

Hsp56: A Novel Heat Shock Protein Associated with Untransformed Steroid Receptor Complexes*

(Received for publication, August 28, 1990)

Edwin R. Sanchez

From the Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699

The recently-described p59 protein has been shown to be associated with untransformed steroid receptors present in rabbit uterus and rat liver cytosols (Tai, P. K., Maeda, Y., Nakao, K., Wakim, N. G., Duhring, J. L., and Faber, L. E. (1986) *Biochemistry* 25, 5269-5275; Renoir, J.-M., Radanyi, C., Faber, L. E., and Baulieu, E.-E. (1990) *J. Biol. Chem.* 265, 10740-10745), while a smaller version of this protein (p56) interacts with glucocorticoid receptors in human IM-9 cell cytosols (Sanchez, E. R., Faber, L. E., Henzel, W. J., and Pratt, W. B. (1990) *Biochemistry* 29, 5145-5152). In addition to interacting with glucocorticoid receptors, the p56 protein of IM-9 cell cytosol is also found as part of a large heteromeric complex that contains both the 70-kDa and 90-kDa heat shock proteins (hsp70 and hsp90, respectively). Given this association of p56 with the two major stress proteins, I have speculated that p56 may itself be a heat shock protein. In this paper, the effect of heat stress on the rate of synthesis of p56 is determined. Intact IM-9 cells were exposed to 37 or 43 °C for 4 h, followed by pulse-labeling with [³⁵S]methionine. Analysis of whole cytosolic extracts by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography reveal an increased rate of radiolabeling for hsp70, hsp90, hsp100, and hsp110, but no heat-inducible protein of smaller relative molecular mass is detected. However, immune-purification of p56 from normal and heat-stressed cytosols with the EC1 monoclonal antibody results in the presence of a 56-kDa protein that exhibits an increased rate of synthesis in response to heat stress. The results of two-dimensional gel Western blots employing the EC1 antibody demonstrate that this heat-inducible protein is indeed the EC1-reactive p56 protein and that the induction effect is not due to unequal yields of p56 during immune-purification. Heat stress has no effect on the composition of the p56·hsp70·hsp90 complex, except that the complex derived from heat shocked-cells contains both the constitutive and heat-inducible forms of hsp70. Induction of p56 also occurs in IM-9 cells subjected to chemical stress (sodium arsenite). It is proposed that p56 is a steroid receptor-associated heat shock protein which can now be termed hsp56. Like hsp90, hsp56 likely serves in

some vital cellular role apart from any specific function it provides in steroid receptor action.

It is becoming increasingly apparent that the macromolecular structure of the untransformed¹ state of steroid hormone receptors is much more complex than a model which simply includes receptor and hsp90² (see Ref. 1 for review). Reports from several laboratories have demonstrated that, in addition to hsp90, untransformed steroid receptor complexes contain a variety of associated proteins. In a protocol employing immune-purification under low stringency wash conditions, the glucocorticoid receptor complex from mouse L-cell cytosols was found to contain multiple copies of hsp90 and several isomorphs of a 55-kDa protein (2). Similarly, untransformed progesterone receptor complexes have been shown to contain not only hsp90 but also hsp70 (3, 4). In addition, these complexes contain undefined proteins with molecular masses of 54, 50, and 23 kDa (5). A more recent report has shown that the untransformed state of mouse glucocorticoid receptor expressed in stably transfected Chinese hamster ovary cells is a complex that contains both hsp90 and hsp70 (6). Interestingly, *in vitro* transformation of transfected glucocorticoid receptor (6) and progesterone receptor (3) complexes to the DNA-binding state results in dissociation of hsp90, as expected, but in retention of hsp70, suggesting that even the DNA-binding states of these receptors are heteromeric complexes.

A report by Tai *et al.* (7) has provided evidence that another protein with a molecular mass of 59-kDa (p59) is part of untransformed steroid receptor complexes. This observation was made through the use of a monoclonal antibody (EC1) against p59, which shifts untransformed glucocorticoid, progesterone, androgen, and estrogen receptor complexes during sucrose gradient centrifugation. The association of p59 with steroid receptors has recently been confirmed by two other laboratories. Renoir *et al.* (8) used chemical cross-linking techniques to demonstrate the presence of p59 in a variety of steroid receptor complexes derived from rabbit uterus, while Sanchez *et al.* (9) showed that the EC1 antibody reacts with the 9 S glucocorticoid receptor complexes derived from human IM-9 cells but not with the 4S monomeric state of the receptor. In the latter study, the human form of this protein had a somewhat smaller molecular mass and was designated p56. These authors also employed a two-dimensional gel analysis of proteins associated with "free" p56 (not receptor-bound), and it was found that p56 existed in a complex that contained both hsp70 and hsp90 along with several unidentified proteins. Given that p56 is found bound to a complex containing the two major stress proteins, I have speculated that p56 may itself be a heat shock protein. In this paper, the rate of synthesis of p56 is examined in human IM-9 cells exposed to

¹ The term "untransformed" refers to the so-called inactive or non-DNA-binding form of steroid hormone receptors, while "transformed" refers to receptors that are in the DNA-binding state.

² The abbreviations and trivial names used are: hsp90, hsp70, and hsp56, the 90-, 70-, and 56-kDa heat shock proteins; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TES, 2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]ethanesulfonic acid.

* This investigation was supported by start-up funds from the Medical College of Ohio, and by a Research Starter Grant from the Pharmaceutical Manufacturers Association. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

heat shock and chemical stress. It is demonstrated that [³⁵S] methionine labeled p56 is not detectable in aliquots of whole cytosols derived from control or stressed cells. However, immunoadsorption of p56 with the EC1 monoclonal antibody and analysis by one- and two-dimensional gels shows a clear increase in the rate of radiolabel incorporation for p56 protein derived from cells subjected to thermal or chemical shock. Based on these results, it is proposed that this newly described steroid receptor associated protein is a novel low abundance heat shock protein which can now be termed hsp56.

EXPERIMENTAL PROCEDURES

Materials

[³⁵S]Methionine ("Trans-label") was obtained from ICN Radiochemicals. Autoradiography enhancer ("Enlightening") was obtained from Du Pont-New England Nuclear. Sodium arsenite, TES, Hepes, protein A-Sepharose, nonimmune mouse IgG, horseradish peroxidase conjugates of goat anti-mouse and goat anti-rabbit IgG, newborn calf serum, and RPMI 1640 powdered media were obtained from Sigma. Immobilon P membranes were obtained from Millipore. Acrylamide, ultrapure urea, and ampholytes were obtained from British Drug House. The EC1 monoclonal antibody against p56 (7) was kindly provided by Dr. Lee Faber (Medical College of Ohio). The AC88 monoclonal antibody against hsp90 (10) was kindly provided by Dr. David O. Toft (Mayo Clinic). The rabbit antiserum to hsp70 was kindly provided by Dr. Etorre Apella (National Cancer Institute). This antiserum was made against a C-terminal peptide of human hsp70 by Ehrhart *et al.* (11) and is known to cross-react with hsp90.

Methods

Cell Culture and Stress Treatment—Human IM-9 lymphoblasts were grown in RPMI 1640 medium containing 10% iron-supplemented newborn calf serum. Maintenance and non-stressed cells lines were incubated in a humid 37 °C chamber containing 5% CO₂. Heat shock treatment of IM-9 cells was achieved by shifting replica flasks to a second 5% CO₂ incubator set at 43 °C. Typical duration of heat shock treatment ranged from 3.5 to 4 h. IM-9 cells were also stressed by addition of sodium arsenite at a final concentration of 100 μM. Both the arsenite-treated and nontreated cells were incubated at 37 °C for 2 h.

[³⁵S]Methionine Labeling and Cytosol Preparation—Immediately following stress treatment, the medium was removed and replaced with methionine-free medium containing 2% dialyzed calf serum and [³⁵S]methionine at a final concentration of 5 μCi/ml. In the case of heat shock experiments, labeling was performed at the appropriate temperature for 45 min. Sodium arsenite-treated and control cells were labeled at 37 °C for 45 min. All subsequent steps were carried out on ice (0–4 °C). Cells were washed 3 times by pelleting and resuspension in Hanks' buffered saline, followed by resuspension in 1.5 pellet volumes of 10 mM Hepes, 1 mM EDTA, pH 7.4. After swelling, the cells were ruptured by Dounce homogenization and centrifuged at 100,000 × *g* for 1 h. The supernatant fluid ("cytosol") was then either used immediately or frozen at –80 °C.

Immunoadsorption—Aliquots (50–100 μl) of IM-9 cell cytosol were diluted 1:1 with TEG buffer (10 mM TES, 1 mM EDTA, 50 mM NaCl, 10% (w/v) glycerol, pH 7.6) and antibodies were added according to the following final concentrations: 50 μg/ml for each of nonimmune mouse IgG, EC1, and AC88; 5% (v/v) of anti-hsp70 serum. Each mixture was incubated for 2 h on ice, followed by the addition of 125 μl of a 20% solution (v/v) of protein A-Sepharose. After rotation for 2 h at 4 °C, the Sepharose pellets were washed 4 times by resuspension in 1-ml aliquots of TEG buffer. The immunoadsorbed material was then eluted with 2 × SDS sample buffer containing β-mercaptoethanol for analysis by SDS-PAGE, or with O'Farrell's lysis buffer for analysis by two-dimensional PAGE.

Gel Electrophoresis, Autoradiography, and Immunoblotting—SDS-polyacrylamide gel electrophoresis was carried out in 7–14% slabs according to the method of Laemmli (12) with minor modifications. Gels destined for autoradiography were impregnated with enhancer prior to drying. Exposure of the dried gels to film was done at –80 °C in x-ray film cassettes each of which was lined with two intensifying screens of identical make and age. Isoelectric focusing was performed according to the procedure of O'Farrell (13) employing ampholines of pH range 4–8. Two-dimensional gel Western blots were performed as

previously described (9). Briefly, proteins from the second dimension gels were transferred to Immobilon P membranes and the membranes were incubated with the appropriate probe antibodies according to the following final concentrations: EC1, 2 ng/ml; anti-hsp70 serum, 1:5000 (v/v). The membranes were then incubated with peroxidase-conjugated goat anti-mouse IgG (Fig. 2) or with a mixture of peroxidase-conjugated anti-mouse and anti-rabbit IgG (Fig. 3). Each counter antibody was used at a 1:1000 dilution and all incubations of the membranes were carried out at 4 °C under gentle rocking. In the experiments of Figs. 2 and 3 each blotted membrane was also subjected to autoradiography under the conditions described above except that treatment with the autoradiography enhancer was omitted.

RESULTS AND DISCUSSION

Our observation (9) that the p56 protein in IM-9 cell cytosols is part of a large complex containing the two major heat shock proteins (hsp70 and hsp90) has been corroborated by another laboratory.³ In this case, a monoclonal antibody against hsp90 was used to immunoadsorb mouse Hepa cell cytosol. Among the five proteins other than hsp90 that were immune-specifically adsorbed, two have been identified. They are hsp70 and a p56 protein which reacts with the EC1 antibody. Thus, our work and that of Perdew strongly suggest that p56, hsp70, and hsp90 exist in cytosols as a heteromeric complex. However, given the vast stoichiometric excess of both hsp70 and hsp90 to p56 (9), it is likely that only a fraction of the two heat shock proteins take part in this complex. Nonetheless, this curious association of p56 to both hsp70 and hsp90 has led to the speculation that p56 may itself be a heat shock protein. One fact that has further fueled this notion is that the p56 protein appears to be evolutionarily conserved. Comparison of our N-terminal amino acid sequence for the human p56 protein (9) with sequences independently obtained by David Toft (Mayo Medical School) and Michel Renoir (INSERM, Paris)⁴ has demonstrated 83% homology between the human and rabbit forms of this protein. As heat shock proteins are among the most highly conserved proteins in all of biology (14), it seemed reasonable to ask if p56 is a heat shock protein.

By definition heat shock proteins are proteins that have an increased rate of synthesis in response to stress. The nature of the stress can take many forms, such as chemical poisoning and heavy metal toxicity, but the most commonly used form is heat shock. Accordingly, I have taken human IM-9 cells and split them into two groups. One group was cultured at normal temperatures (37 °C), while the other was incubated at 43 °C for 4 h. Each group was then pulse-labeled with [³⁵S] methionine and cytosols were prepared. When equal amounts of normal and stressed cytosols are subjected to SDS-PAGE and autoradiography, the results seen in lanes 1–4 of Fig. 1 are obtained. In this case, only the major known heat shock proteins are identifiable on the basis of their higher levels of [³⁵S]methionine incorporation. These are hsp110, hsp100, hsp90, and hsp70. However, if these cytosols are immunoadsorbed with antibodies directed against p56, hsp70, and hsp90, along with the appropriate immune control (nonimmune mIgG), the results see in lanes 5–12 of this figure are obtained. As expected, use of the AC88 anti-hsp90 antibody results in the immune-specific presence of hsp90 and a clear demonstration of its response to heat stress (compare lanes 9 and 10). Similar conclusions can be drawn when an antiserum to hsp70 is employed (lanes 11 and 12), except that this antiserum, in addition to hsp70, also immune-specifically adsorbs hsp90, hsp100, and possibly hsp110. The anti-hsp70 serum was made to a C-terminal peptide of human hsp70 which is

³ G. H. Perdew, submitted for publication.

⁴ D. Toft and M. Renoir, personal communications.

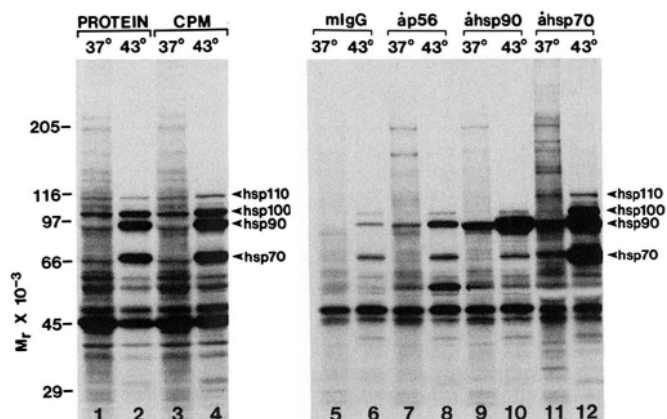


FIG. 1. Heat shock treatment of IM-9 cells results in increased rates of synthesis for the major heat shock proteins and p56. IM-9 cells were cultured at 37 and 43 °C for 4 h and pulse-labeled with [³⁵S]methionine. Cytosols were prepared and analyzed by SDS-PAGE and autoradiography. Lanes 1-4, aliquots of whole cytosols based on equal total protein (PROTEIN) or equal total radioactivity (CPM). Lanes 5-12, 50- μ l aliquots of cytosol immunoadsorbed with nonimmune antibody (mIgG), EC1 antibody against p56 ($\hat{a}p56$), AC88 antibody against hsp90 ($\hat{a}hsp90$), or antiserum against hsp70 ($\hat{a}hsp70$) which also reacts specifically with hsp90. Arrow indicates heat-induced form of p56.

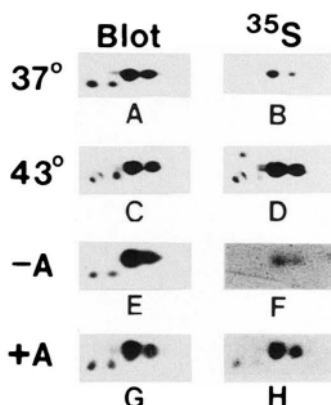


FIG. 2. Two-dimensional gel Western blots of hsp56 induced by heat shock and chemical stress. Aliquots (100 μ l) of ³⁵S-labeled cytosols from normal (37 °, -A), heat-shocked (43 °) and arsenite-treated (+A) cells were immunoadsorbed with equal amounts of EC1 antibody against p56. After washing, the immunoadsorbed material was eluted and analyzed by two-dimensional gel Western blotting using the EC1 antibody as probe (Blot). Autoradiography was then performed for each blot (³⁵S) under identical exposure conditions and times.

known to contain sequence homology with C terminus of hsp90 (11). Not surprisingly, this antiserum has been shown to cross-react with hsp90 (11). Thus the immune-specific presence of hsp90 seen in lanes 11 and 12 was not unexpected. On the other hand, the reasons for the co-immunoadsorption of hsp100 with the anti-hsp70 serum remain unclear. More interesting, however, is the fact that use of the EC1 antibody to immunoadsorb control and heat shocked cytosols has resulted in the immune-specific presence of a labeled 56-kDa protein (compare lanes 6 and 8) that is clearly synthesized at a higher rate in response to stress (compare lanes 7 and 8).

To identify unambiguously this "heat shock" protein as the EC1-reactive p56 protein and to eliminate the possibility that this result was actually due to unequal yields of p56 during immunoadsorption, the experiments of Fig. 2 were performed. In this case, p56 protein was again immunoadsorbed from cytosols made from normal and stressed cells pulse-labeled

with [³⁵S]methionine. This was followed by two-dimensional gel Western-blots in which EC1 was used as the probe antibody. Panels A and C of Fig. 2 show the relative amounts of p56 obtained from normal and heat-shocked cells as detected by the EC1 antibody. Clearly the immunoadsorption procedure has resulted in roughly equal amounts of p56 protein from both control (panel A) and heat shocked (panel C) cytosols. Each of these blots was subjected to autoradiography, taking care that the exposure conditions and times were identical. In this case, the results of the autoradiography show an approximate order of magnitude increase in radiolabel incorporation for the heat-shocked form of p56 (panel D) over the unshocked form (panel B). When IM-9 cells are subjected to chemical stress, the results seen in panels F-H of Fig. 2 are obtained. In this experiment, IM-9 cells were treated with sodium arsenite for 2 h at 37 °C, while control cells were left untreated. As was the case for heat shock treatment, immunoadsorption of stressed and unstressed cytosols with EC1 antibody resulted in similar yields of p56 protein (panels E and G) but in an increased rate of synthesis for p56 derived from arsenite-treated cells (panel H) over control cells (panel F).

The experiments of Figs. 1 and 2 clearly demonstrate that the EC1-reactive p56 protein will increase its rate of synthesis in response to stress. Thus, by the standard criteria, p56 becomes another member in the family of heat shock proteins and it is proposed that it now be referred to as hsp56. If hsp56 is indeed a heat shock protein then one must wonder why it has gone undiscovered to this point. The most likely explanation is suggested by the results seen in Fig. 1. In this experiment, IM-9 cells were pulse-labeled for 45 min, yet an [³⁵S]methionine-labeled 56-kDa protein could not be detected in aliquots of either control or heat shocked cytosols. As pulse labeling is the routine for investigations in the field of cellular heat shock responses, and as we have previously determined that the p56 protein is of relatively low cellular abundance (9), it is probable these two events have combined to cause hsp56 to be overlooked as a heat shock protein.

To determine what effect, if any, heat stress has on the maintenance or composition of the hsp56·hsp70·hsp90 complex, the experiment of Fig. 3 was performed. As in the

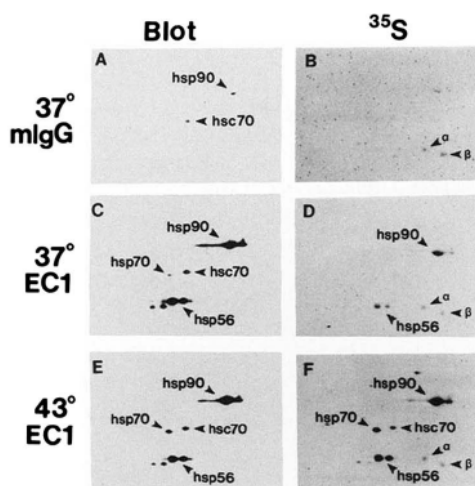


FIG. 3. Effect of heat shock on the hsp56·hsp70·hsp90 complex. Aliquots (100 μ l) of ³⁵S-labeled cytosols from control (37 °) or heat-shocked (43 °) cells were immunoadsorbed with equal amounts of nonimmune mIgG or EC1 antibodies. After washing, the immunoadsorbed material was analyzed by two-dimensional gel Western blotting (Blot) in which both the EC1 antibody against p56 and the anti-hsp70 serum against hsp70 and hsp90 were employed as probes. Each blot was then subjected to autoradiography (³⁵S).

previous figures, IM-9 cells were once again subjected to heat shock and pulse-labeled with [³⁵S]methionine. The p56 complex was then immunoadsorbed with EC1 antibody and analyzed by two-dimensional gel Western blotting in which both the EC1 antibody and the anti-hsp70 serum (which detects both hsp70 and hsp90) were used as probes. The results of the Western blotting clearly show that heat stress has little effect on the composition of the p56 complex, except that the complex derived from heat-shocked cells now contains increased amounts of heat-inducible hsp70, as well as the constitutively expressed hsc70 (compare panels C and E). Comparison of the autoradiograms made from these blots (panels D and F) shows a marked increase in the rate of radiolabel incorporation in response to heat stress for hsp56 as well as hsp70 and hsp90. In contrast, the rate of heat shock induction for hsc70 is quite low as expected for this protein (15), and there is no discernable induction in the rates of synthesis of both α - and β -tubulin, which are present in these autoradiograms only as a result of nonspecific adsorption (panel B).

Since N-terminal sequencing of hsp56 has not demonstrated sequence homology to known proteins (9), it is likely that hsp56 represents a novel heat shock protein and its role in the stress response should be of considerable interest to cell biologists. At the moment, little is known about the function of hsp56 in either steroid receptor action or the heat shock response. There are, however, three features of this protein that may provide clues to its cellular function: 1) it is of low abundance in cellular extracts; 2) it is constitutively expressed; and 3) it is found in oligomeric protein complexes (7-9). A recently discovered heat shock protein, hsp60, is a mitochondrial protein that has been implicated in the folding of proteins and in the assembly of complexes following protein import into the mitochondria (16, 17). Although the published sequence of hsp60 (17) does not demonstrate any relatedness to the N terminus of hsp56, comparisons of their known features reveal some interesting similarities. Like hsp56, hsp60 is also of low cellular abundance, is constitutively expressed in nonstressed cells, and it is found as part of a large heteromeric complex (18, 19). Hsp60 and the structurally related groEL heat shock protein of *Escherichia coli* have been classified as "chaperonins" based on their involvement by unknown mechanisms in the assembly of oligomeric protein complexes (20). It is thought that the ability of hsp60 to assemble protein complexes is in part due to the protein folding activity of hsp60, which in turn is known to require ATP hydrolysis (21). The presence of hsp56 in untransformed steroid receptor complexes (7-9), and the fact that conversion of steroid receptors to the DNA-binding state requires subunit dissociation (22, 23), suggest the possibility that hsp56 may

be involved in either assembly or disassembly of these complexes.

Acknowledgments—I am grateful to Lee Faber, David Toft, and Etorre Appella for providing the antibodies to hsp56, hsp90, and hsp70, respectively. In addition, I want to thank Lee Faber and Michel Renoir for providing manuscripts prior to publication.

REFERENCES

- Pratt, W. B., and Sanchez, E. R. (1988) in *Hormones and Cancer 3: Proceedings of the 3rd International Congress on Hormones and Cancer* (Bresciani, F., King, R. J. B., Lippman, M. E., and Raynaud, J. P., eds) pp. 16-22, Raven Press, New York
- Bresnick, E. H., Dalman, F. C., and Pratt, W. B. (1990) *Biochemistry* **29**, 520-527
- Kost, S. L., Smith, D., Sullivan, W., Welch, W. J., and Toft, D. O. (1989) *Mol. Cell. Biol.* **9**, 3829-3838
- Estes, P. A., Suba, E. J., Lawler-Heavner, J., Elashry-Stowers, D., Wei, L. L., Toft, D. O., Sullivan, W. P., Horowitz, K. B., and Edwards, D. P. (1987) *Biochemistry* **26**, 6250-6262
- Smith, D. F., Faber, L. E., and Toft, D. O. (1990) *J. Biol. Chem.* **265**, 3996-4003
- Sanchez, E. R., Hirst, M., Scherrer, L. C., Tang, H.-Y., Welsh, M. J., Harmon, J. M., Simons, S. S., Ringold, G. M., and Pratt, W. B. (1990) *J. Biol. Chem.* **265**, 20123-20130
- Tai, P. K. K., Maeda, T., Nakao, K., Wakim, N. G., Huhning, J. L., and Faber, L. E. (1986) *Biochemistry* **25**, 5269-5275
- Renoir, J.-M., Radanyi, C., Faber, L. E., and Baulieu, E.-E. (1990) *J. Biol. Chem.* **265**, 10740-10745
- Sanchez, E. R., Faber, L. E., Henzel, W. J., and Pratt, W. B. (1990) *Biochemistry* **29**, 5145-5152
- Sullivan, W. P., Vroman, B. T., Bauer, V. J., Puri, R. K., Riehl, R. M., Pearson, G. R., and Toft, D. O. (1985) *Biochemistry* **24**, 4214-4222
- Erhart, J. C., Dithu, A., Ullrich, S., Appella, E., and May, P. (1988) *Oncogene* **3**, 595-603
- Laemmli, U. K. (1970) *Nature* **227**, 680-685
- O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007-4021
- Lindquist, S., and Craig, E. A. (1988) *Annu. Rev. Genet.* **22**, 631-677
- Pelham, H. R. B. (1986) *Cell* **46**, 959-961
- Cheng, M. Y., Hartl, F.-U., Martin, J., Pollack, R. A., Kalousek, F., Neupert, W., Hallberg, E. M., Hallberg, R. L., and Horwich, A. L. (1989) *Nature* **337**, 620-625
- Reading, D. S., Hallberg, R. L., and Meyers, A. M. (1988) *Nature* **337**, 655-659
- McMullin, T. W., and Hallberg, R. L. (1988) *Mol. Cell Biol.* **8**, 371-380
- Hutchinson, E. G., Tichelaar, W., Hofhaus, G., Weiss, H., and Leonhard, K. R. (1989) *EMBO J.* **8**, 1485-1490
- Hemmingsen, S. M., Woolford, C., van der Vies, S. M., Tilly, K., Dennis, D. T., Georgopoulos, C. P., Hendrix, R. W., and Ellis, R. J. (1988) *Nature* **333**, 330-334
- Ostermann, J., Horwich, A. L., Neupert, W., and Hartl, F.-U. (1989) *Nature* **341**, 125-130
- Mendel, D. B., Bodwell, J. E., Gametchu, B., Harrison, F. W., and Munck, A. (1986) *J. Biol. Chem.* **261**, 3758-3763
- Sanchez, E. R., Toft, D. O., Schlesinger, M. J., and Pratt, W. B. (1985) *J. Biol. Chem.* **260**, 12398-12401