A proteasome regulator, termed PA28, has been shown to modulate peptidase activities of the proteasomes in vitro. Two different but homologous PA28 molecules, designated as PA28α and PA28β, have been cloned. Both α and β polypeptides of PA28 are found in PA28 complexes isolated from cells, indicating that both are constituents of functional PA28 complexes. Using antisera specific to PA28α, PA28β, and epitope-tagged PA28 molecules, we show that expression of PA28α and PA28β is coordinately induced by various cytokines in different cell lines and that PA28 subunits and proteasomes have almost identical half-lives. In addition, we show that PA28 complexes are associated with 20 S but not 26 S proteasomes in vivo. Moreover, we demonstrate that PA28 complex is a heterohexamer composed of both α and β subunits with a stoichiometry of αβα in an alternating order.

The proteasome is a multicatalytic protease complex found in eukaryotes, prokaryotes, and archaea (1–4). The complex has a molecular mass of 700 kDa and is composed of 28 subunits ranging in molecular masses from 20 to 35 kDa (2, 5). The X-ray crystallographic analysis of the 20 S proteasome from the archaeabacterium Thermoplasma acidophilum was recently reported (6). In eukaryotes, proteasome activities are modulated by specific regulatory proteins that form complexes with proteasomes (7–10). Two regulatory complexes, the ATPase complex (11, 12) and PA28 (9, 10), have been studied to some extent.

The ATPase complexes associate with 20 S proteasomes in an ATP-dependent manner, resulting in the 26 S proteasomes (5, 7, 11, 13). This 26 S proteasome is involved in the degradation of protein substrates in a ubiquitin-dependent manner (2, 6). The structure of PA28 complexes isolated from cells, indicating that both are constituents of functional PA28 complexes. Using antisera specific to PA28α, PA28β, and epitope-tagged PA28 molecules, we show that expression of PA28α and PA28β is coordinately induced by various cytokines in different cell lines and that PA28 subunits and proteasomes have almost identical half-lives. In addition, we show that PA28 complexes are associated with 20 S but not 26 S proteasomes in vivo. Moreover, we demonstrate that PA28 complex is a heterohexamer composed of both α and β subunits with a stoichiometry of αβα in an alternating order.

**EXPERIMENTAL PROCEDURES**

**DNA Constructs and Transfection—**The cDNAs encoding for PA28α and PA28β were isolated from an Hela cell cDNA library by polymerase chain reactions using specific primers. PA28α cDNA was used as a template in a polymerase chain reaction to generate a recombinant cDNA, PA28α-Flag, coding for a complete PA28α protein with an additional DYKDDDDK (Flag epitope) (20) sequence at the COOH terminus. Similarly, PA28β-DRα encoding the complete protein of PA28β with an additional cytoplasmic sequence derived from HLA-DRα, DKVGRKSNAAERRGPL, was constructed. All of the above recombinant cDNAs were sequenced and cloned into the expression vector pPD10–1 (5, 21). Transfections were carried out using the calcium–phosphate method as described previously (22).

**Cell Culture, Antibodies, and Cytokines—**Hela (ATCC CCL185) and Jurkat (ATCC TIB152) cells were grown in Dulbecco’s modified Eagle’s medium (Life Sciences) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Raji (ATCC CCL86) and T2 (ATCC CRL-1992) cells were cultured in RPMI 1640 medium. Human interferon γ (IFN-γ) (and tumor necrosis factor α were obtained from Boehringer Mannheim, and human interferon α (IFN-α) was purchased from Genzyme (Cambridge, MA). Anti-PA28α and anti-PA28α antisera were raised by immunizing rabbits with peptides corresponding to the carboxyl-terminal 21 amino acid residues of PA28α and PA28β, respectively. Anti-DRα and anti-proteasome antisera have been described (23, 24). Anti-Flag monoclonal antibody was purchased from Kodak. Antibodies were titrated and saturating amount of antibodies were used in all experiments.

**Metabolic Labeling, Immunoprecipitation, and Immunoblotting—**Metabolic labeling of cells was carried out as described (25). When necessary, cells were induced with cytokines for the indicated time. Cells were then labeled and subsequently chased in the presence of culture medium for the times indicated in individual experiments. Cells were solubilized either in 1% Nonidet P-40 (Nonidet P-40) in phosphate-buffered saline or in RIPA buffer (50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate) with a mixture of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, and 1 μM of each aprotinin, leupeptin, and pepstatin A). Immunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), nonequilibrium pH gradient gel electrophoresis (using Ampholine pH 3.5–10),
and fluorography were performed as described (25). For immunoblotting analysis, proteins in each cell fraction were separated by SDS-PAGE, transferred to an Immobilon-P membrane, and then probed with anti-PA28\(^\alpha\) antisemur. The bound antibodies were detected by horseradish peroxidase-coupled anti-rabbit immunoglobulin (Promega).

Subcellular Fractionation, Gel Filtration, and Fast Protein Liquid Chromatography—HeLa cells were metabolically labeled for 30 min followed by a 2-h chase and homogenized as described (25). The homogenates were first spun for 30 min at 13,000 rpm to collect the nuclei. Cytosolic and crude microsomal membrane fractions were further separated by centrifugation at 100,000 \(\times g\) for 30 min. PA28 and proteasomal complexes were immunoprecipitated from each fraction and analyzed by SDS-PAGE. For gel filtration and fast protein liquid chromatography, HeLa cells were lysed in phosphate-buffered saline containing 1% digitonin. The resulting lysates of 0.1 ml were fractionated by using a gel filtration column (Superose 6, Pharmacia) and 0.25 ml/min/fraction was collected. Each fraction was divided equally into two aliquots, immunoprecipitated with appropriate antibodies, and analyzed by SDS-PAGE.

RESULTS

PA28\(^\alpha\) and PA28\(^\beta\) Are Coordinately Up-regulated by Different Cytokines—In the context of identifying IFN-induced genes by subtractive hybridization and differential display, we observed that both mRNAs for PA28\(^\alpha\) and PA28\(^\beta\) were up-regulated by IFN (data not shown). This finding is in agreement with the report that both mRNAs of PA28 are IFN-\(\gamma\)-inducible (19). However, it was reported that the protein level of PA28\(^\alpha\) was increased by IFN-\(\gamma\) but PA28\(^\beta\) was not (26), which raised the question whether PA28 complexes occur in several structural forms. To compare the induction levels of the two polypeptides we raised PA28\(^\alpha\)- and PA28\(^\beta\)-specific antibodies and immunoprecipitated PA28 from IFN-\(\gamma\)-induced and non-induced HeLa cells (Fig. 1). In non-induced cells, two protein spots were observed by two-dimensional gel electrophoresis after immunoprecipitation with anti-PA28\(^\alpha\) (not shown) or anti-PA28\(^\beta\) (Fig. 1A, right panel) antisera. These two proteins were not present in 20 S proteasomes (Fig. 1A, left panel) even after IFN treatment (not shown). By two-dimensional gel electrophoresis and immunoblotting using specific antisera to PA28, PA28\(^\alpha\), or PA28\(^\beta\) we identified the upper protein spot as PA28\(^\beta\) and the lower one as PA28\(^\alpha\) (not shown) which is in agreement with the predicted molecular masses and isoelectric points (19). Since the anti-PA28\(^\beta\) antisemur does not recognize PA28\(^\alpha\) in immunobots (see below), we conclude that the anti-PA28\(^\beta\) antisemur immunoprecipitated a complex of both polypeptides. Upon IFN-\(\gamma\)-induction, both polypeptides were induced to a similar extent (Fig. 1B), consistent with Northern blot analysis (19). Phosphorimaging analysis indicated that the rates of synthesis of PA28\(^\alpha\) and PA28\(^\beta\) are increased ~10 times following exposure to IFN-\(\gamma\) for 24 h (Fig. 1B, lane 3).

Interestingly, the level of IFN-\(\gamma\) induction for the major histocompatibility complex-encoded proteasomal subunits LMP2 and LMP7 (Fig. 1B, lane 6) was almost identical to those of PA28, indicating similar regulation. This assumption was further supported when we compared the induction of LMPs and PA28s in different cell lines and with different cytokines (Fig. 2). We treated HeLa cells, the B-cell line Raji, and the T cell line Jurkat with IFN-\(\alpha\), IFN-\(\gamma\), or tumor necrosis factor \(\alpha\) for 24 h prior to labeling and immunoprecipitation with anti-proteasome (Fig. 2A) or anti-PA28\(^\beta\) (Fig. 2B) antisera. In all cell lines tested, a 6–10-fold induction of both LMPs and PA28s was observed after incubation with IFN-\(\alpha\) or IFN-\(\gamma\) but not with tumor necrosis factor \(\alpha\). Furthermore, IFN-\(\alpha\) exerted a stronger effect than IFN-\(\gamma\) on the induction of both PA28s and LMPs in Raji cells but not in Jurkat or HeLa cells again indicating a similar regulation. Most importantly, both PA28\(^\alpha\) and PA28\(^\beta\) were always found to be induced to a similar extent in all cell lines and with all cytokines tested suggesting that the synthesis and assembly of both subunits is tightly co-regulated.

**Fig. 1.** A, two-dimensional gel electrophoresis analysis of PA28 molecules. PA28 molecules and proteasomes were immunoprecipitated from cell lysates prepared from HeLa cells after labeling for 30 min followed by a 2-h chase with anti-PA28\(^\beta\) (right panel) or anti-proteasome (left panel) antisera, respectively. The immunoprecipitates were resolved by two-dimensional gel electrophoresis according to charge and molecular mass. The identities of PA28\(^\alpha\) and PA28\(^\beta\) were confirmed by two-dimensional gel electrophoresis and immunoblotting with specific anti-PA28\(^\alpha\) or anti-PA28\(^\beta\) antisera (not shown). LMPs and PA28 proteins are indicated with symbols: square, LMP2 (pl = 4.4); circle, LMP7 (pl = 7.5); open triangle, PA28\(^\alpha\); solid triangle, PA28\(^\beta\). The corresponding positions for PA28 proteins which are absent in anti-proteasome immunoprecipitates are also shown. The observed values for isoelectric points (pl) and molecular masses of PA28\(^\alpha\) (pl = 5.8; molecular mass = 27.6 kDa) and PA28\(^\beta\) (pl = 5.3; molecular mass = 28.2 kDa) are in good agreement with predicted pl and molecular mass values of PA28\(^\alpha\) (pl = 5.7; molecular mass = 28.7 kDa) and PA28\(^\beta\) (pl = 5.3; molecular mass = 27.1 kDa), respectively. B, PA28\(^\alpha\) and \(\beta\) are up-regulated by IFN-\(\gamma\). PA28 molecules and proteasomes from HeLa cells, untreated (0 h), or IFN-\(\gamma\)-treated (1 or 24 h) before labeling for 30 min and chasing for 2 h, were immunoprecipitated with antisera against proteasomes (right) or PA28\(^\beta\) (left) and subjected to PAGE. The arrows indicate proteasomal subunits LMP2 and 7 whose intensities were also increased to similar levels of PA28s after treatment with IFN-\(\gamma\).

PA28\(^\alpha\) and PA28\(^\beta\) Have a Turnover Rate Similar to the Proteasome—The above findings prompted us to investigate whether the degradation rate of both PA28 polypeptides would be similar. HeLa cells were grown with or without IFN-\(\gamma\) for 24 h, followed by pulse labeling and chased for various times prior to immunoprecipitations. Similar to the proteasome (left panel), both PA28\(^\alpha\) and PA28\(^\beta\) (right panel) were degraded with an apparent half-life of ~33 h as calculated after scanning the fluorogram (Fig. 3A). In the presence of IFN-\(\gamma\) the half-lives of both complexes was ~40 h (Fig. 3B). Thus, the PA28 complex is long lived similar to the proteasome and both polypeptides of PA28 are coordinately degraded.

Subcellular Localization of PA28s—Proteasomes have been detected in various subcellular compartments including the cytoplasm, nucleus, and microsomes (3, 5). To determine whether PA28s display a subcellular distribution pattern similar to proteasomes we performed immunofluorescence microscopy of HeLa cells. As described for proteasomes (27), both PA28\(^\alpha\) and PA28\(^\beta\) were present in both the nucleus and the cytoplasm (not shown). To further examine the intracellular distributions of PA28s, metabolically labeled HeLa cell homogenates were fractionated by differential centrifugation fol-
followed by immunoprecipitation with anti-PA28α (not shown), anti-PA28β, or anti-proteasome antisera (Fig. 4A). Similar to proteasomes, PA28αs were predominantly present in the cytosolic fraction with a significant portion in the nuclear and microsomal fractions. To study the steady state protein distribution of PA28αs we analyzed the same fractions by immunoblotting with anti-PA28α (not shown) or anti-PA28β (Fig. 4B) antisera. Again, a significant amount of PA28α was present in both nuclear and microsomal fraction. In contrast to the analysis by immunoprecipitation, only PA28β was recognized by anti-PA28β antisera in immunoblot indicating that this antisera does not cross-react with PA28α. Scanning of the band intensity revealed that the relative amounts of PA28αs in nucleus, cytoplasm, and microsomes are approximately 1:10:2, respectively. Moreover, the fact that both subunits always occurred at the same ratio in each subcellular fraction suggested that they are in a fixed ratio within the PA28 complex. Taken together, these data clearly show that PA28β is physically associated with PA28α. In comparison to the proteasome, the relative amounts of PA28 complexes are similar in the different subcellular fractions (5). Thus, the subcellular distributions of PA28 complexes and proteasomes are similar.

PA28 Complex Is a Hetero-hexamer with an αββα Stoichiometry—To gain insight into assembly and structure of the PA28 complex, we have constructed epitope-tagged PA28α-Flag and PA28β-Drα, which can be specifically monitored using Flag or Drα-specific monoclonal antibodies. These constructs were transiently transfected into HeLa cells and their association with endogenous PA28 subunits was monitored by immunoprecipitation. As shown in Fig. 5, metabolically labeled HeLa cells, mock transfected (lanes 1–4), PA28α-Flag-transfected (lanes 5–8), or PA28β-Drα-transfected (lanes 9–12) were either lysed with 1% Nonidet P-40, which leaves the PA28 complex intact (lanes 1, 5, and 9) or in RIPA buffer (lanes 2, 6, and 10), which disrupts the complex, since only PA28β was immunoprecipitated by anti-PA28β antisera under these conditions (lane 2).

Similarly, anti-Flag, as well as anti-Drα antibodies, immunoprecipitated only their respective antigen in RIPA-buffer (lanes 6 and 10, for control, see lanes 3 and 4) which shows an increase in molecular mass due to the epitope tag. Overexpression of the transfected PA28 subunits was evident, since labeling intensities of the transfected subunits were greater than those of the endogenous subunits (e.g. compare lane 2 with 10) despite the fact that only approximately 15% of the cells were transfected in these transient expression experiments (not shown). Furthermore, both antibodies immunoprecipitated only one additional protein (lanes 7 and 11), which had a molecular mass similar to the PA28β or PA28α subunit, in Nonidet P-40 lysates prepared from PA28α-Flag or PA28β-Drα-transfected cells, respectively. The identity of these coprecipitated proteins as PA28β or PA28α was confirmed by reprecipitations using anti-PA28β or anti-PA28α antisera (lanes 8 and 12, respectively). Thus, the transfected epitope-tagged PA28 subunits associated exclusively with their homologous subunit endogenously synthesized in HeLa cells, but not with the corresponding wild-type subunit. The most likely explanation for this observation is that the transfected subunits displaced the corresponding endogenous wild-type subunit from the complex during assembly due to overexpression. Moreover, a slight reduction of the endogenous PA28α subunit is observed from newly assembled complexes upon transient transfection with the PA28α-Flag subunit (compare lane 5 with lane 1). This level of reduction correlated well with the expression level of the transfected epitope-tagged subunit depending on transfection efficiency. Similar changes were also observed for transfected PA28β subunits in several experiments (not shown). Taken together with
performed by using anti-PA28β antibody. The finding that PA28 complex consists of alternating α and β subunits. Therefore, we interpret these data as evidence that the PA28 complex forms an alternating hexamer or a heptamer (9, 10, 18). Our findings that PA28 complex appears in two major peaks (upper panel) or anti-PA28β (lower panel) antisera. The fraction peaks for PA28 regulators associated with 20 5 proteasomes and free PA28 regulators were 52 and 64, respectively. Only odd numbered fractions were shown in these representative fluorograms. The exposure time for proteasomes or PA28 regulators immunoprecipitates were 6 and 24 h, respectively. Arrow at bottom indicates that the fraction (fraction number 64) containing free PA28 regulators corresponds to an average molecular mass of 170 kDa estimated from protein markers (Low and High Molecular Weight Gel Filtration Calibration Kits, Pharmacia). The protein markers are ribonuclease A, 13.7; chymotrypsinogen A, 25.0; ovalbumin, 43.0; albumin, 67.0; aldolase, 158.0; catalase, 232.0; ferritin, 440.0; thyroglobulin, 669.0; and blue dextran 2000 kDa.

The finding that PA28α complexes with PA28β, these data excluded the possibility that the PA28 complex might exist as homo-oligomers. A similar observation from LMP transfection experiments (23) has led us to the conclusion that both LMP subunits assembled into defined slots in the proteasomal particle. Therefore, we interpret these data as evidence that the PA28 complex consists of alternating PA28α and PA28β subunits.

Since the PA28 complex has a ring-like structure of 6- or 7-fold symmetry as demonstrated by electron microscopy (18), our data that PA28 complex forms an alternating PA28α and PA28β structure would predict that both subunits appear in equimolar amounts in the PA28 complex. We tested this assumption by densitometric quantitation of the fluorograms. The amounts of PA28α and PA28β in either anti-PA28α (not shown) or anti-PA28β (e.g., Figs. 3 and 6) immunoprecipitates were quantitated and their stoichiometric ratio was calculated under the consideration that PA28α and PA28β contain 6 and 5 methionine residues, respectively. As summarized in Table I, the observed stoichiometric ratio of PA28α to PA28β was 1.04:1, indeed indicating that PA28 complex contains α and β subunits in an equal molar ratio. Furthermore, in PA28α-Flag transfection experiments the amount of PA28α protein immunoprecipitated with PA28β was reduced, but the amount of PA28β-associated PA28α-Flag compensated for the decreased amount of PA28α (e.g., lane 5 in Fig. 5). Thus, the quantitation of the PA28 bands obtained from epitope-tagged PA28 transfection experiments (e.g., Fig. 5) showed that the overall ratio between PA28α subunit and PA28β subunit remained unchanged (Table I).

Previous results suggested that the PA28 complex is either a hexamer or a heptamer (9, 10, 18). Our findings that PA28α and PA28β subunits incorporate into PA28 complexes in equimolar amounts and in alternating order suggested that the most likely structure for PA28 complex would be a hexamer. To obtain independent proof for this assumption we fractionated HeLa cell lysates by gel filtration using a Superose 6 column under high resolution fractionation conditions (see "Experimental Procedures"). Each fraction was equally divided into two aliquots and immunoprecipitated with either anti-proteasome or anti-PA28β antisera (Fig. 6). The PA28 complexes appeared in two major peaks (lower panel). The fraction peak (fraction number 64) containing the PA28 complex corresponded to a molecular mass of 170 kDa. Since the predicted molecular mass for a heptameric PA28 is too large to reconcile with the finding that PA28 complexes with PA28β.
Structure and Regulation of a Proteasome Regulator PA28

The stoichiometric ratio of the PA28α:PA28β complex was determined by densitometric scans of fluorograms (e.g., Figs. 3 and 6). The ratio reported here equals the amount of PA28β × 5 divided by the amount of PA28α × 6, since PA28α and PA28β polypeptides contain 6 and 5 methionine residues, respectively. The value is the mean ± S.D. of seven independent experiments.

Table I

| Predicted ratio (PA28α:PA28β) | Observed ratio |
|-------------------------------|---------------|
| 1.0                           | 1.33          |
| 1.35                          | 0.75          |
| 1.04 ± 0.04                   | 0.99 ± 0.05   |

The stoichiometric ratio of the PA28α:PA28β complex was determined by densitometric quantitation using antisera which specifically recognize PA28α or PA28β and coprecipitate PA28α and PA28β, respectively. The value is the mean ± S.D. of three independent experiments.

The previous observations that PA28 consists of two distinct but homologous polypeptides, designated as PA28α and PA28β (9, 16), raised the question whether these two polypeptides form homo- or hetero-oligomers. We have addressed this question by studying the assembly of PA28 in vivo using antisera which specitically recognize PA28α or PA28β and coprecipitate PA28α and PA28β, respectively. When the induction and turnover of the PA28 complex was examined in different cell lines and different cytokines, we observed that both subunits were coordinately induced. Similarly, they were degraded simultaneously. In addition, in immunoprecipitation experiments following subcellular fractionations or gel filtration fractionations both subunits were consistently coprecipitated at a constant molar ratio. We conclude that PA28α subunit physically associates with PA28β subunit and no homo-oligomers are formed. Moreover, the observation that the fraction of transfected PA28 subunits which did associate with other subunits always associated with the other homologous PA28 subunits, e.g., transfected PA28β subunit always associates with endogenous PA28α subunit strongly suggests that the two subunits alternate in the complex. Thus the PA28 complex most likely has an even-numbered subunit stoichiometry, which was indeed confirmed by the data obtained from densitometric scanning of our immunoprecipitates. Taken together with the finding that the complex has a molecular mass of 170 kDa, these data suggest that within the cell the native PA28 complex exists in only one structure which is hexameric and contains both α and β subunits in an alternating order.

Electron microscopic studies revealed that the two regulatory complexes of the proteasome, ATPase complexes and PA28 regulators, bind to one or both ends of the proteasome (18, 28). This observation raises the question whether in vivo PA28 regulator and ATPase complex compete for the same sites at the 20 S proteasome. In vitro the ATPase complex has a higher affinity to the 20 S proteasome than the PA28 regulator, but in vivo PA28 regulators bind to free 20 S proteasomes without interfering with the binding of ATPase complexes with 20 S proteasomes (5). Although asymmetric proteasomes, capped with ATPase complexes on one end and PA28 regulators on the other, may exist, this complex was not detected in our gel filtration experiments (e.g. in Fig. 6). The observation that cleavage of the carboxyl-terminal portion of PA28α subunit abolishes binding of PA28 regulator to the proteasome (29) would suggest that the carboxyl-terminal portion of PA28α subunit may contain the proteasome recognition site, whereas the potential conformational changes of the proteasome may be mediated by the PA28β subunit (see below). It remains to be determined which α-type subunits of the proteasome are involved in this binding.

A number of observations including the crystal structure of the archaebacterial proteasome (6) have indicated that the proteasome has a 7-fold symmetry. How such a 7-fold symmetric structure interacts with the hexameric PA28 regulator poses an interesting topological problem. To our knowledge the binding of a hexameric structure to a heptameric structure is unprecedented. For instance, the bacterial chaperonin GroEL, which promotes protein folding and has a strikingly similar architecture to the proteasome, binds at its ends to the cofactor GroES which is also a heptamer (30, 31). However, the proteasome in mammalian cells is not strictly symmetric, since each ring consists of seven different but related subunits (2). Thus, the symmetric PA28 hexamer de facto interacts with an asymmetric surface of the proteasome. Similarly, the GroES heptamer has been shown to be conformationally plastic and the GroEL binding determinant of GroES is disordered in the crystal in its six subunits (31). These differences may reflect the unique functional requirements of the distinct processes these particles carry out, the hepta- or hexamer structures of GroES and PA28 regulator might be reflective of the fundamental size difference between GroEL and proteasome.

It remains to be shown how PA28 regulator is involved in the proteasome-mediated protein degradation. One likely mechanism that might contribute to rendering the PA28 regulators dedicated to optimizing the proteasomal system is that upon PA28 binding to 20 S proteasomes PA28 regulators affect the catalytic properties of the proteasomes. The finding that in vitro PA28 can accelerate the rate of hydrolysis of peptides by proteasomes would be consistent with this view. Since the PA28 regulator does not seem to be a protease, it is plausible that conformational changes of proteasomes resulting from binding of PA28 regulator would allow the proteolytic enzyme to be more active. Alternatively, conformational changes of proteasomes could temporarily create a bigger orifice in the top of the proteasome and allow degradation products to be released out of the inner chamber of the proteasomes. Thus, PA28 regulators may accelerate proteolysis by clearing the proteasome of prodelytic debris.

Acknowledgments—We thank C. Glass, P. Sempe, and E. Tisdale for critical reading of the manuscript, A. Huvar for technical assistance, and G. N. DeMartino for sharing unpublished data. The technical assistance of the vivarium, protein, and DNA facilities of the R. W. Johnson Pharmaceutical Research Institute is gratefully acknowledged.

REFERENCES
1. Mykles, D. L. (1989) Arch. Biochem. Biophys. 274, 216–228
2. Rohnstein, M., Hoffman, L., and Dubiel, W. (1993) J. Biol. Chem. 268, 6065–6068
3. Rivett, A. (1993) Biochem. J. 291, 1–10
4. Orlofski, M. (1990) Biochemistry 29, 10289–10297
5. Yang, Y., Früh, K., Ahn, K., and Peterson, P. A. (1995) J. Biol. Chem. 270, 27687–27694
