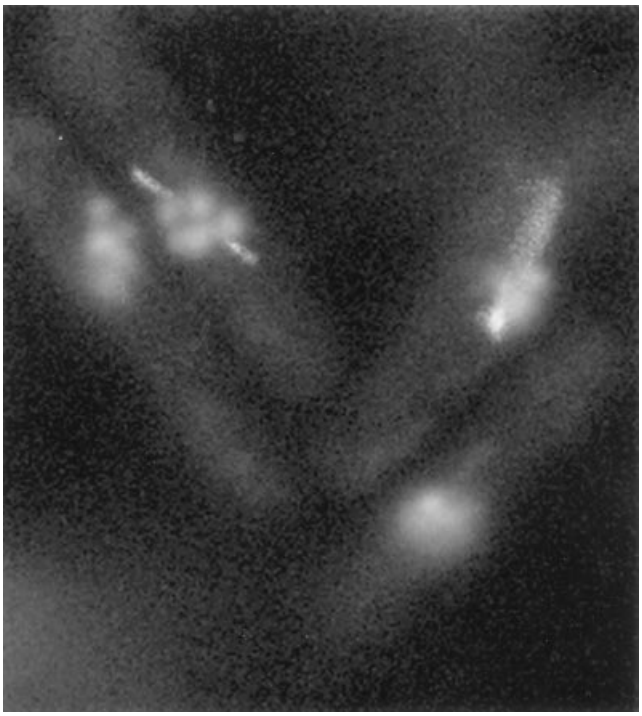

Figure 2. *mts3-1* Cells fixed after incubation for 4 h at the restrictive temperature of 36 °C, stained with the anti β-tubulin antibody TAT1 to visualize the mitotic spindle and with the DNA-specific stain DAPI. Cells arrest with the short spindle typical of the metaphase stage of mitosis.

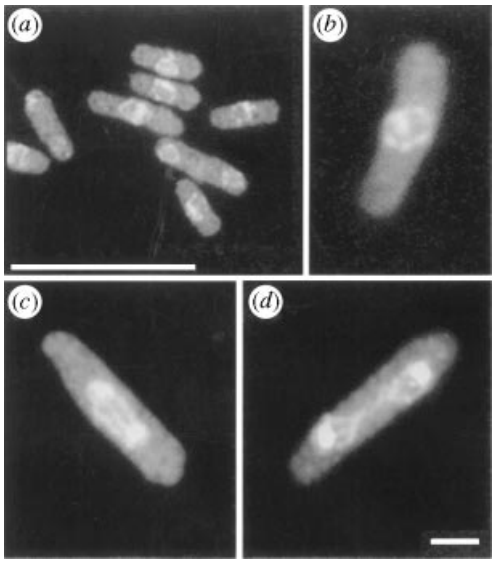

Figure 3. Wild-type *S. pombe* cells were fixed and stained with the anti-Mts4 antibody and the DNA-specific stain DAPI. (*a*) A field of cells (scale bar, 100 mm); (*b*) an interphase cell at higher magnification; (*c*) early-anaphase cell; (*d*) late-anaphase cell (scale bar, 10 mm). Taken from Wilkinson *et al*. (1998).

is ubiquinated by the SCF complex (Skp1–Cullin-1–F-box). Mutants in the SCF complex are no longer able to degrade Sic1 and the cells arrest at the G1/S phase of the cell cycle (Patton *et al*. 1998). In fission yeast, the homologous cdk inhibitor, Rum1, is also ubiquinated by a fission-yeast SCF complex. However, in fission-yeast SCF mutants, although the Rum1 protein is stabilized the cell cycle is not arrested, but the mutant cells do show other phenotypes such as an increase in ploidy (Kominami & Toda 1997). The metaphase arrest arises because a specific substrate, Cut2 in fission yeast and its homologue Pds1 in budding yeast, has to be degraded by the ubiquitin pathway for anaphase to begin (Ciosk *et al*. 1998).

Essentially, all the *mts* mutants have similar conditional lethal phenotypes. The *mts3-1* mutant, however, displays one additional phenotype at the restrictive temperature compared with the other mutants. It endoreplicates to arrest with a 4*n* amount of DNA. This phenotype is probably explained by the fact that the cdk inhibitor Rum1 is stabilized in the *mts3-1* mutant and overexpression of Rum1 has been previously shown to result in endoreplication (Moreno & Nurse 1994). Interestingly, in *mts3-1* mutants incubated at the restrictive temperature, the Rum1 protein is stabilized whereas in the *mts2-1* mutant it is not. Perhaps this indicates that some substrate specificity resides in the subunits of the 19S complex (Kominami & Toda 1997).

When the 26S proteasome was isolated from the *mts2-1*, *mts3-1* and *pad1-1* mutant strains it was found that in each case the 26S complex showed a temperature-sensitive defect in the degradation of ubiquitin conjugates. In the 26S proteasomes purified from the *mts3-1* and *mts1-1* strains the corresponding proteins could not be found in the complexes incubated at the restrictive temperature; this deficiency probably accounts for the loss of activity. However, in the complex purified from the *mts2-1* strain the Mts2 protein was found at the restrictive temperature, implying that the complex is not defective in assembly but perhaps in some other function such as substrate recognition (Seeger *et al*. 1996; Penney *et al*. 1998).

To try to understand how the different subunits interact in the regulatory complex, we have used the mutants to demonstrate specific genetic interactions. Two genetic tests were used: partial suppression of the temperature-sensitive phenotype by overexpression of a suppressor cDNA from the strong *nmt1*$^{+}$ promoter and synthetic lethality. The interactions we have found are summarized in figure 1. We feel that these interactions give information on the position of the subunits in the 19S regulatory complex. Of particular interest is the observation that the non-ATPase subunit Mts4 interacts specifically with the ATPase subunit Mts2 (Wilkinson *et al*. 1997). To date, this is the only description of an ATPase subunit interacting with a non-ATPase subunit. Moreover, we have shown that the two proteins physically interact with each other *in vitro*. The importance of this interaction becomes clear when combined with the recent results described by Glickman *et al*. (1998*b*). This study revealed that the 19S regulatory complex can dissociate into two subcomplexes, which they call the base and the lid (Glickman *et al*. 1998*b*). The base is composed of the six ATPases and the non-ATPases Rpn1 (Mts4), Rpn2 and Rpn10; the lid contains the remaining eight non-ATPase subunits. We consider that our observations are consistent with the Mts4 subunit being part of the 'hinge' linking the base with the lid subcomplexes and thus that it plays an important role in determining the overall structure of the 19S regulatory complex itself.

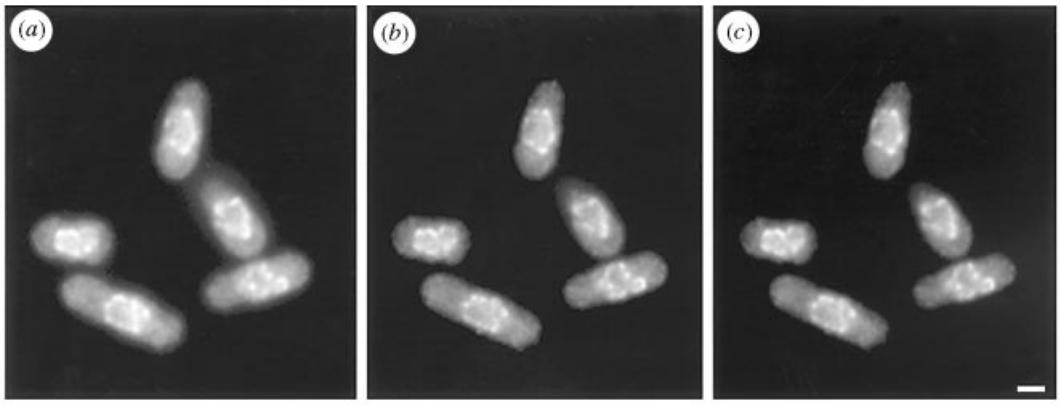

Figure 4. Co-localization of Mts4 with Pad1. The strain containing the HA-tagged *pad1*$^+$ fusion gene was fixed and stained with (*a*) an antibody against the Mts4 protein and (*b*) an anti-HA monoclonal antibody. The two images were merged and the resulting image (*c*) demonstrates that both signals co-localize. Taken from Wilkinson *et al*. (1998).

3. LOCALIZATION OF THE 26S COMPLEX DURING MITOSIS AND MEIOSIS

The ubiquitin pathway is known to degrade proteins that reside in a number of different compartments such as the nucleus, endoplasmic reticulum (ER) and cytosol. An important question, therefore, was to ask where the complex itself was localized in the cell. In an initial attempt to address this point we used specific antibodies against the Mts4 protein in immunofluoresence experiments (Wilkinson *et al*. 1997, 1999). Wild-type cells were fixed with *p*-formaldehyde and stained by using the mts4 antibody. Surprisingly, we found that the signal due to the Mts4 antibody was localized to a discrete area of the cell (figure 3). The staining appeared punctate around the DNA when visualized by the DNA-specific stain DAPI. It therefore appeared to be associated with the nuclear membrane. Further evidence that this was the case was obtained by observing the signal in cells undergoing mitosis. In fungi the nuclear membrane does not break down during cell division and they display what is known as a closed mitosis. In figure 3(*c*,*d*) cells in both early and late anaphase are shown. Once again the Mts4 signal was found to be associated with the nuclear membrane. As little background staining was observed, we assumed that the majority of the Mts4 protein was found at this site. Two control experiments were carried out to confirm that the localization we observed was due to the Mts4 protein. First, when pre-immune sera were used in immunofluoresence experiments no such staining was observed. The second control was to use a strain in which the *mts4*$^+$ gene had been deleted. When the Mts4 antibody was used to stain these cells no signal was observed. These results demonstrate that the specific localization we observed was due to the Mts4 protein (Wilkinson *et al*. 1999).

As a further control we looked at the immunolocalization of another 19S subunit, the Padl protein (Shimanuki *et al*. 1995; Spatoro *et al*. 1997; Penney *et al*. 1998). The *pad1*$^+$ gene was fused in frame to the HA epitope tag. The fusion construct was used to replace the wild-type gene; this replacement resulted in a strain in which the HA-tagged *pad1*$^+$ was under control of the normal wild-type *pad1*$^+$ gene promoter. As *pad1*$^+$ is an essential gene, the observation that the HA-tagged strain grew as wild-type

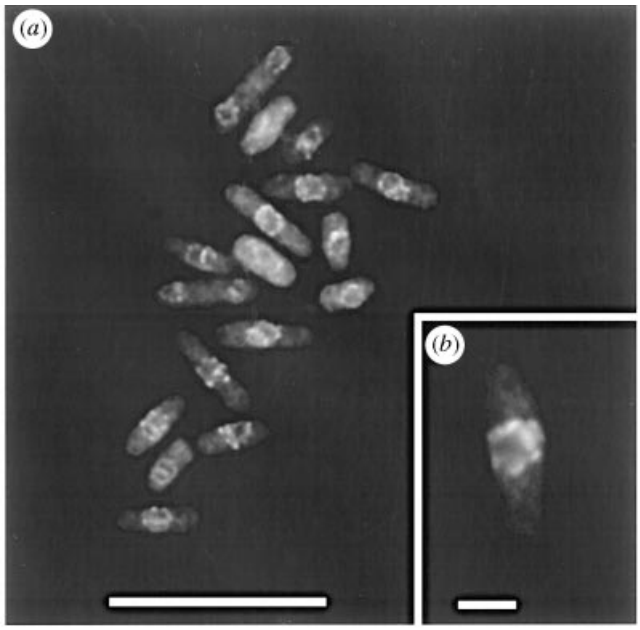

Figure 5. Wild-type cells stained with DAPI and the anti-20S antibody. (*a*) Scale bar, 100 mm; (*b*) scale bar, 10 mm. Taken from Wilkinson *et al*. (1998).

led us to conclude that the HA–Padl fusion protein was fully functional. Staining cells containing the *pad1*$^+$–HA gene with monoclonal antibodies against the HA tag, we found that the Padl protein showed the same staining pattern as found with the Mts4 protein. Furthermore, when we simultaneously stained these cells with rabbit polyclonal antiserum against the Mts4 protein we showed that both staining patterns completely overlapped (figure 4). As all the Mts4 protein and the HA–Padl protein are found associated with the 19S complex (Wilkinson *et al*. 1997; Penney *et al*. 1998), this finding suggested that the majority of the 19S complex was associated with the nuclear membrane.

As stated earlier, the 26S proteasome is composed of two discrete particles, the 20S catalytic core and the 19S regulatory particle. We therefore wanted to investigate whether the 20S particle was localized to the same place as the 19S particle. We therefore stained wild-type *S. pombe* cells with an antiserum raised against the whole *S. pombe* 20S proteasome complex (Seeger *et al*. 1996). A

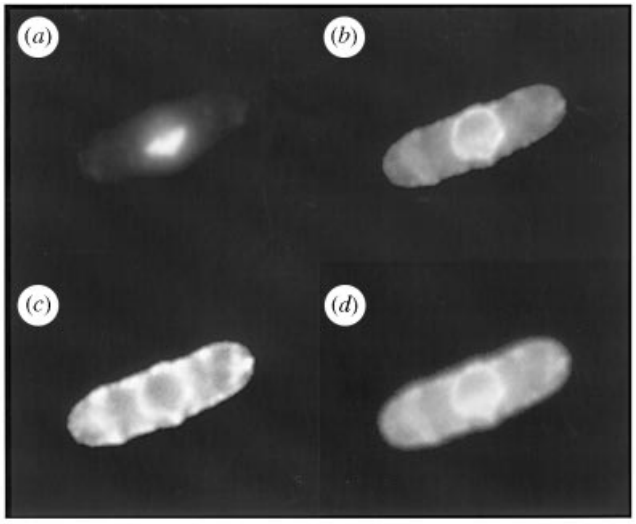

Figure 6. Wild-type cells stained with antibodies against the BiP ER protein and the Mts4 protein. (*a*) DNA stained with DAPI; (*b*) cell stained with monoclonal antibody against HA tag; (*c*) cell stained with anti-BiP antiserum; (*d*) merged image.

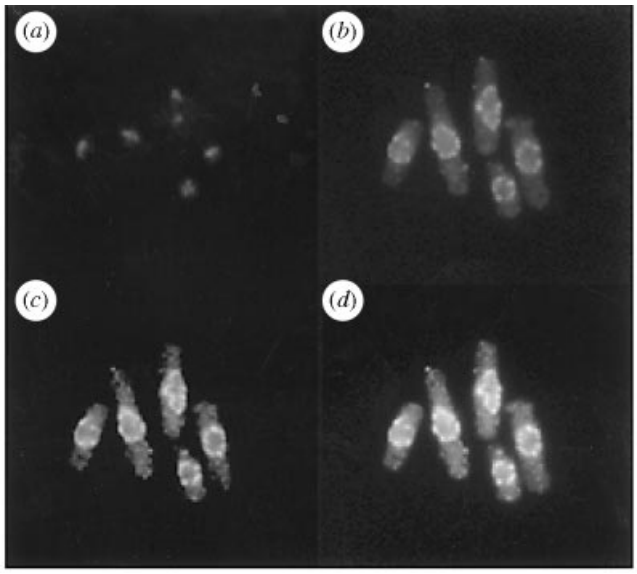

Figure 7. Co-localization of Mts4 and the nuclear pore complex. Cells were fixed and stained with (*a*) DAPI, (*b*) the mAB414 anti-nuclear pore monoclonal antibody or (*c*) the Mts4 antiserum. (*d*) The images were merged to show that the Mts4 and the nuclear pore signals co-localize. Taken from Wilkinson *et al*. (1998).

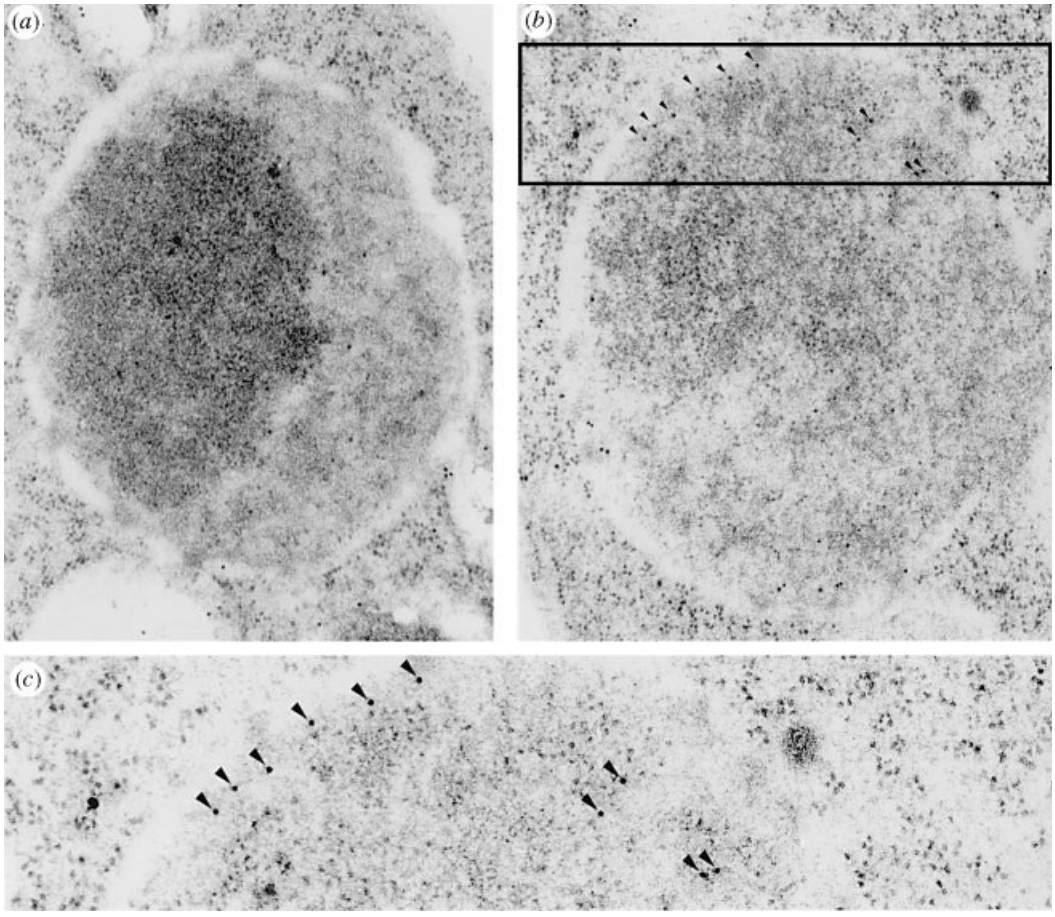

Figure 8. Electron micrograph localization of the Pad1 protein to the inside of the nuclear envelope. Immunogold staining of a section through (*a*) a wild-type or (*b*,*c*) a Pad1–HA-containing cell with the use of an anti-HA antibody. The localization of the HA protein tag was visualized by using a secondary antibody conjugated to 10 nm colloidal gold. The Pad1–HA protein was found to be in the innermost side of the nuclear envelope (arrowheads in *b* and *c*). Taken from Wilkinson *et al*. (1998).

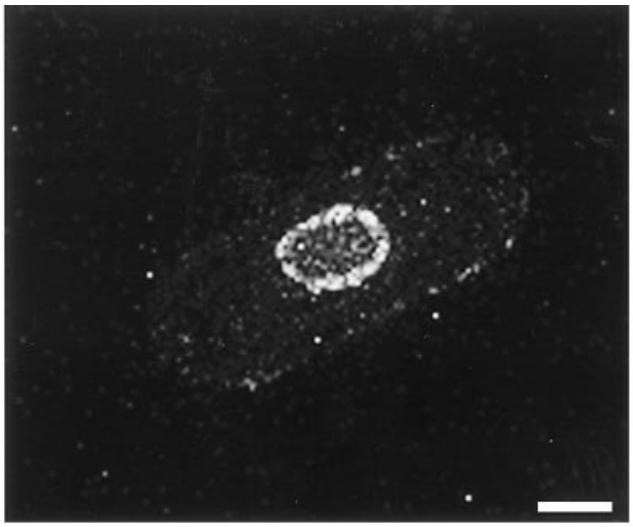

Figure 9. The GFP signal observed by fluorescence microscopy from a live cell expressing the Pad1–GFP fusion protein. Scale bar, 10 mm. Taken from Wilkinson *et al.* (1998).

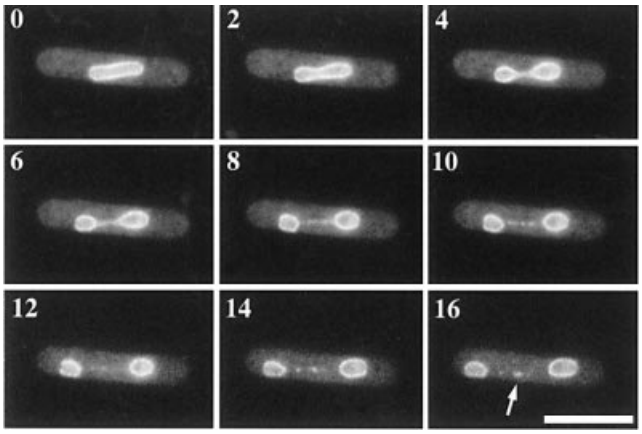

Figure 10. The GFP signal obtained as a cell progressed through mitosis. Images shown were obtained at the times shown in minutes. Scale bar, 10 mm. Taken from Wilkinson *et al.* (1998).

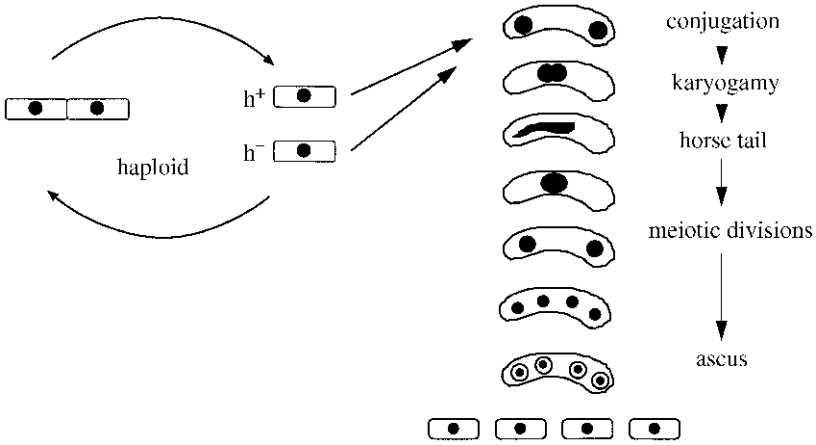

Figure 11. The different stages of the sexual cycle in fission yeast.

staining pattern similar to that seen with the Mts4 antiserum was observed (figure 5). This observation demonstrated that in fission yeast most of the 20S and 19S particles are associated together as the 26S proteasome and do not appear as separate pools.

We have shown that the 26S proteasome is localized to a discrete area of the cell around the nuclear periphery. We were interested in determining exactly what area of the cell this represented. For example, was the complex found on the outside of the nuclear membrane (potentially able to associate with the ER), or inside the membrane, or in the inner side of the nuclear membrane inside the nucleus, or perhaps some combination of all of these? Initial attempts were made to resolve this question by immunoflorescence microscopy with the use of specific antibodies against ER components or nuclear pore subunits. Cells were fixed for immunofluorescence and stained with monoclonal antibody against the nuclear pore component (Rout & Blobel 1993) or an antibody against the BiP ER protein (Pidoux & Armstrong 1992). As can be seen in figure 6, the proteasome signal co-localized with both the ER and the nuclear pore signal (figure 7). It was therefore not possible to resolve this issue by immunofluorescence microscopy.

To answer this important question we decided to carry out electron microscopic immunogold analysis with the HA-tagged *pad1*$^{+}$ strain. Using monoclonal antibodies against the HA epitope, we found that the HA signal localized specifically to the nuclear periphery at the innermost side of the nuclear membrane (figure 8*b*,*c*). No such staining was found in control cells containing the *pad1*$^{+}$ without the HA tag. Furthermore, serial sections through a cell gave identical results.

4. ANALYSIS OF THE DISTRIBUTION OF THE 26S PROTEASOME IN LIVE CELLS BY MEANS OF A GFP-TAGGED PAD1 SUBUNIT

We then decided to investigate the distribution of the 26S proteasome *in vivo* by means of a subunit fused to the green fluorescent protein (GFP) tag. We constructed a

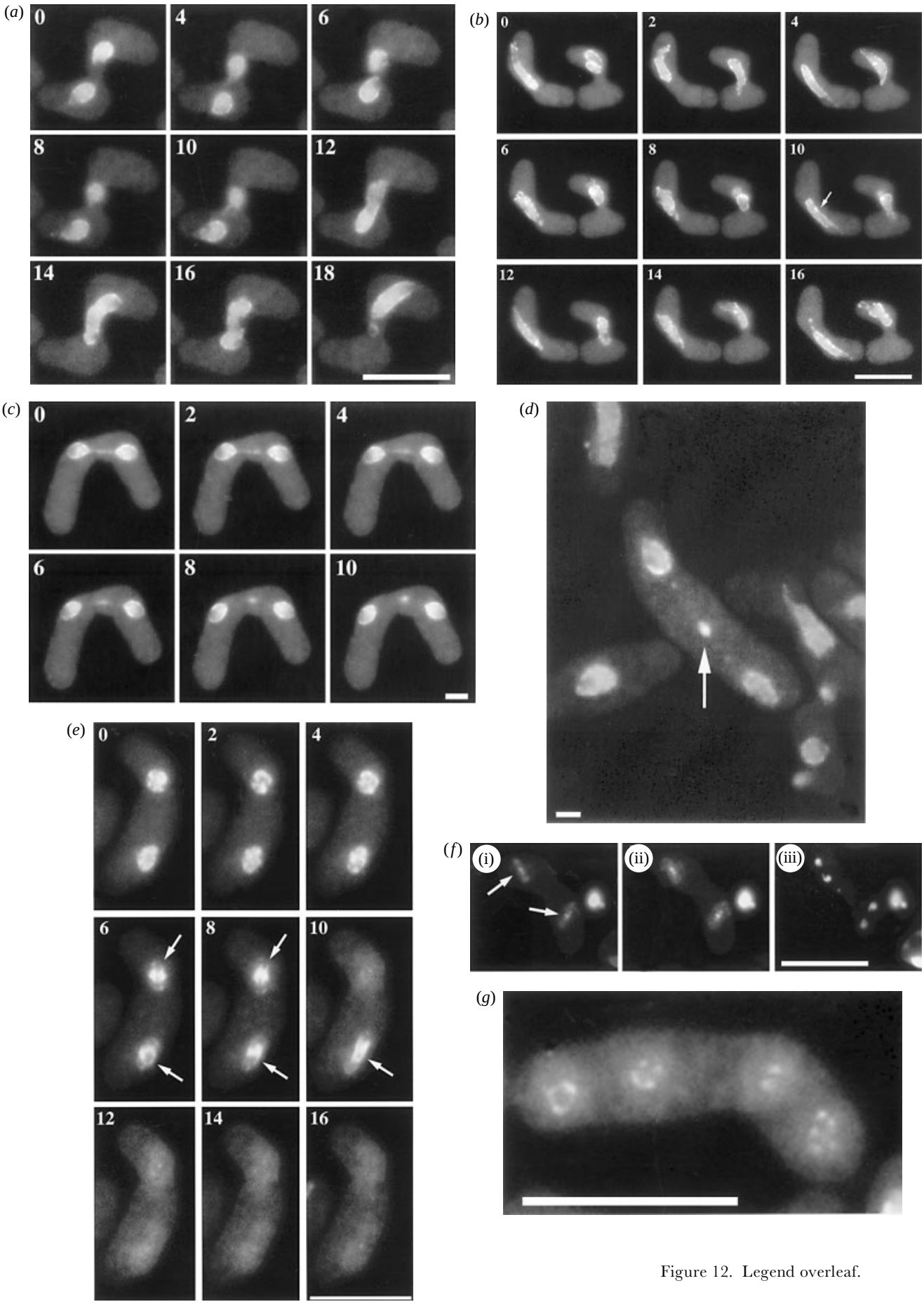

Figure 12. Legend overleaf.

strain with the GFP tag fused to the 3′-end of the *pad1*$^+$ gene. When used to replace the wild-type *pad1*$^+$ gene the strain grew at a rate indistinguishable from that of the wild-type, showing that the Pad1–GFP fusion protein was fully functional. When observed microscopically the GFP signal was similar to that observed by immunofluorescence and described earlier (figure 9). The availability of a GFP-tagged proteasome subunit enabled the localization of the complex to be followed in live cells. First, we investigated the distribution through mitosis. As can be seen in figure 10 the GFP signal was limited mainly to the nuclear periphery.

In addition, the localization of the complex throughout the meiotic cell cycle was observed. The *S. pombe* mating pathway is shown in figure 11. Under conditions of low nitrogen two yeast cells of opposite mating type will fuse by the process of conjugation (cell fusion) followed by karyogamy (nuclear fusion). This is followed by premeiotic DNA synthesis, which is followed by a period of rapid nuclear movement during the prophase stage of meiosis I, termed 'horsetail movement'. This is rapidly followed by the meiosis I division, meiosis II division and formation of the spores. As can be seen in figure 12 the GFP signal is found to be throughout the nucleus, but not in the nucleolous, during karogamy (figure 12*a*) and horsetail movement (figure 12*b*). Interestingly, there was an intense dot of staining routinely observed during horsetail movement. The dot was found to be associated with the nuclear periphery but appeared not to be associated with the spindle pole body, which can be easily identified as it is located at the leading edge of the movement. During meiosis I the staining was found to be localized to the nuclear periphery once again (figure 12*c*). During late anaphase, however, a dot of staining midway between the dividing nuclei was found (see arrow in figure 12*d*). Presumably this represents a large local concentration of proteasomes, the function of which remains unclear.

During meiosis II (figure 12*e*) a dramatic redistribution of GFP staining was observed. As the nuclei separated away from each other during anaphase the GFP signal was found to concentrate in the region between the dividing nuclei (figure 12*f*). At late anaphase this signal dispersed rapidly and appeared to diffuse among the whole emerging ascus (figure 12*g*). On spore formation the GFP signal appeared once more around the nuclear periphery in a pattern typical of mitotic cells.

Our data imply that the 26S proteasome occupies a discrete intracellular compartment throughout mitotic growth, around the nuclear periphery. The simplest model to account for this localization is that at least one subunit in the 26S proteasome is responsible for interacting with some cellular component to account for the observed localization. By using the Pad1–GFP-tagged strain, it should be possible to isolate mutants that no longer show this localization. If such mislocalization mutants can be isolated it will be of particular interest to investigate whether they encode essential non-proteasomal genes and whether they arrest at the metaphase stage of mitosis. This would have the implication that the localization is important for 26S proteasome function.

5. POSSIBLE MECHANISM OF SUBSTRATE PRESENTATION

The distribution of the 26S proteasome observed during mitosis and meiosis raises important questions about the action of the complex. From these studies it appears that in fission yeast the proteasome occupies a discrete intracellular compartment, the nuclear periphery inside the nucleus. If this is the case it implies that substrates would have, by some mechanism either active or passive, to be brought to the complex for degradation. How could this occur? One possible mechanism could involve the polyubiquitin binding subunit of the 19S complex, Rpn10 (S5a, Mcb1). This subunit is the only 19S subunit that is not stoichiometric compared with the other subunits. After glycerol-gradient centrifugation it is present in fractions with low and high molecular mass. It is present at a far greater abundance than other subunits in 26S proteasomes purified from humans, budding yeast and *Drosophila* cells (Deveraux *et al.* 1994; Van Nocker *et al.* 1996*b*; Haracska & Udvardy, 1995). Therefore one possible model is that this subunit is able to bind polyubiquitin-tagged substrates and bring them to the 26S proteasome for destruction. However, this simple model cannot be the whole story, as the budding yeast subunit has been identified and, unlike most other 19S subunits, encodes a non-essential gene. Therefore, to explain the viability of the rpn10 strain we would have to assume that this protein is redundant, implying that there is a family of such polyubiquitin-binding proteins. However, as there are no homologues of the protein in the *Saccharomyces cerevisiae* genome, such proteins would have to have a significantly different primary structure.

6. LOCALIZATION OF THE 26S PROTEASOME IN OTHER ORGANISMS

The lesson from fission yeast is that it appears that the 26S proteasome occupies a fixed position in the cell; this observation raises the possibility that the substrate comes to the complex. A recent study of the localization of the 26S proteasome in budding yeast during the mitotic cycle has also demonstrated a perinuclear localization (Enenkel *et al.* 1999). Could such a mechanism also exist in higher organisms? A number of different studies have addressed the question of where the 26S proteasome is localized in higher eukaryotes, with conflicting results. Some reports provide evidence that the complex is located in both the nucleus and the cytoplasm (Rivett *et al.* 1992; Peters *et al.* 1994); others, that it is associated with the ER (Yang *et al.* 1995). Still other reports state

Figure 12. The GFP signal obtained in live cells progressing through the different stages of mating. In each case the time is in minutes. (*a*) Karyogamy. (*b*) Horsetail movement. (*c*,*d*) Meiosis I; the arrow in (*d*) indicates the bright spot of staining between the two nuclei. (*e*) Meiosis II; the arrow indicates the bright staining observed as cells proceed through meiosis II. (*f*) DNA and GFP staining of cells in meiosis II: (i) merged image; (ii) GFP; (iii) DNA-specific stain Hoechst 33342. (*g*) Ascus. Taken from Wilkinson *et al*. 1998.

that the complex is concentrated in the nucleus (Stauber *et al.* 1987) or the cytoplasm (Kloetzel *et al.* 1987). Changes in localization have been reported during mitosis (Kawahara & Yokosawa 1992; Amsterdam *et al.* 1993) and also during development (Akhayat *et al.* 1987). One explanation to reconcile these conflicting results is that the 26S complex is found in different locations depending on the cell type.

7. SIGNIFICANCE OF THE NUCLEAR PERIPHERY LOCALIZATION

What is the reason for the concentration of 26S proteasomes around the nuclear periphery in fission yeast? One reason could be to take advantage of the massive amount of transport that occurs continuously between the nucleus and the cytoplasm. Our knowledge of this transport has increased dramatically in recent years (Weis 1998). Potentially, receptors involved in such transport could also act as shuttling factors bringing substrates to the proteasome. Experiments are in progress to try to answer some of these important questions.

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Discussion

M. Hochstrasser (*University of Chicago, Illinois, USA*). Although most of the immunofluorescence signal appears to come from the nuclear periphery, there is a significant signal in the rest of the cell. Can you estimate what fraction of the total proteasome is in the nuclear periphery?

C. Gordon. We have tried to address this with fractionation studies, which are relatively difficult to do in fission yeast. However, the recent paper by Enenkel *et al.* (1998) demonstrates that the proteasome distribution in budding yeast is very similar to that which we observed in fission yeast; they estimate from cell fractionation experiments that 80–90% of the total proteasome is found in the nuclear periphery. We have preliminary fractionation data which is consistent with this estimate.

K. A. Nasmyth (*Research Institute of Molecular Pathology, Vienna, Austria*). How many clear cytoplasmic substrates for the proteasome are there? This might give some estimate of the biological activity of the fraction in the cytoplasm.

C. Gordon. I wouldn't say that all the proteasome is in the nucleus. There could be a fraction in the cytoplasm.

K. A. Nasmyth (*Research Institute of Molecular Pathology, Vienna, Austria*). The literature is conflicting depending on which lab has done the experiment and which cell type is examined. Some groups see the proteasome in the nucleus, some in the cytoplasm, some groups see it in both, so there could be some cell-type dependence. However, it does now seem that the localization in both *S. cerevisiae* and *S. pombe* is the same which is reassuring.

A. J. Rivett (*University of Bristol, Bristol, UK*). In most animal cells the proteasome does appear to be found in both the nucleus and the cytoplasm.

A. Hershko (*Technion—Israel Institute of Technology, Haifa, Israel*). I would just like to point out that reticulocytes have no nuclei but do contain plenty of proteasomes, so this is a clear case of a non-nuclear proteasome distribution.