Dynamic Association of Proteasomal Machinery with the Centrosome

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Abstract. Although the number of pathologies known to arise from the inappropriate folding of proteins continues to grow, mechanisms underlying the recognition and ultimate disposition of misfolded polypeptides remain obscure. For example, how and where such substrates are identified and processed is unknown. We report here the identification of a specific subcellular structure in which, under basal conditions, the 20S proteasome, the PA700 and PA28 (700- and 180-kD proteasome activator complexes, respectively), ubiquitin, Hsp70 and Hsp90 (70- and 90-kD heat shock protein, respectively) concentrate in HEK 293 and HeLa cells. The structure is perinuclear, surrounded by endoplasmic reticulum, adjacent to the Golgi, and colocalizes with γ-tubulin, an established centrosomal marker. Density gradient fractions containing purified centrosomes are enriched in proteasomal components and cell stress chaperones. The centrosome-associated structure enlarges in response to inhibition of proteasome activity and the level of misfolded proteins. For example, folding mutants of CFTR form large inclusions which arise from the centrosome upon inhibition of proteasome activity. At high levels of misfolded protein, the structure not only expands but also extensively recruits the cytosolic pools of ubiquitin, Hsp70, PA700, PA28, and the 20S proteasome. Thus, the centrosome may act as a scaffold, which concentrates and recruits the systems which act as censors and modulators of the balance between folding, aggregation, and degradation.

Key words: proteasome • protein misfolding • inclusions • CFTR • centrosome

Many pathologies result from the inability of a mutant protein to properly fold (Thomas et al., 1995). It is therefore important to understand the mechanisms by which misfolded proteins are recognized, processed, and ultimately degraded by the cell. The 26S proteasome catalyzes the intracellular degradation of damaged or misfolded proteins, the rapid degradation of short-lived regulatory proteins such as those involved in cell cycle control and signaling, as well as the generation of antigenic peptides for presentation to MHC-I (Coux et al., 1996). Degradation of most substrates by the proteasome requires the covalent conjugation of ubiquitin, an event catalyzed by the sequential action of three enzymes (Ciechanover, 1994; Hochstrasser, 1996). The ubiquitination machinery acts progressively, resulting in a polyubiquitin chain that serves as a signal for degradation of the modified protein by the 26S proteasome.

The 26S proteasome is composed of two major subcomplexes. The 20S proteasome, a 700-kD cylinder, constitutes the catalytic core of the protease (Coux et al., 1996; Baumeister et al., 1998). Structural (Lowe et al., 1995; Groll et al., 1997) and biochemical evidence (Wenzel and Baumeister, 1995) indicate the catalytic sites are located within a hollow cavity of the cylinder. Access to these sites is achieved through narrow openings at the ends of the cylinder which may be gated by regulatory proteins. This topology limits substrates to short peptides and completely unfolded proteins.

The regulatory complex of the 26S proteasome is a 20-subunit, 700-kD activator called PA700 or 19S cap (Coux et al., 1996), which associates with either (or both) end(s) of the 20S proteasome to form the 26S complex...
Several other CF-causing folding mutations (Gregory et al., 1995; Jensen et al., 1995; Ward et al., 1995) have been shown to play a role in both preventing protein aggregation and promoting folding or degradation of a wide range of proteins. Recently, the ubiquitination of certain proteins has been shown to depend upon Hsp70, implicating it in the targeting of misfolded or mutant proteins for degradation by the proteasome (Bercovich et al., 1997). In this regard, Hsp70 has been shown to interact transiently with immature, incompletely folded CFTR (Yang et al., 1993). While this paper was under review, a report appeared that demonstrated that the Hsp90 inhibitor geldanamycin prevents the association of immature CFTR with the chaperone, blocks its maturation, and enhances its degradation by the proteasome (Loo et al., 1998).

In spite of the large body of evidence demonstrating the proteasome machinery’s involvement in the degradation of a diverse range of substrates, it is unclear whether its functions are distributed homogeneously throughout the cell or if they are segregated. We present evidence herein of the centrosome as a unique location in which the proteasome machinery, Hsp70, and Hsp90 are concentrated in resting HEK 293 and HeLa cell lines. In addition, the centrosome, a structure thought previously to function primarily during cell division, responds to inhibition of the proteasome and increases in the level of misfolded proteins by expanding and recruiting cellular pools of the proteasome components and Hsp70.

Materials and Methods

Materials

Plasmids pCMVNot6.2 and pCMVNot6.2ΔF containing expressible human CFTR cDNAs were the generous gift of Dr. Johanna Rommens (The Hospital For Sick Children, Toronto). Two anti-human (COOH terminus) CFTR antibodies were used: mouse monoclonal antibody 24-1 from Genzyme Diagnostics and rabbit polyclonal antibody R3194 (Zeng et al., 1993). Mouse monoclonal anti-α-tubulin and anti–γ-tubulin antibodies and rhodamine-conjugated wheat germ agglutinin were purchased from Sigma Chemical Co. Mouse monoclonal anti-Hsp70 and anti-Hsp90 antibodies were from Abfinity Bioreagents, Inc. Goat polyclonal anti-aldolase was from Biodesign International. Mouse monoclonal anti-lamin B2 antibody was from Zyomed Laboratories, Inc. Mouse monoclonal anti-β-cop antibodies contained in media from secreting hybridoma cells were the generous gift of Dr. G. George Bloom (Department of Cell Biology, University of Texas Southwestern Medical Center). Fluorescein-conjugated concanavalin A was from Molecular Probes, Inc. Fluorescently labeled secondary antibodies were from Jackson ImmunoResearch Laboratories, Inc. Lactacycin was purchased from Calbiochem. All other materials were of the highest quality commercially available.

Generation and Characterization of Antisera

Polyclonal anti-PA 28 (M a et al., 1993), anti-PA 28 ( Song et al., 1996), and anti-20S proteasome (M a et al., 1994) were generated as described. Polyclonal antibodies were prepared in chickens against highly purified bovine PA 700. Chicken IgG was purified from egg yolks of immunized birds. The antibodies specifically recognized multiple subunits of the PA 700 complex, including p112, p97, p58, p56, and p45, upon Western blot analysis of crude cell lysates and could specifically immunoprecipitate PA 700 from solution using an agarose bound anti-IgY antibody. Poly-
Preparation of P20SS Mutant Expression Construct

Oligonucleotide-directed mutagenesis as described (A ndrews and Lesley, 1998) was used to generate the mutant CFTR from the parent expression vector pCMVNot6.2. In brief, mutants were selected based upon the incorporation of a second-site mutation in β-lactamase which alters its sub-
strate specificity leading to resistance of transformed bacteria to ceto-
taxime and ceftriaxone in addition to ampicillin. The sequence of the mutagenic primer used to create P20SS was 5′-CGTGGTGAATGGCT-
TCTTTGCAAGTGGC-3′. Incorporation of the mutation was verified by DNA sequencing. Transfection-quality plasmid DNA was prepared using reagents supplied by Qiagen Inc.

Cell Culture and Transfection

Wld-type and mutant CFTR cDNAs were transfected into human embry-
onic kidney (HEK) 293 or HeLa cells (American Type Culture Collec-
tion) using the FuGene Mammalian Transfection Reagent (Boehringer Mannheim). Cell lines were maintained in DMEM supplemented with 10% FCS, 50 µg/mL streptomycin, and 50 units/mL penicillin.

Preparation of Centrosomes

Centrosomes were isolated from HEK 293 and HeLa cells by discontinu-
grade gradient ultracentrifugation according to the method of Moudjou and Bornens (1998). In brief, cells in the exponential phase of growth were treated with 1 µg/mL cytochalasin D and 0.2 µM nocodazole for 1 h. Cells were collected by trypsinization and centrifugation and the resulting pellet was washed in TBS followed by 0.1% TBS/0.1% sucrose. Cells were resus-
pended in 2 mL of 0.1% TBS/5% sucrose followed by addition of 8 mL lysis buffer (1 mM Heps, pH 7.2, 0.5% NP-40, 0.5 mM MgCl2, 0.1% β-mercap-
toethanol, 1 µg/mL leupeptin, 1 µg/mL pepstatin, 1 µg/mL aprotinin, and 1 mM PMSE). The suspension was gently shaken and passed five times through a 10-ml narrow-mouth serological pipette to lyse the cells. The lysate was spun at 2,500 g for 10 min to remove swollen nuclei, chromatin aggregates, and unlysed cells. The resulting supernatant was filtered through a nylon

 SDS-PAGE and Western Blotting

Density gradient fractions were diluted into 1 mL of 10 mM Pipes, pH 7.2, and centrosomes were sedimented at 14,000 rpm in a microfuge for 15 min at 4°C. Centrosome pellets were resuspended in Laemmli sample buffer containing 5% β-mercaptoethanol and electrophoresed on 10% SDS-
PAGE gels. Proteins were transferred onto nitrocellulose membranes in the presence of Towbin transfer buffer (25 mM Tris, 192 mM glycine, and 20% methanol, pH 8.3). Membranes were blocked in TBS-T (Tris-buffered saline; 1% Tween-20) containing 10% nonfat dry milk for 1 h and then incubated in fresh blocking buffer containing primary antibody at the desired concentration. Membranes were washed several times in TBS-T followed by incubation with the appropriate horseradish peroxidase-conjugated secondary antibody. Immune-reactive proteins were visualized with enhanced chemiluminescence.

Inhibition of 20S Proteasome

The proteolytic activity of the endogenous proteasomal pool was inhibited by the addition of the potent fungal product lactacystin. The inhibitor was added to 293 cells 48-72 h after transfection at a concentration of 10 µM. Treatment of cells was carried out for 2 to 12 h and was immedi-
ately followed by several washes with PBS before fixation for immunocy-
tochemistry.

Immunocytochemistry

For immunofluorescent subcellular localization of CFTR (Lee et al., 1997), transiently transfected cells attached to glass cover slips were rinsed three times with PBS followed by fixation and permeabilization with 1 mL of ice-cold methanol for 10 min at -20°C. Upon removal of methanol, cells were again rinsed three times with PBS and incubated 10 min in 1 mL of PBS supplemented with 50 mM glycine (this and all subsequent manipulations were carried out at room temperature). This buffer was then re-
moved and nonspecific sites were blocked with 0.1 mL of blocking medium (PBS supplemented with 5% goat serum, 1% BSA, and 0.1% gelatin) for 1 h in a humidified chamber. After blocking, the medium was replaced with 0.1 mL of blocking medium containing a 1:100 dilution of the respec-
tive primary antibody for 1 h. Cells were next washed three times with blocking medium and incubated for 1 h with blocking medium containing a 1:100 dilution of the appropriate fluorescently labeled secondary anti-
body. For double-labeling experiments, these primary and secondary in-
cubations were repeated with antibodies against the second protein of in-
terest. For lectin affinity staining involving either fluorescently tagged wheat germ agglutinin or concanavalin A, these reagents were added at either a 1:250 dilution with the secondary antibody, or at 1 µg/mL in PBS for 30 min before the initial fixation and permeabilization, respectively. Fluorescent images were obtained using a Bio-Rad MRC 1024 confocal mi-
oscope. Expression levels of cells expressing CFTR were divided into two categories, those which required a gain of 1,150 or higher to visualize (low expressers) and those which required a gain setting of 950 or less (high expressers). In each case the Iris was held constant at 3.5. CMV-driven CFTR expression levels can be categorized as either low or high. A t the high expression level, some cells form CFTR inclusions without lacticystin treatment. This is observed for the folding mutants as well as for wild-type CFTR. This is likely due to an overload of the cellular fold-
ing machinery and may account, in part, for the low (∼30%) maturation efficiency observed for wild-type CFTR overexpressed in cultured cells (Ward and Kopito, 1994). We have therefore restricted our study to cells expressing low levels of CFTR.

Subcellular Morphometry

Due to periodic irregularities in the shape of the perinuclear structure, morphometric analysis describing their apparent diameter, as delineated by confocal imaging of immunosulfated HEK 293 cells, was limited to cells containing reasonably symmetric centrosomes. Measurements of diameter were performed using software supplied by the manufacturer. Three fields (×400) of each experiment were randomly selected and the diameters were measured from all the cells in each field. In the case of elliptically shaped structures, the geometric mean of the diameters were used. Data presented are reported as the mean ± SEM.

Analysis of Soluble and Insoluble Cellular Fractions

2.5 × 106 HeLa cells per 3-cm dish were either mock-transfected or trans-
fected with P20SS mutant CFTR expression plasmid. 48 h post-transfec-
tion, one of each was treated with 10 µM lacticystin for 12 h. Cells were washed with PBS, collected by trypsinization, and pelleted in a microfuge at 4°C. Each pellet was washed twice with PBS and resuspended in 100 μL PBS supplemented with Complete protease inhibitor cocktail (Boehringer Mannheim). A 1-fs lysis by passage 10-20 times through a 27-gauge needle, lysates were spun at 14,000 g for 1 h at 4°C. The supernatants were collected and the pellets were washed in supplemented PBS followed by centrifugation at 14,000 g for 30 min. A 1-fs aspiration of the wash solu-
tion, the pellets were resuspended in 100 µL supplemented PBS. Both pel-
lets and supernatants were stored at -70°C until analysis. Equal volumes of each pellet and supernatant were analyzed by SDS PAGE through 4-20% gels followed by Western immunoblotting versus the indicated anti-
body as described above.

Results

Concentration of Proteasome Machinery at the Centrosome

A central question with respect to proteasome-mediated degradation of misfolded integral membrane proteins is where in the cell does proteolysis occur. To address this question, we employed immunocytochemistry to delineate the subcellular distribution of several key components of
the proteasome pathway including: the 20S proteasome, the PA 700 and PA 28 activator complexes, ubiquitin, and Hsp70.

In HEK 293 cells, antibodies directed against each of these proteasome components identified multiple distinct subcellular pools. The 20S proteasome (Fig. 1, A and D), the PA700 complex (Fig. 1, B and E), and ubiquitin (Fig. 1 G) are each clearly discernible in both a nuclear pool and a cytoplasmic pool. In contrast, PA28-associated immunofluorescence was identified mainly in the nucleus (Fig. 1, C and F), and Hsp70 was primarily found in a reticular pattern in the cytosol (Fig. 1 H). In addition to these general distributions, staining with each of these specific antibodies revealed the existence of a unique perinuclear site in which all of the studied components concentrate (Fig. 1, A–H). The structure is surrounded by but does not colocalize with staining against the ER lumenal chaperone BiP (Fig. 1 F), and is proximal to the Golgi apparatus as illustrated by staining with the lectin, WGA (Fig. 1, D and E). These same subcellular distributions were also observed in HeLa, COS, and CHO cells suggesting generality of the observation (data not shown).

Further investigation of this perinuclear structure using double-labeled immunofluorescence revealed exact colocalization of the 20S proteasome, PA700, PA28, ubiquitin, and Hsp70 with γ-tubulin, an established centrosomal marker (Mitchison and Kirschner, 1984; Oakley and Oakley, 1989). The majority of γ-tubulin staining was restricted to the centrosome, although an expected faint diffuse cytosolic staining was also observed corresponding to the soluble fraction of this protein. These data implicate the centrosome as a unique site for the colocalization and concentration of the proteasomal machinery and certain cell stress chaperones under basal conditions, suggesting a novel function for the centrosome.

The specificity of these proteasomal antibodies for immunocytochemistry is supported by several control experiments. First, using indirect immunofluorescence and confocal microscopy, staining with non- and preimmune rabbit sera or secondary antibody alone revealed virtually

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**Figure 1.** Localization of proteasome components at the centrosome. HEK 293 cells were immunostained with antibodies against (A and D) 20S proteasome and γ-tubulin (A) or fluorescently labeled WGA (D). (B and E) PA700 and γ-tubulin (B) or fluorescently labeled WGA (E). (C and F) PA28 and γ-tubulin (C) or BiP (F). (G) Ubiquitin (Ub) and γ-tubulin, and (H) Hsp70 and fluorescently labeled WGA. Confocal images were obtained as described under Materials and Methods. Specificity of antisera was investigated by staining HEK 293 cells with (I) polyclonal chicken anti-PA700, or (J) polyclonal chicken anti-PA700, preabsorbed at room temperature against purified bovine PA700. Confocal fluorescent images were obtained on the same day at identical magnification, iris settings, and gain. Note: For clarity of presentation in each panel, immunofluorescence of the proteasomal machinery, Hsp70, and Ub is consistently presented in green while the counter staining for γ-tubulin, WGA, and BiP is consistently presented in red. Arrows in panels A–H indicate locations of perinuclear proteasome-enriched centrosomes. Scale bars indicate size in μm.
PA28, were also observed in lighter gradient fractions, where faint γ-tubulin immunoreactivity was detected. This observation may be explained either by a heterogeneous population of centrosomes or lower-affinity binding of the chaperones and PA28 to the centrosome relative to that of 20S and PA700. Sucrose fractions were devoid of other subcellular markers, including Bip (ER), aldolase (cytosol), Lamin B1 (nucleus), and β-cop (Golgi), establishing the purity of the centrosome preparations.

Expansion of the Centrosome in Response to Proteasome Inhibition

Treatment of cells with lactacystin, a potent and specific inhibitor of the proteasome (Fenteany et al., 1995) resulted in a significant increase in size of the centrosome. Representative images of cells treated and untreated with lactacystin, were stained for PA28 and the lectin Con A (Fig. 3, A and B). Similar results were obtained for cells stained for 20S proteasome, Ub, PA 700, Hsp70, and γ-tubulin. These results were quantified by morphometric analysis of centrosome diameters (identified by fluorescent staining for γ-tubulin or perinuclear proteasomal components). Comparison of lactacystin-treated and untreated HEK 293 cells revealed a twofold increase in mean diameter in response to the proteasome inhibition (Fig. 3 C). These data suggest that the centrosome is a dynamic structure, capable of expansion in response to inhibition of the proteasome. This expansion may be due to a build up of misfolded proteins, which would otherwise be degraded by the proteasome concentrated at the centrosome. To further test this hypothesis, we expressed in HEK 293 cells wild-type CFTR and two variants known to misfold, namely ΔF508 and P205S, and examined their effect on the centrosome and the proteasome machinery.

Expansion in Response to Expression of Mutant Protein

Consistent with previous reports (Cheng et al., 1990), immunolocalization of CFTR in transiently transfected HEK 293 cells clearly differentiates the wild-type CFTR subcellular locale from that of the folding mutants. An antisem directed against the COOH terminus of CFTR recognized wild-type CFTR at the plasma membrane of transfected cells (Fig. 4 A) and at an early stage of maturation that colocalized with the ER-resident chaperone Bip (Fig. 4 B). The ER is the site of initial membrane translocation and integration of nascent polytopic membrane proteins. In sharp contrast, ΔF508 and P205S mutant CFTR are detected predominantly in the ER of transfected cells as illustrated by the ER pattern of CFTR staining (Fig. 4, C and E) and the complete colocalization with Bip staining (Fig. 4, D and F).

Cells expressing low levels of the wild-type CFTR do not significantly perturb either the PA28 distribution or size of the centrosome, although in highly expressing cells a fraction of CFTR colocalizes within this structure as seen in Fig. 5 A. Similar results were obtained with other proteasome components studied (data not shown). In striking contrast, expression of P205S (Fig. 5 B) or ΔF508 (data not shown) expands the centrosome in a manner similar to that observed when cells are treated with lactacystin alone (Fig. 3). This observation provides further support for the...
accumulation of misfolded proteins at the site of the centrosome even when the proteasome is functional.

**Recruitment of Proteasomal Components to a Centrosome-Associated Inclusion**

In cells overexpressing mutant CFTR (P205S) and treated with lactacystin, we observed the formation of large, perinuclear aggregates of misfolded CFTR which appear to arise from the centrosome as indicated by colocalization with γ-tubulin (Fig. 6). Identical results were observed with ΔF508 expressing cells (data not shown). In addition, a remarkable and comprehensive recruitment of the cytosolic pools of the 20S proteasome (Fig. 7 A), PA 700 (B), ubiquitin (D), and Hsp70 (E) to the site of CFTR aggregate formation was observed. This recruitment is coincident with a depletion of the cytosolic pools implicating this as the source. PA 28 is also recruited to the inclusion in response to the build up of misfolded CFTR (Fig. 7 C). Morphometric analysis of the CFTR aggregate formed in lactacystin treated cells is presented in Fig. 7 F. A composite of data from Fig. 3 E describing centrosome expansion due to proteasome inhibition alone relative to nontreated cells is included for comparison. The diameter of the aggregate formed in lactacystin-treated cells expands to four to six times the size of the centrosome from which it is apparently derived.

Next, to further investigate the observed redistribution of the proteasomal machinery and Hsp70 in response to the formation of lactacystin-induced intracellular inclusions of misfolded CFTR, we separated cell lysates into soluble and insoluble cellular fractions. In mock-transfected cells, γ-tubulin was observed primarily in the soluble fraction (Fig. 8). However, in cells expressing P205S mutant CFTR and treated with lactacystin, γ-tubulin was observed distributed between the soluble and insoluble fractions. PA28, PA700, and Hsp70 redistributed in a manner parallel to γ-tubulin (Fig. 8). An intermediate degree of redistribution was observed for mock-transfected cells treated with lactacystin and untreated mutant transfected cells (data not shown).

**Discussion**

The work reported here describes the immunocytochemical (Fig. 1) and biochemical (Fig. 2) identification of the centrosome as a unique subcellular location in which, under basal conditions, components of the proteasome proteolytic pathway and certain relevant heat shock chaperones concentrate. This localization was observed under normal growth conditions in HEK 293, HeLa, COS, and CHO cells. Moreover, the association of the proteasome with the centrosome does not require an intact F-actin nor microtubular network as indicated by their presence in the purified centrosomal fractions obtained from nocodazole/cytchalasin D–treated cells (Fig. 2), indicating that their localization at this site is not simply a result of clustering at the minus-ends of microtubules. This does not, however, exclude the possibility that the cytoskeleton may be required for trafficking to and from this location.

When the cellular level of misfolded protein is high, either due to the overexpression of a misfolded mutant protein (such as ΔF508 or P205S CFTR) or the inhibition of the proteasome, the cell responds by expanding the diame-
ter of the centrosome up to twofold (Figs. 3 and 5). Assuming a spherical, three-dimensional shape, this would translate to more than a fourfold increase in its volume. Under high loads of misfolded substrate and/or insufficient proteasome activity, the centrosome, Hsp70, and the proteolytic machinery undergo correspondent redistribution to a sedimentable fraction (Fig. 8). The presence of the perinuclear proteasome concentration in control cells argues that the simplest explanation for this expansion involves the targeting of misfolded proteins to this central-ized locale for rapid and efficient degradation. Consistent with this hypothesis, the centrosomal-associated proteasomal machinery is active (Fabunmi, R.P., W.C. Wigley, P.J. Thomas, and G.N. DeMartino, unpublished observations). When degradation is insufficient the misfolded proteins accumulate at and proximal to this site eventually forming a large inclusion. In light of this finding and the proximity of the centrosome to the Golgi and lysosomes, care should be taken when interpreting subcellular localization of overexpressed proteins.

It is particularly interesting that under the highest loads of misfolded protein, when the proteasome is inhibited and a mutant protein is overexpressed, the cell responds by extensively recruiting additional proteasomal machi-
based motors in the recruitment process. Consistent with this notion is the observation that the nuclear pools of proteasomal component remain unperturbed (Fig. 7). Further experiments will be required to determine if the recruitment is an active process or simply due to diffusion although the latter possibility is unlikely because molecules the size of the 26S proteasome are too large to simply diffuse through the cytosol (Janson et al., 1996).

Similar questions exist as to the targeting of the substrates to this site. While this manuscript was under review, an interesting and complementary study reported the formation of CFTR aggregates at the centrosomes (Johnston et al., 1998). Formation of the aggregates required an intact microtubular system implicating a motor-based translocation of substrate to the centrosome, analogous to the potential mechanism of proteasome recruitment suggested here. Interestingly, fibrillar extensions, as observed by CFTR and proteasomal staining (Figs. 6 A and 7), radiate from the centrosomally localized aggregates. These fibrils are sensitive to treatment with nocodazole suggesting their microtubular origin. However, the centrosomal localization of the aggregates and associated proteasomal components persist in lactacystin treated cells after nocodazole treatment. The mechanisms employed for assembly and, potentially, disassembly of these structures deserves further study. Regardless of the means of assembly, it is clear that this structure concentrates and recruits proteins that would be expected to perform...
were lysed and separated into supernatant (S) and pellet (P) frac-
tions after treatment with lactacystin for 12 h and untreated mock-transfected control cells were lysed and separated into supernatant (S) and pellet (P) frac-
tions as described in Materials and Methods. Fractions were sepa-
ated by SDS-PAGE and analyzed by Western blotting versus each of the indicated antibodies.

a censor function by monitoring and perhaps controlling the balance between folding, degradation, and aggregation of nascent membrane proteins in the cell.

Understanding the composition and assembly of the proteasome-enriched centrosomes should provide new insight into the mechanisms of quality control employed by eukaryotic cells. For example, it is unclear if PA700, free or in complex with the 20S proteasome, participates directly in the recognition of the misfolded CFTR, altered localization, or the formation of the inclusions. However, it is possible that free PA700 uses its poly-Ub binding do-

The accumulation of overexpressed mutant CFTR at the centrosome upon inhibition of proteolysis suggests that it may serve as a terminal point in the pathway of misfolded polypeptides suggesting that they either assemble or nucleate at this location. It is interesting to note that similar inclusions (Russell, 1890; Valetti et al., 1991) have been previously observed in heat-stressed (Vidair et al., 1996), protease-inhibited cells (Wójcik et al., 1996; Johnston et al., 1998), and a growing family of pathologies related to protein misfolding such as Alzheimer’s, Huntington’s, amyotrophic lateral sclerosis, and type-1 spinocerebellar ataxia (SCA1) (Bruijn et al., 1998; Cummings et al., 1998; Sisodia, 1998). The detailed structural and functional relationships among these inclusions are unclear and warrants further investigation.

The centrosomal localization of the proteolytic machinery under basal conditions described in this study may play an important role in the degradation of proteins involved in progression through the cell cycle (King et al., 1996). In

![Figure 8](image-url)


Mitchison, T., and M. Kirschner. 1984. Microtubule assembly nucleated by iso-