Various factors are involved in the heat shock-induced inhibition of protein synthesis. Changes upon heat shock in phosphorylation, leading to inactivation, of eukaryotic initiation factors (eIFs) eIF2 and eIF4E have been shown for several cell types. However, in mammalian cells these changes occur at temperatures of 43 °C or higher while protein synthesis is already affected at milder heat shock temperatures. In searching for the cause for the inhibition of protein synthesis, the regulation of eIF2 and eIF4E by additional factors was analyzed. In this respect, the activity of eIF2B was measured during and after heat shock. A very clear correlation was found between the activity of this guanine exchange factor and the levels of protein synthesis, also at mild heat shock conditions. Changes in the phosphorylation of eIF4E and of the eIF4E-binding protein PHAS-I were also analyzed. Surprisingly, in H35 cells as well as in some other cell lines, PHAS-I phosphorylation was increased by heat shock, whereas in others it was decreased. Therefore, decreasing the eIF4E availability under stressful conditions does not seem to be a general mechanism to inhibit protein synthesis by heat shock. Regulation of eIF2B activity appears to be the main mechanism to control translation initiation after heat shock at mild temperatures.

In incubating mammalian cells at elevated temperatures inhibits the translation of mRNAs (1–4). The translational lesion in heat-shocked cells principally occurs at the initiation step of translation (1, 5–7; for review, see Ref. 8). In the initiation process, the 7-methyl guanosine cap at the 5' end of the mRNA is bound by a complex of eukaryotic initiation factors (eIFs), including the cap-binding protein eIF4E and the RNA helicase eIF4A. Unwinding of the RNA enables recognition of the AUG initiation codon by the 43 S ribosomal complex and delivery of initiator methionyl tRNA. Then the large ribosomal subunit joins the complex, and peptide synthesis begins (8).

Both the concentration and activity of protein synthesis initiation factors and the primary sequence of the 5'-untranslated region of an mRNA can affect the rates of eukaryotic translation initiation. With respect to the heat shock-induced modifications of eukaryotic protein synthesis initiation factors, it is of interest that the activity of many initiation factors can be regulated by phosphorylation. It has been shown that changes in the phosphorylation state of various of these eukaryotic initiation factors, such as eIF2, eIF2B, eIF4B, and eIF4E, could coincide with changes in protein synthesis (9, 10).

The proteins eIF2 and eIF2B act to bring Met-tRNA to the 40 S ribosomal subunit: eIF2 by binding to Met-tRNA and GTP, and eIF2B by replacing GDP on eIF2 for GTP. Previous studies from several laboratories have focused on heat shock-induced phosphorylation of eIF2α (11–13). However, it was found that less severe heat shocks that inhibit protein synthesis by more than 70% do not elicit this phosphorylation. Duncan and Hershey (13) suggested that eIF2B might be a key player in the inhibition of protein synthesis under mild heat shock conditions. In this paper, we have studied the effect of heat shock temperatures in the range of 41–44 °C on eIF2B activity.

Phosphorylation of eIF4E appears to be required for efficient cap binding and formation of the 48 S preinitiation complex (14–16). Previous studies have demonstrated that heat shock leads to eIF4E dephosphorylation in various cell types (13, 17–19). However, in mammalian cells this phenomenon of dephosphorylation, similar to phosphorylation of eIF2α, mainly occurs at temperatures of 43 °C or higher while protein synthesis is inhibited already at lower temperatures (13). Therefore, eIF4E dephosphorylation cannot be the main determinant of the inhibition of protein synthesis as induced by heat shock. However, alternative types of regulation of eIF4E activity that could be involved in the decrease of protein synthesis in heat-shocked cells have become available by recent studies, using growth factors and insulin to stimulate protein synthesis. Besides phosphorylation-dephosphorylation of eIF4E, eIF4E activity can be regulated through eIF4E-binding proteins (4E-BPs) (20, 21). The 4E-BPs compete for a binding site on eIF4E with eIF4G, the largest subunit of the eIF4F cap-binding complex (22, 23). Binding of the 4E-BPs to eIF4E is regulated by phosphorylation of the binding proteins; enhanced phosphorylation leads to dissociation of the eIF4E-4E-BP complex, increasing the amount of eIF4E available for eIF4F formation (21, 24). We have studied the effects of heat shock on one of the 4E-BPs, PHAS-I, or 4E-BP1.

The strong changes in protein synthesis during mild heat shock of H35-Reuber hepatoma cells are associated with the rapid inactivation of eIF2B while an unexpected phosphorylation of PHAS-I was measured due to heat shock. This suggests that at these temperatures protein synthesis is mainly regulated by this eIF2B, and not by the activity of cap-binding complexes.

**EXPERIMENTAL PROCEDURES**

*Cells and Culture Conditions—Rat hepatoma Reuber H35 cells were grown in Leibowitz (L15) medium (Flow/ICN Laboratories) containing...*
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RESULTS

Protein Synthesis—Heat shock of cells leads to a rapid decrease in protein synthesis, mainly caused by inhibition of translation initiation processes (2, 7). The subsequent time needed for recovery of protein synthesis is correlated with the duration and severity of the stress conditions (30, 31). Rat H35-Reuber hepatoma cells were used to study protein synthesis during and after heat shock and to study the effects of these heat shocks on the activity or phosphorylation of various translation initiation factors. The sensitivity of protein synthesis of H35 cells for exposure to elevated temperatures (range 41–44 °C) and the ability to recover at 37 °C from inhibition of protein synthesis are shown in Fig. 1A. In these experiments, cells were kept at the indicated temperatures for the appropriate time and incubated with [35S]methionine/cysteine for the last 15 min.

All used temperatures caused a rapid and drastic inhibition of protein synthesis of at least 80% when protein synthesis was measured during the last 15 min of the heat shock period. Even stronger inhibition was found 30 min after the stress at 43 and 44 °C while cells that were incubated at 41 or 42 °C already started to recover during this period. Cells that were exposed to mild heat shock temperatures had regained control levels of protein synthesis after 4 h while protein synthesis in cells that were shocked at 43 or 44 °C was still impaired after that time period.

eIF2B Activity—Because changes in the activity of GTP-GDP exchange factor eIF2B will influence translation initiation on all mRNAs, this five-subunit complex is a good candidate to be affected by heat shock. Therefore, we have determined whether changes in eIF2B activity coincide with the changes in [35S]methionine/cysteine incorporation after heat shock treatment. eIF2, bound in vitro to [3H]GDP, was added to cell lysates from cells that were heat treated similarly to the samples in Fig. 1A. Active eIF2B in the cell extracts will exchange the labeled GDP for unlabeled GTP, which can be measured by retention of labeled complexes on nitrocellulose filters.

A very good correlation exists between eIF2B activity and overall protein synthesis in heat-shocked H35 cells (Fig. 1B). The lower heat shock temperatures of 41 and 42 °C caused a rapid and transient inhibition of eIF2B activity of approximately 70%. Cells shocked at either 41 or 42 °C showed recovery of eIF2B activity immediately after the heat shock while eIF2B activity in cells exposed to 43 or 44 °C only recovered partially over a 4-h interval.

Phosphorylation of eIF2α—Inactivation of eIF2B can occur by several mechanisms, of which phosphorylation of eIF2α and subsequent sequestering of eIF2B is the best-known. We tried to determine the extent of eIF2α phosphorylation by isoelectric focusing and Western blotting. Although these blots indicated that eIF2α phosphorylation did not occur at the lower temperatures, we could not produce a consistent result. Fortunately, we obtained an antibody from Dr. Gary Kause that specifically recognizes phosphorylated eIF2α. With this antibody, the phosphorylation state of eIF2α after heat shocks at 40.5–44 °C was determined by SDS-PAGE and Western blotting (Fig. 2, bottom). To this end, equal amounts of eIF2α were loaded in the separate lane and amounts of the lysates were analyzed with an antibody that recognizes both forms of eIF2α (top). At 40.5 and 41 °C, no change in eIF2α phosphorylation occurred (lanes 2 and 3) while at 41 °C a major decrease in eIF2B activity was measured (Fig. 1). Further increase of the heat shock temperature resulted in a gradual increase in eIF2α phosphorylation that reached its maximal level at 42.5 °C.
Phosphorylation of eIF2—Besides a reduction of protein synthesis, the heat shock response is characterized by the onset of translation of the heat shock mRNAs. Cap-binding proteins are thought to play a role in this process, and, for example in HeLa cells, diminished phosphorylation of eIF4E was found (13). However, this reduction was found at temperatures of 43 °C and higher. Therefore, we studied the possible involvement of eIF4E-binding proteins in inhibition of protein synthesis of H35 cells. Since cell types show different sensitivities to heat stress, we have first determined whether H35 cells showed similar eIF4E dephosphorylation characteristics as HeLa cells, which have been studied thoroughly (13) (Fig. 3).

After 30 min at 43 and 44 °C eIF4E was almost completely dephosphorylated (Fig. 3, lanes 4 and 5). No decrease in eIF4E phosphorylation was found at 41 °C (lane 2) while at 42 °C eIF4E phosphorylation was reduced by approximately 50%. Nevertheless, the 41 °C did result in a severe inhibition of protein synthesis (Fig. 1). Reduced protein synthesis at mild heat shock temperatures is apparently not due to dephosphorylation of eIF4E, similar to the results obtained with HeLa cells (13).

Phosphorylation of PHAS-I—In parallel with changes in the phosphorylation state of eIF4E, the activity of eIF4E can also be controlled by changes in the activity of the eIF4E-binding protein, PHAS-I. Binding of PHAS-I to eIF4E results in reduced availability of eIF4E for eIF4F complex formation, which is needed for cap-dependent translation (32). The binding activity of PHAS-I is controlled by changes in the phosphorylation state of PHAS-I because the dephosphorylated forms of PHAS-I bind eIF4E more tightly. Therefore, a reduced PHAS-I phosphorylation is expected in heat-shocked cells with inhibited protein synthesis. The distribution of PHAS-I among its phosphorylated forms was determined during heat shock and recovery (Fig. 4A).

Surprisingly, heat shock led to a strong increase in the phosphorylation state of PHAS-I. PHAS-I phosphorylation was increased slightly at 42 °C while at 43 and 44 °C the highly phosphorylated forms of PHAS-I were the predominant forms as compared with the control cells (Fig. 4A, lane 16). Phosphorylation of PHAS-I continued after the cells were returned to 37 °C (compare lanes 2 and 3, 7 and 8, and 12 and 13) while during further recovery dephosphorylation occurred (lanes 4...
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Figure 3. Phosphorylation state of eIF4E after heat shock. Cells were incubated for 30 min at the indicated temperatures before harvesting in 75 μl of ISB. Samples of 25 μl were analyzed by one-dimensional isoelectric focusing and Western blotting. The phosphorylated and unphosphorylated forms of eIF4E are indicated on the right. The percentage of phosphorylated eIF4E as determined by quantification with a densitometer (Molecular Dynamics) is given at the bottom of the figure.

Figure 4. PHAS-I phosphorylation during and after heat shock. A, cells were heat-shocked for the times indicated above the figure. Cells were harvested during or after the heat shock in 75 μl of LSB. Samples of 15 μl were separated by SDS-PAGE and analyzed by Western blotting as described under "Experimental Procedures." The isoforms of PHAS-I are indicated on the right. B, the γ-form of PHAS-I represents a highly phosphorylated species of PHAS-I. After a heat shock for 30 min at 43 °C, cells were harvested and extracts were prepared as described under "Experimental Procedures." Extracts were incubated in the absence or presence of calf intestinal alkaline phosphatase as indicated and analyzed by SDS-PAGE and Western blotting.

and 5, and 9 and 10). A minor shift from the β-form to the more phosphorylated γ-form was found at 41 °C (not shown). The increase in phosphorylation as well as the time needed for dephosphorylation was correlated with the heat shock temperature.

The inhibition of protein synthesis (Fig. 1) and the enhanced phosphorylation of PHAS-I (Fig. 4A) in heat-shocked cells is opposite to enhanced phosphorylation of PHAS-I as found after growth factor and insulin stimulation of protein synthesis. This suggests that protein synthesis is not regulated by phosphorylation of PHAS-I under these conditions and that PHAS-I phosphorylation serves other, unknown purposes than regulation of eIF4E availability.

To establish the nature of the γ-form of PHAS-I, that was found at all three temperatures in Fig. 3A, cell extracts were treated with the alkaline phosphatase CIP (Fig. 4B). Thus, three forms of PHAS-I were detected in extracts from untreated cells: α, β, and γ (lane 1). In heat-shocked cells, the γ-form was found (lane 3), as in Fig. 3A. Treatment of these extracts with CIP resulted in conversion of γ- and β-forms to the α- and β-forms of PHAS-I (lanes 2 and 4), confirming that γ-PHAS-I was a phosphorylated form of PHAS-I. The γ-form of PHAS-I was also detected in insulin-treated rat muscle cells (34). The absence of conversion of the phosphorylated β-form (20) to the unphosphorylated α-form is not understood. Similar experiments with potato acid phosphatase also failed in dephosphorylating this β-form (not shown).

Four other cell lines, A14, CHO, CCL39, and PC12, were used to investigate whether the fourth form of PHAS-I was unique for rat hepatoma H35 cells or whether this highly phosphorylated form was also found in other cells after heat shock (Fig. 5). In mouse fibroblastic A14 cells and rat pheochromocytoma PC12 cells, heat shock also resulted in strong phosphorylation of PHAS-I as in H35 cells. Therefore, heat shock-induced phosphorylation of PHAS-I is not restricted to H35 cells but occurs in cell lines from different origins. However, phosphorylation of PHAS-I was not found in two Chinese hamster cell lines, CHO and CCL39, upon heat shock. In these cell lines, dephosphorylation of this eIF4E binding protein occurred. These unexpected results will be discussed later.

Dissociation of eIF4E-PHAS-I Complexes—Phosphorylation of PHAS-I has been correlated with increased protein synthesis after insulin administration (34). Under these conditions eIF4E could not longer bind to PHAS-I, making eIF4E available for translation initiation. In contrast, heat shock increased phosphorylation of PHAS-I in H35 cells and severely inhibited protein synthesis (Figs. 1 and 4A). In Fig. 6, we determined whether heat shock induced the dissociation of eIF4E-PHAS-I complexes after heat shock of H35 cells. For comparison, CHO cells were used in which heat shock induced PHAS-I dephosphorylation.

H35 and CHO cells were heat-shocked at 44 °C for 30 min and returned to 37 °C for 1 or 6 h after the heat shock. In both cell lines, a decrease of protein synthesis to 10–20% of the control cells was found after heat shock as well as in cells that were replaced at 37 °C for 1 h. After recovery for 6 h, protein synthesis in both cell lines was 70–80% compared with the untreated cells. Apparently, both cell lines have similar kinetics for the levels of protein synthesis after heat shock. Fig. 6A, top panel, shows the pattern of PHAS-I phosphorylation in the various samples. Heat shock induced a strong increase in PHAS-I phosphorylation (as shown in Fig. 4) immediately after the heat shock (lanes 2 and 3) while a recovery period of 6 h resulted in reduced PHAS-I phosphorylation (lane 4), comparable with the control cells (lane 1). The opposite effect, although less pronounced, was found in CHO cells; heat shock resulted in dephosphorylation of PHAS-I while, after a 6-h recovery period, a slight increase in PHAS-I phosphorylation was detectable.

Complexes of eIF4E and PHAS-I from these samples were purified by m7GTP-Sepharose affinity chromatography (Fig. 6A, bottom panel). Similar amounts of eIF4E were recovered in all samples showing that m7GTP-binding activity of eIF4E was not changed drastically due to heat shock (18). However, in H35 cells, the ability of PHAS-I to bind eIF4E was dramatically reduced after heat shock (lanes 2 and 3). Apparently, phosphorylation of PHAS-I after heat shock (lanes 1 and 2) abolished complex formation between PHAS-I and eIF4E. After the recovery period, in which PHAS-I dephosphorylation occurred, an increase in eIF4E-PHAS-I complex formation was found (lane 4). A strong increase in the binding of PHAS-I to eIF4E was found in CHO cells (lanes 6 and 7). The amount of associated PHAS-I after recovery (lane 8) was somewhat higher than expected.

Fig. 6B shows a schematic representation of the quantification of the results in Fig. 6A. Phosphorylation of PHAS-I (black bars) increased from 37 to 85% in H35 cells, with a concomitant
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The contradictory results on PHAS-I phosphorylation after heat shock occurs via a pathway that also regulates p70S6K activity and PHAS-I phosphorylation. H35 cells were heat-shocked at 43 °C and allowed to recover for the indicated times either in the absence (lanes 1–6) or presence (lanes 7–11) of rapamycin (25 ng/ml). The cells were harvested in 75 µl of buffer A as described under “Experimental Procedures.” Aliquots of the obtained cell lysates were incubated with reticulocyte 80 S ribosomes in the presence of [γ-32P]ATP as described under “Experimental Procedures.” The samples were analyzed by SDS-PAGE and autoradiography. The part of the film with the phosphorylated S6 protein is shown.

FIG. 7. Heat shock-induced activation of p70S6K. A, p70S6K activity and PHAS-I phosphorylation. H35 cells were heat-shocked at 43 °C and allowed to recover for the indicated times either in the absence (lanes 1–6) or presence (lanes 7–11) of rapamycin (25 ng/ml). The cells were harvested in 75 µl of buffer A as described under “Experimental Procedures.” The part of the film with the phosphorylated S6 protein is shown.

Dissociation on eIF4E-PHAS-I complexes (gray bars). In the heat-shocked cells, an approximately 10-fold decrease in PHAS-I binding was found. In the cells that were allowed to recover for 6 h, PHAS-I phosphorylation and its association with eIF4E were similar to the situation found in the non-shocked cells. The approximately 40% dephosphorylation of PHAS-I found after heat shock of CHO cells, resulted in a 7-fold increase of PHAS-I binding to eIF4E.

p70S6K Activity—The contradictory results on PHAS-I phosphorylation after heat shock resembles the findings on the effect of heat shock on phosphorylation of S6, one of the proteins of the small ribosomal subunit. Phosphorylation as well as dephosphorylation of this protein under stress conditions has been reported (35–41). As activation of p70S6K and PHAS-I phosphorylation seem to be regulated by similar signaling pathways (28, 42–45), we have studied the effect of heat shock on p70S6K, one of the kinases that phosphorylates S6. To determine a possible correlation between PHAS-I phosphorylation and p70S6K activity after heating H35 cells, cells were shocked in the absence or presence of rapamycin, a specific inhibitor of the FRAP pathway (46, 47). The activity of p70S6K was determined under these conditions (Fig. 7A).

The activity of p70S6K in H35 cells was enhanced during heat shock at 43 °C (Fig. 7A, lanes 2–6). Maximal S6 phosphorylation was found at the end of the 30-min heat shock period (lane 3). At this point, activity was enhanced 2.5-fold compared with cells that were not shocked (lane 1). During recovery, p70S6K activity was reduced, coinciding with dephosphorylation of PHAS-I. In the presence of rapamycin, phosphorylation of PHAS-I did not occur during or after heat shock (Fig. 7A, lanes 7–11). These results indicated that PHAS-I phosphorylation after heat shock occurs via a pathway that also regulates p70S6K activity.

In Fig. 4A, it was shown that PHAS-I phosphorylation diminished during the recovery period when the cells were shocked at mild temperatures. To further establish a positive correlation between p70S6K activity and PHAS-I phosphorylation, we have determined the p70S6K activity after recovery at 37 °C of heat-shocked cells (Fig. 7B).

At mildly elevated temperatures, 41 and 42 °C, the kinase lost its activity during the recovery period while at 43 °C, and more pronounced at 44 °C, p70S6K was still activated 4 h after the heat shock. The duration of p70S6K activation was remarkably similar to the duration of the increased PHAS-I phosphorylation status (Fig. 4A), suggesting a common activator of p70S6K and PHAS-I phosphorylation.

DISCUSSION

In search of factors whose activity might be compromised by heat shock, we found that eIF2B activity was rapidly inhibited upon heat shock. Within 15 min, even low heat shock temperatures reduced the activity of this factor to 40%. Recovery of
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protein synthesis coincided with recovery of eIF2B activity at the four temperatures that were tested (Fig. 1). Low heat shock temperatures induced a transient inhibition of translation and eIF2B activity while more severe heat shocks compromised protein synthesis and eIF2B activity for longer periods.

The inhibition of eIF2B activity at 41 °C was not caused by changes in eIF2α phosphorylation (Fig. 2). Mild heat shock temperatures also did not elicit a change in eIF2α phosphorylation in HeLa cells (13, 48), and in heat-shocked Drosophila cells, decreased protein synthesis without detectable increase in eIF2α phosphorylation has been observed also (19). An approximately 2-fold increase in eIF2α phosphorylation at temperatures of 41.5 °C or higher has been shown for Ehrlich cells (49), very similar to the results for H35 cells in Fig. 2. Our findings show that mild heat shock temperatures diminish eIF2β activity by a mechanism that is independent of eIF2α phosphorylation. Presumably, more severe heat shocks inactivate eIF2B by the same mechanism; however, under these conditions, eIF2α phosphorylation also occurs. Studies on the heat shock-induced inactivation of eIF2B are in progress.

To shed more light on the mechanism of preferential translation of mRNAs under stressful conditions, we have studied the effect of heat shock on the 4E-binding protein PHAS-I. Surprisingly, phosphorylation of PHAS-I was increased due to heat shock in rat hepatoma cells (Fig. 4A) but also in rat pheochromocytoma cells and in a cell line derived from mouse NIH-3T3 cells (Fig. 5). This phosphorylation resulted in dissociation of eIF4E-PHAS-I complexes (Fig. 6). Heat shock appeared to be a very strong activator of PHAS-I phosphorylation in these cell lines as a fourth phosphorylated form of PHAS-I, denoted as γ', was found routinely after heat shock. Treatment of extracts with alkaline phosphatase resulted in the disappearance of the γ and γ'-forms of PHAS-I (Fig. 4B), confirming that the γ'-form of PHAS-I is, next to the γ-form, a highly phosphorylated form of this protein.

Apparently, heat shock is an inducer of PHAS-I phosphorylation. Even low heat shock temperatures of 41 and 42 °C led to a shift to the more phosphorylated PHAS-I forms. This shift of PHAS-I to the γ'-form has not been noticed before in experiments on induction of cells with growth factors. In Figs. 4–7, samples were run on gels with a length of 10 cm while routinely minigel systems with 5-cm gels were used. The higher resolution on the longer gels could explain why the γ'-form of PHAS-I was detected in most lanes.

The possible function of PHAS-I phosphorylation during heat shock is intriguing. Phosphorylation of PHAS-I may influence translation of certain mRNAs. It has been shown that PHAS-I specifically regulates cap-dependent translation (21, 32), and it plays a role in the translation of mRNAs encoding for ribosomal proteins and elongation factors (50, 51). The heat shock-induced phosphorylation of PHAS-I might reflect the need for translation of specific mRNAs, such as the heat shock messengers. However, the heat shock messengers are thought to be translated under conditions in which eIF4E is limiting (reviewed in Ref. 3) while PHAS-I phosphorylation would actually lead to a situation in which more “free” eIF4E is present (Fig. 6). Independent regulation of eIF4E phosphorylation and PHAS-I phosphorylation has been described for 293 cells infected with adenovirus (52). It was suggested that eIF4E and its binding proteins can act in concert or in opposition to regulate cap-dependent translation (52). Our results with H35 and CHO cells imply that different signaling pathways were induced by heat shock in these cell lines. Interestingly, there is a large difference between H35 and CHO cells in the amount of PHAS-I that is bound to eIF4E under normal growth conditions. Whether this and the difference in heat shock-induced signaling pathway reflects differences in the regulation of cap-dependent and heat shock mRNA translation in these cell lines remains to be solved. Evidently, our results show that in various cell lines, PHAS-I activity does not govern the overall levels of protein synthesis after heat shock. Furthermore, its role in preferential translation of heat shock mRNAs is doubtful.

We have tried to determine whether specific heat shock-induced signaling transduction pathways were involved in the activation of eIF2B and phosphorylation of PHAS-I. Changes in glycosyn thase kinase 3 and casein kinase II, kinases that may regulate eIF2B activity (53, 54) were not found (not shown). The stress-activated p38 kinase was not involved as its specific inhibitor SB203580 did not influence heat shock-induced inhibition of eIF2B activity (not shown). Also, staurosporin, wortmannin, or rapamycin, inhibitors of various signaling pathways, did not affect the inhibition of eIF2B activity by heat shock (not shown). However, it should be noted that SB203580 reduced eIF2B activity in all extracts, either from control or heat-shocked cells. This suggests a possible role of p38 kinase in regulating eIF2B activity under normal growth conditions.

Phosphorylation of PHAS-I, as found in H35 cells, was likely achieved via a FRAP-dependent pathway, as shown by S6 kinase assays and by the inhibitory effect of rapamycin on the heat shock-induced PHAS-I phosphorylation (Fig. 7A). Higher temperatures led to a prolonged activation of p70S6K (Fig. 7B) and a further increase in PHAS-I phosphorylation (Fig. 4A). These results together strongly suggest a common heat shock-induced activator of these processes.

The conclusion from Figs. 5 and 6 is that stress has opposite effects on PHAS-I phosphorylation depending on the cell type. The apparently conflicting results on S6 phosphorylation or dephosphorylation under stress conditions that have been reported (35–41) are probably caused by analogous opposite effects dependent on cell type. In CHO and CCL39 cells p70S6K activity was also diminished after heat shock (not shown), coinciding with the decreased phosphorylation of PHAS-I (Fig. 5) while in H35 cells the opposite results were obtained. Apparently, heat shock induces FRAP activity in some cell lines leading to p70S6K activation and PHAS-I phosphorylation while in other cells FRAP activation does not occur, or might be reduced, after heat shock resulting in reduced p70S6K activity and PHAS-I dephosphorylation.

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