Role of Extracellular Molecular Chaperones in the Folding of Oxidized Proteins

REFOLDING OF COLLOIDAL THYROGLOBULIN BY PROTEIN DISULFIDE ISOMERASE AND IMMUNOGLOBULIN HEAVY CHAIN-BINDING PROTEIN *

Frédéric Delom, Bernard Mallet‡, Pierre Carayon, and Pierre-Jean Lejeune
From the Unité 555 INSERM and Laboratoire de Biochimie Endocrinienne et Métabolique, Faculté de Médecine, Université de la Méditerranée, 13385 Marseille Cedex 5, France

The process of thyroid hormone synthesis, which occurs in the lumen of the thyroid follicles, results from an oxidative reaction leading, as side effects, to the multimerization of thyroglobulin (Tg), the prothyroid hormone. Although hormone synthesis is a continuous process, the amount of Tg multimers is relatively constant. Here, we investigated the role of two molecular chaperones, protein disulfide isomerase (PDI) and immunoglobulin heavy chain-binding protein (BiP), present in the follicular lumen, on the multimerization process due to oxidation using both native Tg and its N-terminal domain (NTD). In vitro, PDI decreased multimerization of Tg and even suppressed the formation of NTD multimers. Under the same conditions, BiP was able to bind to Tg and NTD multimers but did not affect the process of multimerization. Associating BiP with PDI did not enhance the ability of PDI to limit the formation of multimers produced by oxidation. However, when BiP and PDI were reacted together with the multimeric forms and for a longer time (48 h), BiP greatly increased the efficiency of PDI. Accordingly, these two molecular chaperones probably act sequentially on the reduction of the intermolecular disulfide bridges. In the thyroid, a similar process may also be effective and participate in limiting the amount of Tg multimers present in the colloid. These results suggest that extracellular molecular chaperones play a similar role to that occurring in the endoplasmic reticulum and, furthermore, take part in the control of multimerization and aggregation of proteins formed by oxidation.

In the thyroid gland, the functional unit is the follicle, which is composed of a monolayer of epithelial cells (the thyrocytes) delimiting a closed space, the follicular lumen, which is filled with colloid. Thyrocytes are polarized cells that synthesize the prothyroid hormone: the thyroglobulin (Tg). This protein, which is the basic component involved in thyroid hormone synthesis, is secreted into the follicular lumen. Like most newly synthesized proteins, Tg follows the usual pathway of chaperone-assisted folding in the endoplasmic reticulum (ER) involving calnexin, calreticulin, Grp78 (BiP), Grp94, PDI, and Grp170 (1–5). Among these molecular chaperones, PDI certainly plays a fundamental role due to the presence of ~110 cysteine residues in the monomer Tg (330 kDa, 12 S) (6). In normal conditions, properly folded Tg is dimerized (2 × 330 kDa, 19 S) without any interchain disulfide bonds, then mature Tg is secreted into the follicular lumen where Tg19S undergoes the major post-translational modification accompanying the production of thyroid hormones. Hormone synthesis takes place at the apical surface of the thyrocyte via the iodination of some tyrosine residues (among 132 tyrosine residues for Tg19S) and the subsequent coupling of a very limited number of iodotyrosine residues to form triiodothyronine (T3) and thyroxine (T4). The iodination and coupling process depend on a H2O2-generating system (NADPH-oxidase) and thyroperoxidase. During this oxidative reaction, besides participating in hormone synthesis, Tg19S is also modified by the formation of interchain covalent cross-links, generating high molecular mass forms of Tg: the soluble forms, known as Tg27S and Tg37S (4 × 330 kDa and 8 × 330 kDa, respectively), and the Tg aggregates, which constitute about 30% of the total Tg in the follicular lumen (7). Tg aggregates differ from soluble Tg multimers in that they contain about 40% more iodine but almost no hormone (8). In addition, Tg aggregates are formed by several types of covalent cross-links: disulfide, dityrosine, and γ-glutamyl-lysine bridges (7–10). The process of di-, tri-, and multimerization of Tg19S is also observed in vitro as the result of iodination and coupling. The major difficulty with Tg is its large size, which precludes detailed analysis in terms of the structure-activity relationships. Therefore, to circumvent this problem, we have often used a Tg peptide to study the various process in which the entire molecule is involved. This peptide is obtained from the entire Tg molecule by CNBr treatment. It corresponds to the N-terminal domain (NTD) of the molecule, which contains the main site of hormone synthesis at the Tyr5 (11). The NTD is a dipeptide Asn1-Met127 that is linked via disulfide bridges to Glu128-Met171. It contains two sites of N-glycosylation at Asn57 and Asn91. Using this model, we have established by performing in vitro iodination and coupling that N-glycosylation was essential to both hormone formation (12) and multimerization in which covalent cross-links are involved (13).

In a recent study, we reported that in the follicular lumen BiP, Grp94, and PDI are associated with Tg aggregates. Although these molecular chaperones carry a retention signal KDEL in the ER, several studies establish that in secretory...
cells, some of the molecular chaperones escape to the ER (14–19). In the present study, we investigated the role of PDI and BiP in the process of Tg19S and NTD multimerization during in vitro iodination and coupling. PDI was found to limit the multimerization process more efficiently than BiP. The conjugate action of these two molecular chaperones did not modify the rate of Tg and NTD multimers formed by the oxidative iodination and coupling reaction. On the other hand, when the multimeric forms of Tg were incubated with PDI and BiP for 48 h, the refolding of the multimeric forms of Tg occurred.

**EXPERIMENTAL PROCEDURES**

**Preparation of the Various Forms of Human Tg**—A thyroid gland was obtained from a patient with a colloid goiter who underwent thyroidectomy. After the pathological examination, the gland was frozen in liquid nitrogen and taken to the laboratory where it was immediately lyophilized. An aliquot of the lyophilized gland was fragmented and suspended in 0.1 M phosphate buffer, pH 7.2, for 15 min at +4 °C. After filtration on gauze, the supernatant of the thyroid homogenate was salted out (1.8 M phosphate buffer). After being centrifuged, the precipitate was dissolved with distilled water, dialyzed against 50 mM phosphate buffer, pH 7.2, and chromatographed on a Bio-Gel A-5m (Bio-Rad) column (100 × 5 cm) equilibrated with the same buffer. Briefly, the typical elution profile showed in the void volume of the column, a bulky fraction constituted of Tg aggregates followed by two minor peaks corresponding to Tg37S and Tg27S in that order (fraction “Tg37S+Tg27S”), and a major peak identified as Tg19S (20). Elution fractions corresponding to Tg37S+Tg27S and Tg19S were pooled separately, dialyzed against distilled water, and characterized in terms of their amino acid composition and electrophoretic profile.

**In Vitro Iodination and Coupling of Tg19S**—In vitro iodination and coupling (oxidative system) of Tg19S was carried out as previously described (12) to obtain iodinated and coupled Tg19S. Briefly, 1 mg of glucose, 20 mM of KI, and 5 µg of lactoperoxidase (Sigma) were added to a final volume of 1 ml of 50 mM Tris-HCl buffer, pH 7.2, containing 1 mM of Tg19S. The reaction was initiated by 2.5 µg of glucose oxidase (Sigma), continued for 30 min at 37 °C, and stopped by adding 0.1 M hyposulfite. The same reaction was performed with 4 mM reduced glutathione (Roche Molecular Biochemicals) and 0.5 mM oxidized glutathione, 100 mM CaCl₂, 1 mM ATP, and 1 µl of protease inhibitor mixture (Sigma). Renaturation was initiated by adding iodinated PDI (0.8 µg) and iodinated BiP (0.6 µg), and the sample was incubated at 37 °C. At times corresponding to 1, 12, 24, and 48 h, an aliquot (2 µg) was taken and stored at ~20 °C for immunoblotting analysis. The experiment was repeated with native PDI and BiP (Stress-Gen).

**Results**

**Preparation of the NTD of Tg19S—**Tg19S was treated with cytochrome c. The CNBr peptides were separated by chromatography on a Sephadex G-200 column (Amersham Pharmacia Biotech) in 1 M propionic acid, and the NTD was then purified on a Bio-Gel P-100 column in 0.05 M ammonium bicarbonate as previously described (11). Last, it was dialyzed against distilled water and lyophilized.

**In Vitro Iodination and Coupling of the NTD—**NTD (1 nmol), dissolved in 50 µl of 50 mM Tris-HCl buffer, pH 7.2, was incubated with 7 nmoles of KI and 5 µg of lactoperoxidase, a H₂O₂-generating system consisting of glucose (0.2 mg) and glucose oxidase (0.5 µg). After 30 min of incubation at 37 °C, the reaction was stopped by adding 0.1 M sodium hyposulfite. The same reaction was performed with 4 mM reduced glutathione and 0.5 mM oxidized glutathione in the presence or absence of PDI (Stress-Gen), Victoria, Canada) and/or 100 µM CaCl₂ and 1 mM ATP (Aldrich) in the presence or absence of PDI (Stress-Gen). When PDI and/or BiP were added to the sample, the iodide concentration was increased to 20%, since PDI content was too low to allow efficient iodination and coupling of Tg19S. The reaction was terminated by adding lactoperoxidase (0.8 µg) and lactoperoxidase (0.6 µg), and the sample was incubated at 37 °C. At times corresponding to 1, 12, 24, and 48 h, an aliquot (2 µg) was taken and stored at ~20 °C for immunoblotting analysis. The experiment was repeated with native PDI and BiP (Stress-Gen).

**Other Procedures—**SDS-PAGE was performed under nonreducing conditions using a 5% or 12% acrylamide and a 0.1% SDS gel system. Protein bands were stained with Coomassie Brilliant Blue. Immunoanalysis was performed using a mouse monoclonal antibody directed against a specific region of human Tg (monoclonal antibody 2) (23). The second reagent was either gold-conjugate goat anti-mouse IgG (diluted 1/250), detected with a silver enhancing kit (British Biocell International), or horseradish peroxidase-conjugated goat anti-mouse IgG (diluted 1/250,000), detected with Super Signal West Femto maximum sensitivity substrate (Pierce). Scanning quantitative analysis was subsequently performed using the NIH Image V1.56 software program.

**Results**

**Effects of Iodination and Coupling of the Tg19S and NTD—**After performing in vitro iodination and coupling of Tg19S or NTD samples for 30 min, we noted upon carrying out SDS-PAGE under nonreducing conditions that Tg (Fig. 1A) and NTD (Fig. 1B) were multimerized. With NTD, quantification of the various multimerized forms showed that NTD dimer amounted to about 21% ± 8% (n = 8), and another band around 75 kDa corresponding to NTD trimer was also identified. The percentages of this latter fraction was variable and low (~3%). Tg multimer quantification was more difficult to perform, because the degree of multimerization was so variable that the fractions were distributed along the stacking gel, and only some of them reached the running gel. To evaluate the multimeric forms of Tg, it was therefore necessary to perform separation on a Bio-Gel A-5m column, which has a better resolution than SDS-PAGE (data not shown). Under these conditions, Tg37S+Tg27S amounted to around 25 ± 8% (n = 5). These values are comparable with those observed in vivo (24). When the incubation time was extended and when the iodide concentration was increased, similar results were obtained (data not shown). Since it was difficult to assess these values...
The role of PDI in the oxidation reaction induced by aggregated Tg (8), we studied the recognition that PDI can influence the refolding of denatured (Fig. 2B). These results specifically involved PDI and were not due to the redox system GSH/GSSG (200 \( \mu M \)). Lane 1, NTD iodinated and coupled without PDI; lanes 2–5 correspond to Tg19S iodinated and coupled (see “Experimental Procedures”) in the presence of PDI at PDI/Tg19S molar ratios of 0.2, 0.5, 1.0, and 2.0, respectively. Tg19S and Tg multimers are indicated. In A and B, immunoblots were probed with anti-Tg monoclonal antibody and detected by chemiluminescence.

When performing SDS-PAGE, in the forthcoming experiments we designated as Tg multimers and NTD multimers all the multimerized forms generated during iodination and coupling.

Role of PDI in the Multimerization Process—It is widely recognized that PDI can influence the refolding of denatured and/or reduced proteins in vitro. Because we previously established that PDI is associated with aggregated Tg (8), we studied the role of PDI in the oxidation reaction induced by in vitro iodination and coupling. The presence of PDI in various PDI/ligand ratios (mol/mol) limited the amount of Tg multimers (Fig. 2A) and NTD multimers (Fig. 2B). These results specifically involved PDI and were not due to the redox system GSH/GSSG, since in the absence of PDI, this system did not affect the proportions of the multimers (Fig. 2, A and B, lanes 1).

With NTD, no multimeric form was obtained with PDI at a molar ratio \( \geq 1 \) (Fig. 2B, lanes 4 and 5), whereas PDI had a slightly less effect on Tg, since at a PDI/Tg molar ratio = 2, around 20% of the Tg multimers were always present (Fig. 2A, lane 5). Although most of the cysteine residues are already involved in intradisulfide bridges, this difference was probably due to the number of cysteine residues present in the Tg19S molecule, which may explain why the efficiency of PDI was lower with Tg than with NTD (110 versus 6 Cys). Accordingly, we concluded that PDI was able to limit (Tg) or suppress (NTD) the multimerization process. However, it was not established whether PDI reacted with the multimeric forms, causing them to refold into monomeric forms, or whether it acted on the monomers, thus limiting or preventing the multimerization process.

Action of PDI Is Restricted to the Multimeric Forms—To determine the forms of Tg and NTD recognized by PDI, we used a PDI/ligand molar ratio equal to 0.5, which did not significantly reduce the formation of multimers with either Tg or NTD (see above). After 30 min of incubation for iodination and coupling, the mixture was chromatographed on an anti-PDI-Sepharose column. After extensively washing the column, the specific fractions were eluted by acidic solution (see “Experimental Procedures”). The acid fractions were analyzed by immunoblotting using a Tg monoclonal antibody. Only the multimeric forms of Tg and NTD were identified (Fig. 3). Regarding NTD, no band corresponding to the complex formed by PDI and NTD dimer (150 kDa) was detected; only two small bands corresponding to the molecular mass of the multimeric forms were observed (Fig. 3B, lane 3). This may have been due to the elution conditions at pH 3.0. In fact, at pH levels lower than 6.0, it has been reported that PDI, acting as a molecular chaperone, is dissociated from its ligand (25). On the other hand, the immunopurification was PDI-specific, since the Tg and NTD multimers chromatographed on this column at the same concentrations as those used above were not retained on the anti-PDI-Sepharose column (data not shown).

Interaction of BiP on the Multimerization Process—BiP is known to participate in vivo to the correct folding of Tg in the ER. The action of BiP involves multiple cycles of association and dissociation, requires Ca\(^{2+}\) and ATP, and interacts optimally with hydrophobic peptides, which are normally buried in the hydrophobic core of properly folded proteins (26–28). Moreover, BiP is not directly involved in disulfide bridge formation and, therefore, presumably does not participate in the multimerization process. To confirm this assumption, we performed in vitro iodination and coupling on Tg19S and NTD in the presence of BiP. With both Tg and NTD, BiP showed little, if any, ability to limit the multimerization process whatever the BiP/ligand (mol/mol) ratios tested (Fig. 4). However, this limited ability did not necessarily mean that BiP did not bind to multimeric forms. We reasoned that multimeric forms generated by oxidation reaction might express specific conformational hydrophobic sites recognized by BiP.

BiP Is Bound to the Multimeric Forms—To investigate the possibility that BiP might specifically recognize the multimeric forms, we incubated Tg19S or NTD in the presence of BiP (molar ratio BiP/ligand = 2) and in the presence of 100 \( \mu M \) Ca\(^{2+}\). After iodination and coupling for 30 min at 37 °C, the mixture was chromatographed on an anti-BiP-Sepharose column. After extensive washing of the column, the specific fractions were eluted with an acidic solution (see “Experimental Procedures”). The fractions were neutralized, concentrated, and analyzed by immunoblotting using a Tg monoclonal antibody (Fig. 5). With Tg and NTD, most of the specifically retained material was identified as consisting of multimeric forms, although a small proportion of monomeric forms was also retained.

Role of the Association PDI/BiP on the Oxidative Process of Multimerization and on the Refolding of Tg Multimers—Depending on the position of the N-linked glycans in the nascent protein in the ER, the folding of the glycoprotein is assisted either by BiP or by the calnexin/calreticulin pathway (29). With Tg,
PDI/BiP Mediate Colloidal Thyroglobulin Refolding

FIG. 4. Effects of BiP on the multimerization of Tg19S and NTD. A: lane 1, Tg19S (0.3 μg) was iodinated and coupled without BiP but in the presence of CaCl2 (100 μM) and ATP (1 mM); lanes 2–5, Tg19S iodinated and coupled (see “Experimental Procedures”) in the presence of BiP, corresponding to BiP/Tg19S molar ratios of 0.2, 0.5, 1.0, and 2.0, respectively. Tg19S and Tg multimers are indicated by arrows. B: lane 1, NTD (0.3 μg) was iodinated and coupled without BiP but in the presence of CaCl2 (100 μM) and ATP (1 mM); lanes 2–5, NTD iodinated and coupled (see “Experimental Procedures”) in the presence of BiP, corresponding to BiP/NTD molar ratios of 0.2, 0.5, 1.0, and 2.0, respectively. NTD and NTD multimer are indicated by arrows.

FIG. 5. Relative selectivity of BiP for the multimeric forms. After iodination and coupling of Tg19S (30 μg) or NTD (5 μg) in the presence of BiP (BiP/ligand molar ratio = 1) and Ca2+ (100 μM), the mixture was incubated for 30 min at 37 °C and then immunopurified on an anti-BiP-Sepharose column (volume = 1 ml). A, Tg19S. Lane 1, incubation medium before immunopurification; lane 2, fraction immunopurified. B, NTD. Lane 1, incubation medium before immunopurification; lane 2, fraction immunopurified. In A and B, immunoblots were probed with anti-Tg monoclonal antibody and detected with immunogold conjugate.

DISCUSSION

Under physiological conditions, the native Tg19S secreted into the follicular lumen undergoes the oxidative iodination and coupling reaction leading to hormone formation. This reaction results in a Tg with a high hormonal content but also in the formation of covalent cross-links, which take the form of either di-tyrosine bridges or disulfide bridges (intramolecular or intramolecular cross-links) (8). Soluble multimeric forms of Tg constitute the first step toward the insolubility and aggregation of Tg molecules (7). Several mechanisms may be involved in limiting these aggregates in the follicular lumen, such as that based on the presence of the lysosomal enzymes, cathepsins B, L (31), and K (32), at the surface of the thyrocyte as well as that based on the presence of oxygen free radicals generated by the system H2O2 + peroxidase (8). The results of all the previous studies have suggested that the limitation of the multimeric forms of Tg present into the follicular lumen may be attributable to their degradation. In the present study, it was proposed to test the hypothesis that the molecular chaperones present in the follicular lumen might contribute to both limiting the formation of the multimeric forms of Tg19S produced during the process of hormone synthesis and reducing the proportions of the soluble multimeric forms of Tg after their formation.

Numerous studies have described the role of PDI and/or BiP in the folding of proteins in vitro. However, to our knowledge,
molecular chaperones were used in these studies to rescue the unfolded proteins generated by reduction-denaturation (33, 34) or heating (35). Here we investigated the role of PDI and/or BiP using an original process to avoid the formation of unfolded protein from a folded protein (Tg19S) and the secondary misfolding, which occurs due to the oxidative process of hormone synthesis. For this purpose, we used the entire Tg molecule (Tg19S) and its NTD. In vitro the iodination and coupling reaction multimerized ~20% of all the Tg and NTD, although in the case of Tg, the rate of multimerization was more difficult to assess exactly. In vitro, PDI either limited (in the case of Tg) or prevented (in that of NTD) the formation of multimeric forms. In addition, when PDI was used at various concentrations relative to Tg or NTD, no anti-chaperone activity was evidenced. This finding differs from those of previous studies where the PDI, present at substoichiometric concentrations relative to denatured lysozyme, exhibited anti-chaperone activity (36). This difference is probably due to the fact that none of the substrates (Tg and NTD) used in the present experiments tends to aggregate spontaneously. It is worth noting that PDI acted specifically on the multimeric forms, since the immunopurification procedure performed using a polyclonal antibody directed against PDI yielded no Tg19S forms. The fact that Tg19S was not bound to PDI during the immunopurification step was not in disagreement with Mezghrani et al. (19), since under the conditions used here (subneutral pH buffer) we ruled out the possibility that Tg19S may have bound to PDI via a “receptor-binding domain” expressed only under acidic conditions.

At this stage, it was possible to assume that PDI in the follicular lumen contributes in limiting the formation of the multimeric forms of Tg, which would otherwise be generated by in vitro iodination and coupling. This reaction probably also resulted in the iodination of the PDI present in the incubation medium, and this effect is certainly very similar to what occurs in vivo. In the colloid, the molecular chaperones may also be iodinated as the result of the process of hormone synthesis, like several other colloidal proteins (37). On the other hand, to considerably reduce the formation of the multimeric forms of Tg, we used an amount of PDI corresponding to ~1/5 of the Tg in weight. This amount of PDI may seem rather low for an in vitro study, but it is very high given the physiological concentrations of PDI that are to be found in the follicular lumen (38). The chaperone/ligand molar ratios were low (never above 10). This difference was not due to the iodination of the molecular chaperone but partly to the fact that the chaperone/ligand molar ratios were low (never above 2) in our study and partly to the incubation time being restricted to 30 min. When directly applied to the multimeric forms for a longer time (48 h), BiP actually greatly increases the efficiency of PDI, since Tg37S+Tg27S were almost completely transformed in Tg19S. This result confirmed both that BiP was bound to multimeric forms of Tg and that BiP acted synergistically with PDI to restore the initial structure of the Tg19S. The difference in the efficiency of the association PDI/BiP observed between the inhibition of the formation and the refolding of the multimeric forms was probably due to the kinetic competition occurring between the disulfide bridge formation resulting from the oxidation of Tg19S, reduction of the disulfide bridges by PDI, and the speed with which BiP bound to the multimeric forms. Once formed, most of the disulfide bridges become rapidly buried deeply in the core of the multimeric Tg molecules, which explains why PDI alone had little effect, even after 48 h. On the other hand, Tg multimers may expose specific conformational peptides recognized by BiP (41), but it is very high given the physiological concentrations and for critical reading of the manuscript and to L. Vinet and R. Galibert for their excellent technical assistance.

Acknowledgments—Special thanks to J. L. Franc for helpful discussions and for critical reading of the manuscript and to L. Vinet and R. Galibert for their excellent technical assistance.

REFERENCES

1. Kim, P. S., Bole, D., and Arvan, P. (1982) J. Cell Biol. 118, 541–549
2. Kuznetsov, G., Chen, L. B., and Nigran, S. K. (1994) J. Biol. Chem. 269, 22900–22904
3. Kim, P. S., and Arvan, P. (1995) J. Cell Biol. 128, 29–38
4. Muresan, Z., and Arvan, P. (1997) J. Biol. Chem. 272, 26095–26102
5. Kuznetsov, G., Chen, L. B., and Nigran, S. K. (1997) J. Biol. Chem. 272, 3057–3063
6. Dunn, J. T., and Dunn, A. D. (2000) in Werner and Ingbar’s The Thyroid (Braverman, L. E., and Utiger, R. D., eds) 8th Ed., pp. 91–104, Lippincott Williams and Wilkins, Philadelphia
7. Herzog, V., Berndorfer, U., and Saber, Y. (1992) J. Cell Biol. 118, 1071–1083
8. Delom, F., Lejeune, P. J., Vinet, L., Carayon, P., and Mallet, B. (1999) Biochem. Biophys. Res. Commun. 255, 438–443
9. Baudry, N., Lejeune, P. J., Delom, F., Vinet, L., Carayon, P., and Mallet, B. (1998) Biochem. Biophys. Res. Commun. 242, 292–296
10. Saber-Lichtenberg, Y., Brix, K., Schmitz, A., Heuser, J. E., Wilson, J. H., Lorand, L., and Herzog, V. (2000) FASEB J. 14, 1005–1014
11. Marriq, C., Lejeune, P. J., Venot, N., Rolland, M., and Lissitzky, S. (1986) FEBS Lett. 207, 302–306
12. Mallet, B., Lejeune, P. J., Baudry, N., Niccoli, P., Carayon, P., and Franc, J. L. (1995) J. Biol. Chem. 270, 29881–29888
13. Maret, D., Lejeune, P. J., Baudry, N., Niccoli, P., Carayon, P., and Franc, J. L. (1996) FEBS Lett. 396, 223–226
14. Brunoe, N., and Lombardo, D. (1995) J. Biol. Chem. 270, 13524–13533
15. Brunoe, N., Lombardo, D., and Bendayan, M. (1998) J. Cell Sci. 111, 2665–2679
16. Forza, E., and Lenazza, W. J. (1992) J. Biol. Chem. 267, 3555–3556
17. Freedman, R. B., Hirst, T. R., and Tuite, M. F. (1994) Trends Biochem. Sci. 19, 331–336
18. Gilbert, H. F. (1997) J. Biol. Chem. 272, 28099–28014
19. Mezghrani, A., Courageot, J., Mani, J. C., Pugniere, M., Bastiani, P., and Miquelis, R. (2000) J. Biol. Chem. 275, 1920–1929
20. Feldt-Rasmussen, U. (1978) J. Immunol. Methods 21, 265–303
21. Yamauchi, K., Yamamoto, T., Hayashi, H., Koya, S., Takikawa, H., Teyoshima,
K., and Horiuchi, R. (1987) Biochem. Biophys. Res. Commun. 146, 1485–1492
22. Ting, J., and Lee, A. S. (1988) DNA (N. Y.) 7, 275–286
23. Ruf, J., Carayon, P., Sarles-Philip, N., Kourilsky, F., and Lissitzky, S. (1983) EMBO J. 2, 1821–1826
24. Frati, L., Bilstad, J., Edelhoch, H., Rall, J. E., and Salvatore, G. (1974) Arch. Biochem. Biophys. 162, 126–134
25. McLauhlin, S. H., and Bulleid, N. J. (1998) Biochem. J. 331, 793–800
26. Braakman, I., Helenius, J., and Helenius, A. (1992) Nature 356, 260–262
27. Berns, M. J., Wessely, L. C., and Kauffman, R. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7429–7432
28. Knittler, M. R., and Haas, I. G. (1992) EMBO J. 11, 1573–1581
29. Molimard, M., and Helenius, A. (2000) Science 288, 331–333
30. Mayer, M., Kies, U., Kammermeier, R., and Buchner, J. (2000) J. Biol. Chem. 275, 29421–29425
31. Brix, K., Lemansky, P., and Herzog, V. (1996) Endocrinology 137, 1963–1974
32. Tepel, C., Brimme, D., Herzog, V., and Brix, K. (2000) J. Cell Sci. 113, 4487–4498
33. Radford, S. E., Dobson, C. M., and Evans, P. A. (1992) Nature 358, 302–307
34. Van den Berg, B., Chung, E. W., Robinson, C. V., Mateo, P. L., and Dobson, C. M. (1999) EMBO J. 18, 4794–4803
35. Prim, T. P., Walker, K. W., and Gilbert, H. F. (1996) J. Biol. Chem. 271, 33644–33649
36. Puig, A., and Gilbert, H. F. (1994) J. Biol. Chem. 269, 7764–7771
37. Lemansky, P., Popp, G. M., Tietz, J., and Herzog, V. (1994) Endocrinology 135, 1566–1575
38. Berndorfer, U., Wils, H., and Herzog, V. (1996) J. Clin. Endocrinol. Metab. 81, 1918–1926
39. Gething, M.-J. (1999) Semin. Cell Dev. Biol. 10, 465–472
40. Bland-Elguindi, S., Cwirla, S. E., Dower, W. J., Lipshutz, R. J., Sprang, S. R., Sambrook, J. F., and Gething, M.-J. H. (1993) Cell 75, 717–728
41. Melnick, J., Dui, J. L., and Aron Y. (1994) Nature 370, 373–375
