Expression of antisense RNA against eukaryotic translation initiation factor 4E (eIF-4E) in HeLa cells causes a reduction in the levels of both eIF-4E and eIF-4y (p220) and a concomitant decrease in the rates of both cell growth and protein synthesis (De Benedetti, A., Joshi-Barve, S., Rinker-Schaffer, C., and Rhoads, R. E. (1991) Mol. Cell Biol. 11, 5435–5445). The synthesis of most proteins in the antisense RNA-expressing cells (AS cells) is decreased, but certain proteins continue to be synthesized. In the present study, we identified many of these as stress-inducible or heat shock proteins (HSPs). By mobilities on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by reactivity with monoclonal antibodies generated against human HSPs, four of these were shown to be HSP 90, HSP 70, HSP 65, and HSP 27. The steady-state levels of HSP 90, 70, and 27 were elevated in relation to total protein in AS cells. Pulse labeling and immunoprecipitation indicated that HSP 90 and HSP 70 were synthesized more rapidly in AS cells than in control cells. The accelerated synthesis of HSPs in the AS cells was not due, however, to increased mRNA levels; the levels of HSP 90 and 70 mRNAs either remained the same or decreased after induction of antisense RNA expression. Actin mRNA, a typical cellular mRNA, was found on high polysomes in control cells but shifted to smaller polysomes in AS cells, as expected from the general decrease in translational initiation caused by eIF-4E and eIF-4y depletion. HSP 90 and 70 mRNAs showed the opposite behavior; they were associated with small polysomes in control cells but shifted to higher polysomes in AS cells. These results demonstrate that HSP mRNAs have little or no requirement in vitro for the cap-recognition machinery and suggest that these mRNAs may utilize an alternative, cap-independent mechanism of translational initiation.

The heat shock or stress response, elicited upon exposure of cells to elevated temperatures, alcohol, metal ions or other stress conditions, is characterized by the elevated synthesis of a set of proteins with high evolutionary sequence conservation termed the heat shock proteins (HSPs) (Lindquist, 1986). These proteins are also synthesized under normal conditions (Bienz, 1984; Kurtz et al., 1986), suggesting that they may play a role in normal cellular growth and differentiation. The synthesis of HSPs is tightly regulated by both transcriptional and post-transcriptional mechanisms. Exposure to heat shock results in a virtual shutdown of normal cellular protein synthesis, paralleled by a shift to high level synthesis of the heat shock proteins. It is known that neither degradation of normal mRNAs nor competition by HSP mRNAs can account for this selectivity (De Benedetti and Baglioni, 1986a, reviewed by Lindquist (1987)). The preferential translation of HSP mRNAs may be due to cis-acting elements in the 5'-UTR of HSP mRNA, since large deletions in this region abolish translation of HSP mRNAs at high temperatures (Klemenz et al., 1985; McGarry and Lindquist, 1985; Hultmark et al., 1986).

Previous studies have focused on the translational initiation machinery in an attempt to understand the dual effect of diminished translation of normal mRNAs and enhanced translation of HSP mRNAs. One effect of heat shock is increased phosphorylation of the α subunit of eIF-2, which impairs its activity and consequently causes a reduction in the formation of 43 S initiation complexes (Duncan and Hershey, 1984; De Benedetti and Baglioni, 1986b). Another effect of heat shock is decreased activity of factors involved in the recognition of the mRNA cap and recruitment of mRNA to the ribosome (factors of the eIF-4 group). Duncan and Hershey (1984) demonstrated by in vitro assays that heat shock partially impaired the activities of eIF-3(4+4F) and -4E, but not eIF-4A, -4C, or -4D. Similarly, Maroto and Sierra (1988) demonstrated that the inhibition of translation in Drosophila embryos following heat shock was due to inactivation of cap binding factor(s). Duncan et al. (1987) found that heat shock resulted in an accumulation of the unphosphorylated form of eIF-4E. Other studies have demonstrated that only eIF-4E which is phosphorylated at Ser-53 is active in recruitment of mRNA to the ribosome (reviewed in Rhoads (1991)). Lamphear and Panniers (1990) found that a complex containing eIF-4E (predominantly phosphorylated) and eIF-4y (p220) was able to restore translation in extracts of heat-shocked Ehrlich cells. They also found that eIF-4 from these cells (eIF-4 is a complex of eIF-4E, eIF-4A, and eIF-4y) was dephosphorylated and inactive (Lamphear and Panniers, 1991). In addition, a loss of the eIF-4 complex was detected immunologically in heat-shocked Drosophila embryos (Zapata et al., 1991). The collective evidence suggests that heat shock interferes with the maintenance of eIF-4E in the active phosphorylated state and perhaps with the association of eIF-4E and eIF-4y. These effects could be due to inactivation of eIF-4E, eIF-4y, or kinases responsible for their phosphorylation.

Recently, we developed a mammalian system which is deficient in eIF-4E and eIF-4y (De Benedetti et al., 1991). We expressed antisense RNA complementary to the 5' terminus...
of eIF-4E mRNA from the inducible promoter of an episomally replicating vector in HeLa cells. This caused a decrease in the level of eIF-4E which was proportional to the degree of antisense RNA expression. Unexpectedly, the level of eIF-4\(\gamma\) was decreased in parallel. The rates of cell growth and protein synthesis were similarly decreased, but even when no eIF-4E and eIF-4\(\gamma\) were detectable, protein synthesis continued at approximately 8% of the control rate. Analysis of polypeptide patterns indicated that this residual protein synthesis was due to a small number of proteins. The present study was undertaken in order to identify the proteins whose synthesis escaped the inhibitory effects of eIF-4E and eIF-4\(\gamma\) depletion. We find that many of these are previously characterized.

EXPERIMENTAL PROCEDURES

Treatment of Cells—Control HeLa cells and cells expressing antisense RNA against eIF-4E (AS cells) were maintained as previously described (De Benedetti et al., 1991). For the heat shock treatment, HeLa cells were exposed to 43°C for 1 h and allowed to recover at 37°C for 3 h. The AS cells were grown in medium containing 0.2 mg/ml G418 and treated with TCDD for 0, 24, or 48 h as described by De Benedetti et al. (1991). For the labeling experiments, typically 5 \(\times\) 10^5 cells were incubated with 50 \(\mu\)Ci/ml [35S]methionine (ICN, Irvine, CA).

Western, Northern, and Polysome Analysis—These were carried out as described by De Benedetti et al. (1991). The monoclonal antibodies used were HSP 90 and HSP 27 (StressGen Biotechnologies Corp., Canada), HSP 72/73 (Oncogene Science, Manhasset, NY) and HSP 65 (a gift from Dr. Lee Faber, Medical College of Toledo, Ohio). The cDNA probes used were HSP 89\(\alpha\) (StressGen) and HSP 70 (a gift from Dr. Richard Morimoto, Northwestern University, Evanston, IL).

Immunoprecipitation Analysis—Equal aliquots of protein (15 \(\mu\)g) from each sample were diluted in 3 volumes of buffer containing 50 mM Tris-HCl, pH 7.2, 150 mM NaCl, 0.1% SDS, 1% sodium deoxycholate, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 2 \(\mu\)g/ml each of pepstatin A and leupeptin and allowed to react with the appropriate monoclonal antibodies at room temperature for 20 min. The immune complex was adsorbed overnight at 15°C to either Protein-A or Protein-G coupled to a solid support. After several washes with the same buffer, the antigen-antibody complex was resolved by boiling in SDS sample buffer for 10 min. The immunoprecipitated proteins were then analyzed by SDS-PAGE.

Polysomal RNA Analysis (Slot Blots)—Formaldehyde and SSC were added to each of the sucrose density gradients to achieve final concentrations of 11% and 4 X, respectively (1 X SSC is 0.015 M NaCl, 0.1 M NaCitrate). After heating at 65°C for 15 min, the samples were filtered through MSI Nylon membrane (presoaked in 0.1 M NaCl, 0.1 M Tris-HCl, pH 7.2, 150 mM NaCl, 0.1% SDS, 1% sodium deoxycholate, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 2 \(\mu\)g/ml each of pepstatin A and leupeptin and allowed to react with the appropriate monoclonal antibodies at room temperature for 20 min. The immune complex was adsorbed overnight at 15°C to either Protein-A or Protein-G coupled to a solid support. After several washes with the same buffer, the antigen-antibody complex was resolved by boiling in SDS sample buffer for 10 min. The immunoprecipitated proteins were then analyzed by SDS-PAGE.

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antibody recognized a single major band of the expected size in control cells (panel B). The band was approximately the same in the heat-shocked cells and slightly increased in the AS cells. The amount of HSP 65 appeared to remain constant in the control cells (panel D), but heat shock caused a 2-fold increase and antisense RNA expression, a 5-fold increase.

The results with all four antibodies provided positive identification of major polypeptides surviving in AS cells and indicated that by both electrophoretic mobility and immunoreactivity, they were the same as known HSPs. Furthermore, the levels of these proteins as a percentage of total cellular protein were increased in AS cells in most cases.

**Immunoprecipitation Analysis**—The Western blotting experiments provided information on the total levels of HSPs but not on their rates of synthesis. In order to measure the latter, newly synthesized 35S-labeled proteins were analyzed by immunoprecipitation (Fig. 3). A small amount of HSP 70 was detected by immunoprecipitation in control extracts (panel A). Its synthesis, however, was induced substantially upon heat shock. The level of newly synthesized HSP 70 in the AS cells was also significantly higher than in control cells but not as high as in heat-shocked cells. Labeled HSP 90 was not detectable by immunoprecipitation in control HeLa cells (panel B). Its synthesis was strongly induced by both heat shock and expression of antisense RNA although the level decreased after 48 h of TCDD treatment (lane AS48). The prominent protein migrating at 50 kDa has been previously shown to be immunoprecipitated by HSP 90 antibodies, but its relationship to HSP 90 is not known (Whitelaw et al., 1991). These results indicate that HSP 90 and HSP 70 are synthesized more efficiently in AS cells than in control cells.

**Northern Analysis**—In order to determine whether the accelerated synthesis of HSPs was due to increased levels of HSP mRNA as is the case in heat shock, we examined the mRNAs for HSP 90 and HSP 70 and compared them to β-actin mRNA in control HeLa and AS cells by Northern analysis (Fig. 4). As shown in panel A, the level of HSP 90 mRNA increased approximately 3-fold upon heat shock treatment, as observed previously (Hickey et al., 1989), but did not increase in the AS cells. The levels of HSP 70 and β-actin mRNAs were measured in AS cells as a function of time after the induction
of AS RNA with TCDD (panel B). β-Actin mRNA remained essentially unchanged over the course of TCDD induction. The level of HSP 70 mRNA, however, gradually decreased by about 80% upon treatment with TCDD. Despite this decrease in HSP 70 mRNA, the protein was synthesized more rapidly in AS cells (Fig. 3A). Thus, the elevated synthesis of HSPs in AS cells cannot be attributed to higher mRNA levels.

Polysomal Distribution of mRNA—The foregoing results indicate that HSP mRNAs were more efficiently translated in AS cells, since more protein was synthesized from either the same (HSP 90) or less (HSP 70) mRNA. Since initiation of protein synthesis is rate-limiting under normal conditions, the more efficient utilization of mRNAs should be reflected in a higher rate of initiation and concomitant shift of HSP mRNAs to higher polysomes, assuming that there is not a simultaneous and proportionate increase in elongation rate. To test this prediction directly, we analyzed RNA from polysomal fractions using 32P-labeled cDNA probes to HSP 90, HSP 70, and β-actin mRNA (which should represent the polysomal behavior of a normal cellular message). As reported previously (De Benedetti et al., 1991), polysomes were clearly visible in the control cells (Fig. 5A, HeLa), but in AS cells treated with TCDD for 48 h (AS), they were almost completely disaggregated with accompanying increases in monosomes and ribosomal subunits. The mRNA of β-actin mRNA was associated mostly with large polysomes in control cells (panel B; midpoint = 8–9 ribosomes/mRNA) but was shifted into the nonpolysomal and small polysomal fractions in AS cells. The HSP 90 mRNA showed just the opposite behavior, it was associated with small polysomes in control cells but shifted to larger polysomes in AS cells (Fig. 5C). A similar shift from lighter to heavier polysomes was observed in the case of HSP 70 mRNA (panel D). This confirms that HSP 90 and HSP 70 mRNAs are more efficiently translated in AS cells, despite the almost complete loss of polysomes.

Heat Shock and Recovery in AS Cells—The foregoing results indicate that cells depleted of eIF-4E and eIF-4y are able to translate HSP mRNAs but not normal cellular mRNAs, even though HSP mRNA levels are not increased. Since heat shock leads to increased levels of all HSP mRNAs to varying degrees, with HSP 70 being the most prominent (Wu et al., 1985), one would predict that synthesis of HSPs would be even further enhanced when AS cells were subjected to heat shock. To test this, we compared the pattern of proteins synthesized during heat shock and recovery of control and AS cells (Fig. 6). Cells were incubated at 43 °C for 2 h and then allowed to recover at 37 °C for various time intervals. The cells were labeled with [35S]methionine during the last 45 min of each time interval. Synthesis of HSPs following heat shock was much more prominent in AS than in control cells. In particular, there was a massive increase in HSP 70 synthesis, reflecting the combined effects of an increase in HSP mRNA level due to heat shock and a preferential translation of HSP 70 mRNA due to initiation factor depletion. Normal translation was recovered rapidly in control cells, but interestingly, it was not recovered in AS cells, which continued to translate HS mRNAs almost exclusively. In fact, the AS cells could not recover from exposure to heat shock and began to detach from the plate after 8 h of incubation at 37 °C.

**DISCUSSION**

AS cells, which maintain a normal morphology and polypeptide pattern despite the deficiency of eIF-4E and eIF-4y (De Benedetti et al., 1991), provide a unique mammalian system to study the role of these initiation factors in translation, akin to the yeast cell-free system containing a temperature-sensitive eIF-4E variant (Altmann et al., 1989). Other systems that have been used to study eIF-4 initiation factors

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**FIG. 5. Northern analysis.** Cytoplasmic RNA (10 μg) from HeLa, heat-shocked HeLa, and AS cells treated with TCDD for the indicated number of hours was analyzed by hybridization. A, Northern probed with HSP 90 cDNA. B, Northern probed with both β-actin and HSP 70 cDNA.

**FIG. 6. Heat shock and recovery in control and AS cells.** Control HeLa and AS cells (not treated with TCDD) were heat shocked for 1 h and then allowed to recover as described under "Experimental Procedures." The proteins were labeled with [35S]methionine for the last 45 min of each time interval, extracted, resolved by SDS-PAGE, and detected by fluorography. C, control, non-heat-shocked HeLa cells; HS, cells heat-shocked for 1 h; R1, R2, etc., cells allowed to recover at 37 °C for 1 h, 2 h, etc. The positions of HSP 90, HSP 70, and HSP 27 are indicated.
are poliovirus-infected cells and their lysates, or cell-free translation systems derived from components of poliovirus-infected and normal cells (Etchison et al., 1984; Morley and Hershey, 1990), and extracts from heat-shocked cells of various types (see the Introduction). An advantage of AS cells and their extracts for the analysis of translational initiation is that the numerous additional biochemical and ultrastructural changes occurring in poliovirus-infected cells (Koch and Koch, 1985) and heat-shocked cells (Schlesinger et al., 1982) are not complicating factors. Not only do AS cells allow one to distinguish translational from transcriptional effects, they are especially suitable for studies of mRNA discrimination and cap-dependence of translation since they are depleted in the two polypeptides most closely identified with cap recognition, eIF-4E and eIF-4G, but not the third component of the eIF-4 complex, the RNA helicase eIF-4A (De Benedetti et al., 1991).

Our results using this system suggest that HSP 90 and HSP 70, and possibly all HSP mRNAs, are translated by a cap-independent pathway. We have shown that HSPs are efficiently synthesized in AS48 cells, as indicated by immunoprecipitation of newly synthesized HSP 90 and 70 (Fig. 3), despite the fact that eIF-4E and eIF-4G are undetectable in these cells (De Benedetti et al., 1991). This is not a transcriptional effect since the levels of HSP 90 and 70 mRNAs do not increase (Fig. 4). Furthermore, the HSP 90 and 70 mRNAs are associated with larger polysomes in AS cells than in control HeLa cells unlike typical cellular mRNAs (Fig. 5). Consistent with these in vivo results, Zapata et al. (1991) have recently shown that in vitro translation of HSP 70 mRNA in Drosophila extracts was resistant to inactivation of eIF-4E by the addition of antibodies. Another system which is deficient in eIF-4E is a yeast strain carrying a temperature-sensitive eIF-4E and eIF-4G mutation (Altmann et al., 1989). Extracts from cells exposed to the nonpermissive temperature were translationally inactive and were dependent on exogenous eIF-4E. Interestingly, despite the fact that these extracts could not translate most yeast mRNAs, a prominent band migrating at approximately 70 kDa was synthesized in the absence of added eIF-4E. Although it was not identified, it is conceivable that it was HSP 70.

The reason why HSP mRNAs are translated in AS cells is not clear. According to a model proposed by Lodish (1974), messengers with rate constants of initiation which are higher than average ("strong" mRNAs) will be preferentially translated when the capacity for initiation is globally decreased. The original hypothesis formulated to explain the efficient translation of HSP mRNAs following heat shock required their definition as strong messengers (Hickey and Weber, 1982). The resistance of HSP synthesis to treatment of cells with hypertonic medium seemed to corroborate this idea (Hickey and Weber, 1982). However, the behavior of HSP synthesis during recovery from heat shock cannot be accounted for by this model. De Benedetti and Baglioni (1986a) showed that, after heat shock, a return to normal translation is accompanied by a decrease in HSP synthesis, without an appreciable decrease in HSP mRNA levels. In addition, HSP 70 mRNA is associated with large polysomes during heat shock but with small polysomes after 90 min of recovery. Thus, it would appear that HSP mRNAs are actually outcompeted for translation during recovery from heat shock, contrasting with their definition as strong mRNAs. In the present study, it is noteworthy that HSP mRNAs are associated with small polysomes in control cells. If they were strong mRNAs, as hypothesized, they would be associated with large polysomes like actin mRNA.

A more likely explanation for the preferential translation of HSP mRNAs is to postulate that their mechanism of initiation is qualitatively different from that of normal mRNAs. Thus, cap-dependent and cap-independent mechanisms of mRNA binding to ribosomes may be operative in HeLa cells and may compete for components of the translational machinery. One possibility for the low requirement of HSP mRNAs for eIF-4E and/or eIF-4G is that they contain very little secondary structure and hence do not require ATP-dependent unwinding by the eIF-4 machinery. In the eIF-4E- and eIF-4G-deficient yeast system, alfalfa mosaic virus-4, which has a 5'-UTR of low secondary structure, is only slightly stimulated by eIF-4E addition, in contrast to the mRNA for bacterial chloramphenicol acetyltransferase (Altman et al., 1989). It has been suggested that the 5'-UTRs of Drosophila HSP mRNAs are unstructured (Lindquist, 1987).

Another possible explanation for the preferential translation of HSP mRNAs in AS cells is internal initiation. Most cellular eukaryotic mRNAs are thought to follow a scanning mode of initiation in which the 40 S ribosome binds to the cap or the 5'-end and then scans the mRNA linearly in a 5'-3' direction until it encounters the initiator AUG (Huls and Hershey, 1990)). Translation of picornaviral RNAs, however, follows a cap-independent pathway (reviewed by Jackson (1991)). Such RNAs undergo internal initiation which requires certain structural elements in the 5'-UTR called ribosome landing pads or internal ribosome entry sites. In the case of infection by some picornaviruses, e.g., poliovirus, proteolysis of p220 leads to a situation in which cap-dependent translation is restricted (Etchison et al., 1982). Interestingly, certain cellular mRNAs continue to be translated under these conditions, two of which have been identified as HSP 70 (Munoz et al., 1984) and HSP 78 (Sarnow, 1989). Macejak and Sarnow (1991) confirmed that HSP 78 mRNA can support internal initiation; when expressed in a bicistronic construct, the 5'-UTR of HSP 78 mRNA allowed internal ribosome binding and translation of the distal cistron. The only other cellular mRNA currently thought to be internally initiated is that of the Drosophila antennapedia gene (mentioned in Jackson, 1991). The sequence requirements for internal initiation are not clearly defined, so it is not yet possible to make general conclusions by simple inspection of the untranslated regions of the HSP messengers. A recent report indicates that the oligopyrimidine tract UUUCC may be important in internal ribosome binding to picornaviral mRNAs (Pilipenko et al., 1992). Nonetheless, experiments with bicistronic constructs will be necessary to establish whether all HSP mRNAs are in fact internally initiated.

The experiment on heat shock of AS cells (Fig. 6) gives insight into the mechanism by which protein synthesis is restored in normal cells following heat shock. When the AS cells were heated, the synthesis of HSPs became dominant, whereas the translation of normal mRNAs was severely repressed and was not restored even after an extended period of time at 37°C. It therefore seems likely that the synthesis of new eIF-4E and/or eIF-4G is required for complete restoration of protein synthesis, and that this is prevented in AS cells. Despite the reduction of eIF-4E and eIF-4G, AS cells continue to synthesize HSPs following heat shock until they eventually die, indicating that translation of HSP mRNAs does not require eIF-4E and eIF-4G.

In contrast to this idea, Anthony and Merrick (1991) used bicistronic constructs in in vitro assays and found that eIF-4 facilitated or stimulated internal initiation. Also, a recent report by Thomas et al. (1991) demonstrates that eIF-4 is

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2 P. Sarnow, unpublished results.
essential for or enhances internal initiation in the cowpea mosaic virus middle component RNA. The reason for this apparent discrepancy is not known. It may be due to differences in the experimental systems used or may indicate that HSP mRNAs employ a cap-independent mechanism of initiation which is different from that of picornaviruses.

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**REFERENCES**

Altmann, M., Sonenberg, N., and Trachsel, H. (1989) Mol. Cell. Biol. 9, 4467-4472

Anthony, D. D., and Merrick, W. C. (1991) J. Biol. Chem. 266, 10218-10226

Bienz, M. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3138-3142

De Benedetti, A., and Baglioni, C. (1986a) J. Biol. Chem. 261, 15800-15804

De Benedetti, A., and Baglioni, C. (1986b) J. Biol. Chem. 261, 338-342

De Benedetti, A., Joshi-Barve, S., Rinker-Schaffer, C., and Rhoads, R. E. (1991) Mol. Cell. Biol. 11, 5435-5445

Duncan, R., and Hershey, J. W. B. (1984) J. Biol. Chem. 259, 11882-11889

Duncan, R., Milburn, S. C., and Hershey, J. W. B. (1987) J. Biol. Chem. 262, 380-388

Etchison, D., Milburn, S. C., Edery, I., Sonenberg, N., and Hershey, J. W. B. (1982) J. Biol. Chem. 257, 14806-14810

Etchison, D., Hansen, J., Ehrenfeld, E., Edery, I., Sonenberg, N., Milburn, S., and Hershey, J. W. B. (1984) J. Virol. 51, 832-837

Hickey, E. D., and Weber, L. A. (1982) Biochemistry 21, 1513-1521

Hickey, E. D., Brandon, S. E., Smale, C., Lloyd, D., and Weber, L. A. (1989) Mol. Cell. Biol. 9, 2615-2626

Hultmark, D., Klemenz, R., and Gehring, W. J. (1986) Cell 44, 429-438

Jackson, R. J. (1991) Nature 353, 14-15

Klemenz, R., Hultmark, D., and Gehring, W. J. (1985) EMBO J. 4, 2053-2060

Koch, F., and Koch, G. (1985) The Molecular Biology of Picornaviruses, p. 209, Springer-Verlag, New York

Kozak, M. (1989) J. Cell Biol. 108, 229-241

Kurtz, S., Ross, J., Petko, L., and Lindquist, S. (1986) Science 231, 1154-1157

Lamphear, B. J., and Panniers, R. L. (1986) J. Biol. Chem. 261, 5333-5336

Lamphear, B. J., and Panniers, R. L. (1991) J. Biol. Chem. 266, 2789-2794

Lindquist, S. (1986) Annu. Rev. Biochem. 55, 1151-1191

Lindquist, S. (1987) in Translational Regulation of Gene Expression (Ilan, J., ed) pp. 187-207, Plenum Press, New York

Lodish, H. F. (1974) Nature 248, 385-388

Maciejak, D. G., and Sarnow, P. (1991) Nature 353, 90-94

Maroto, F. G., and Sierra, J. M. (1988) J. Biol. Chem. 263, 15720-15725

McGarry, T. J., and Lindquist, S. (1988) Cell 42, 903-911

Morley, S. J., and Hershey, J. W. B. (1990) Biochimie (Paris) 72, 269-294

Munoz, A., Alonzo, M. A., and Carrasco, L. (1984) Virology 137, 150-159

Pilipenko, E. V., Gmyl, A. P., Maslova, S. V., Svitkin, Y. V., Sinyakov, A. N., and Agol, V. I. (1992) Cell 68, 119-131

Rhoads, R. E. (1991) Curr. Opin. Cell Biol. 3, 1019-1024

Sambrook, J., Fritsch, E. F., and Maniatis, T. (eds) (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sarnow, P. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5795-5799

Schlesinger, M., Ashburner, M., and Tissieres, A. (eds) (1982) Heat Shock from Bacteria to Man, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Thomas, A. A. M., Haer, E. T., Wellink, J., and Voorma, H. O. (1991) J. Virol. 65, 2953-2969

Whitewall, M. L., Hutchinson, K., and Perede, G. H. (1991) J. Biol. Chem. 266, 16436-16440

Whitewall, K., and Morimoto, R. (1985) Mol. Cell. Biol. 5, 339-341

Zapata, J. M., Maroto, F. G., and Sierra, J. M. (1991) J. Biol. Chem. 266, 16007-16014