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Increased rates of decay and reduced levels of accumulation of the major poly(A)-associated proteins of *Dictyostelium* during heat shock and development

(mRNA-binding proteins/heat shock proteins/translational initiation/developmental regulation)

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ABSTRACT **Two major polypeptide species, 31,000 M_r (p31) and 31,500 M_r (p31.5), are associated with the 3′ poly(A) tails of *Dictyostelium* mRNAs. We have measured the accumulation of newly synthesized p31 and p31.5 and the decay of preexisting p31 and p31.5 during heat shock and early development. Only trace amounts of newly synthesized p31 and p31.5 accumulate at elevated temperatures, indicating that these polypeptides are not heat shock proteins. In addition, preexisting p31 and p31.5 are rapidly degraded in heat-shocked cells. This degradation is selective and occurs simultaneously with a sharp drop in the rate of translational initiation. Similarly, in early development, a time when the rate of translational initiation is also sharply reduced, only trace amounts of newly synthesized p31 and p31.5 accumulate and most of the preexisting p31 and p31.5 is rapidly degraded. When translational elongation is inhibited with cycloheximide, preexisting p31 and p31.5 remain stable. Therefore, a correlation seems to exist between the abundance and stability of these poly(A)-associated proteins and the rate of translational initiation. Our results are consistent with the proposed role of the poly(A)–protein complex in translation and do not support the findings of Schönfelder *et al.* [Schönfelder, M., Horsch, A. & Schmid, H.-P. (1985) *Proc. Natl. Acad. Sci. USA* 82, 6884–6888] that the 73,000 M_r HeLa cell poly(A)-binding protein and the major 73,000 M_r mammalian heat shock protein (i.e., hsp70) are identical.**

The precise function of the 3′ poly(A) tract present on most eukaryotic mRNAs has yet to be elucidated. Previous work from this and other laboratories (1–5) has suggested that poly(A) plays a role in the efficient translation of poly(A)$^+$ mRNA and has led to the hypothesis that poly(A) and its associated proteins [i.e., poly(A)-binding proteins] function at the level of translational initiation (4). Our recent finding that the sharp reduction in the rate of translational initiation observed in early developing *Dictyostelium* cells (6–8) occurs in parallel with the rapid and selective degradation of most of the preexisting poly(A)-associated protein (9) provides additional evidence that poly(A)-binding proteins function in translational initiation.

A significantly reduced rate of translational initiation is also observed in heat-shocked HeLa cells (10–12). It was with interest, therefore, that we read the recent report of Schönfelder *et al.* (13), in which it was concluded that the 73,000 M_r HeLa cell poly(A)-binding protein and the major 73,000 M_r mammalian heat-shock protein (i.e., hsp70) are identical. At least two sets of observations that we and others have made seem to be inconsistent with this conclusion. (*i*) The coding sequence of the yeast poly(A)-binding protein gene has been recently published (14, 15), and comparison of this sequence with the earlier published partial coding sequence of the yeast *hsp70* gene (16) indicates that these genes and their encoded polypeptides are not related. (*ii*) Polyclonal antibodies raised against p31 and p31.5, the closely related, major poly(A)-associated proteins of *Dictyostelium*, do not cross-react with the hsp70s found in non-heat-shocked *Dictyostelium* cells (refs. 9, 17, 18; see also Fig. 3). The *Dictyostelium* hsp70s, however, have been shown to be immunologically cross-reactive with the hsp70s of higher organisms (19, 20).

Dictyostelium cells, like HeLa cells, incorporate reduced amounts of exogenous (i.e., labeled) amino acids into protein during heat shock (10–12, 17). We report here that this reduced incorporation can be explained by a sharp reduction in the rate of translational initiation. In addition, we show that preexisting p31 and p31.5 are rapidly and selectively degraded during heat shock. As noted above, the sharp reduction in the rate of translational initiation observed in early developing cells is also accompanied by the selective degradation of most preexisting p31 and p31.5. In this report, we further show that newly synthesized p31 and p31.5 do not accumulate in heat-shocked or developing cells and that these polypeptides remain stable when translational elongation is inhibited with cycloheximide. Our results demonstrate that the poly(A)-associated proteins of *Dictyostelium* are not heat shock proteins and that the stability and abundance of these polypeptides are probably linked to the level of translational initiation.

MATERIALS AND METHODS

Materials. Phenylmethylsulfonyl fluoride, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone, Nonidet P-40, L-methionine, and cycloheximide were purchased from Sigma. *Staphylococcus aureus* nuclease (micrococcal nuclease) was obtained from Boehringer Mannheim. Tran^{35}S-label was purchased from ICN Radiochemicals. All other reagents were obtained from the sources described in ref. 9.

Cell Culture, Labeling, and Chase Conditions. *Dictyostelium discoideum* strain AX-3 was grown axenically and plated for development as described (9). Exponentially growing (i.e., normal) cells were heat shocked by transferring their culture flasks from a shaking waterbath maintained at 22°C to one maintained at 30°C. The protein in normal, heat-shocked, and developing cells was labeled with L-[^{35}S]methionine (50 or 100 μCi/ml; 1 Ci = 37 GBq), except when translational elongation rates were measured (see below). In most experiments, the decay of prelabeled protein in cells subjected to different treatments was monitored. The cells used in these chase experiments were grown in the presence of label for 0.5–3.0 generations (4–24 hr) prior to use. To initiate a chase, the cells were pelleted from the labeling medium and either

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resuspended in fresh growth medium with additional L-methionine (1 mM or 5 mM) or washed and plated for development. If the cells were resuspended in growth medium with 1 mM L-methionine, additional methionine was added at 2-hr intervals to raise the concentration in 1 mM increments.

Cell Fractionation. Whole cell lysates were prepared from normal and heat-shocked cells by pelleting the cells (1000 × *g*, 2.5 min, 4°C) and lysing them by freezing and thawing in 1% Nonidet P-40/12% sucrose/20 mM $NH_4C_2H_3O_2$/0.4 mM $CaCl_2$/0.2 mM EDTA/1 mM phenylmethylsulfonyl fluoride/1 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone/50 mM Hepes, pH 7.5. The cell densities during lysis were ≈1 × 10^8 per ml. Postmitochondrial supernatants were normal and heat-shocked cells for use in immunoprecipitation experiments were prepared as described above except that the cells were lysed by Vortex mixing, instead of freezing and thawing, and the lysates were centrifuged at 12,000 × *g* for 10 min to pellet the nuclei and mitochondria. Postmitochondrial supernatants from developing and cycloheximide-treated cells were prepared as described (9). Ribosome distributions in normal and heat-shocked cells were analyzed by using 15–50% sucrose gradients as described (9) except that the Cemulsol concentration in the lysis buffer was raised to 2% and cycloheximide (50 μg/ml) was added to the lysis buffer and to the cell cultures immediately before harvesting.

Immunoprecipitations and Protein Assays. Immunoprecipitations using rabbit polyclonal anti-p31/p31.5 and *S. aureus* cells were done essentially as described (9). When p31 and p31.5 were precipitated from whole cell lysates, micrococcal nuclease (100 units/ml) was added to the lysates 5 min prior to the addition of antibodies. In addition, the precipitates from whole cell lysates were washed with NET buffer (150 mM NaCl/1 mM EDTA/0.5% Nonidet P-40/0.02% NaN_3/20 mM Tris, pH 7.5) containing 0.4% $NaDodSO_4$. Previous experiments have shown that antigen–antibody complexes remain bound to *S. aureus* in the presence of 0.4% $NaDodSO_4$ (R.E.M., unpublished results). The lysates or extracts used in the immunoprecipitation reactions were adjusted (with lysis buffer) such that the final protein concentration, cell concentration, or number of hot trichloroacetic acid (Cl_3CCOOH)-precipitable (10% Cl_3CCOOH, 90°C, 10 min) cpm/ml in each sample was the same. Equal numbers of Cl_3CCOOH-precipitable cpm/ml were used in most experiments in which the accumulation or decay of p31 and p31.5 in growing and nongrowing cells (i.e., heat-shocked or developing cells) or cells exhibiting different growth rates (i.e., with or without cycloheximide) was directly compared. The immunoprecipitated proteins were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography (9), and the relative amounts of p31 and p31.5 were measured either by densitometry or by scintillation spectrometry of excised, solubilized (in 3.5% Protosol) gel slices. The decay of total labeled protein was measured by determining the number of hot Cl_3CCOOH-precipitable cpm remaining as a function of time per 10^8 cells. In those experiments in which the samples were adjusted to give equal numbers of Cl_3CCOOH-precipitable cpm/ml, the absolute decay rates for p31 and p31.5 were calculated after compensating for the decay of total labeled protein. The method of Lowry *et al.* (21) was used to determine the total protein concentration in specific samples.

Translational Elongation Rate Measurements. Polypeptide chain-elongation rates in normal and heat-shocked cells were measured essentially as described by Fan and Penman (22) and Cardelli and Dimond (7). In these experiments, the cells were labeled with Tran^{35}S-label at a concentration of 100 μCi/ml.

RESULTS

Newly Synthesized p31 and p31.5 Do Not Accumulate in Early Developing Cells. We have shown that most of the preexisting p31 and p31.5 in early developing *Dictyostelium* cells is rapidly and selectively degraded (9). This degradation occurs at a time when the rate of translational initiation is reduced by 3- to 5-fold (6–8). Fig. 1 shows that newly synthesized p31 and p31.5 do not accumulate in early developing cells, strengthening our conclusion that there is a 5-fold net reduction in the levels of these polypeptides early in development (9).

Reduced Rate of Translational Initiation in Heat-Shocked Cells. When the incubation temperature of growing *Dictyostelium* cells is shifted from 22°C to 30°C, the heat-shock response is induced (17). At 30°C, cell division ceases and the overall rate of protein synthesis is reduced (17). Heat-shocked *Dictyostelium* cells incorporate only half as much [^{35}S]methionine into Cl_3CCOOH-precipitable protein as cells labeled at 22°C (R.E.M., unpublished results). A similar reduction in the overall rate of labeled amino acid incorporation has been observed in heat-shocked HeLa cells (10, 11), and this reduction has been shown to result from a decreased rate of translational initiation (10–12). To determine whether the rate of translational initiation is also reduced in heat-shocked *Dictyostelium* cells, we compared the ribosome distributions of normal and heat-shocked cells (Fig. 2). In these experiments, postmitochondrial supernatants from both cell types were centrifuged into 15–50% linear sucrose gradients and the percentage of total ribosomes present as monosomes was calculated.

In normal cells, 8–10% of the ribosomes are present as monosomes (see Fig. 2*A*, 80S peak). In heat-shocked cells, however, 40–45% of the ribosomes are present as monosomes (see Fig. 2*B*). The most likely explanation for the increased number of monosomes in heat-shocked cells is that the rate of translational initiation is sharply reduced at elevated temperatures. When heat-shocked cells are treated with cycloheximide at a concentration of 50 μg/ml, the overall rate of protein synthesis is reduced by >90% (data not shown) and the ribosome distribution appears normal (see Fig. 2*C*). This result indicates that the 80S monosomes of heat-shocked cells are translationally competent and that a larger than normal pool of translatable mRNA is not associated with polysomes during heat shock. In another experiment, we determined that the rates of polypeptide chain elongation in heat-shocked and normal cells are not significantly different (data not shown). We conclude, therefore, that the rate of translational initiation in heat-shocked *Dictyostelium* cells is reduced by 4- to 5-fold, a reduction

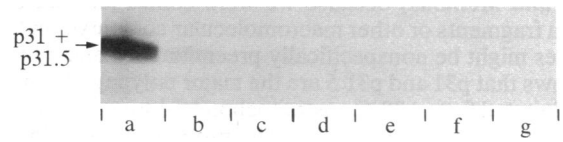

FIG. 1. Accumulation of newly synthesized p31 and p31.5 in developing cells. *Dictyostelium* cells were labeled for 2 hr with [^{35}S]methionine (100 μCi/ml) either during exponential growth or at various times during development. After labeling, the cells were harvested and postmitochondrial supernatants were prepared. The supernatants were adjusted (with lysis buffer) to give equivalent numbers of hot Cl_3CCOOH-precipitable cpm/ml, and the p31 and p31.5 in 200-μl aliquots of these supernatants were immunoprecipitated and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography. The zero time of development is designated as the time at which the cells are first removed from growth medium. Lanes: a, exponentially growing cells; b, 0.5–2.5 hr of development; c, 2.5–4.5 hr of development; d, 4.5–6.5 hr of development; e, 6.5–8.5 hr of development; f, 8.5–10.5 hr of development; g, 10.5–12.5 hr of development.

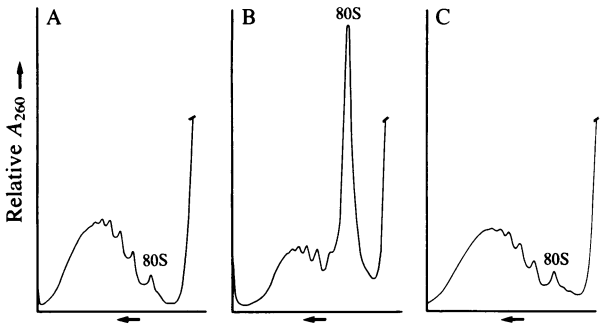

FIG. 2. Reduced translational initiation in heat-shocked cells. Postmitochondrial supernatants were prepared from normal (i.e., 22°C) cells, cells that were heat-shocked for 1 hr (30°C), and cells that were heat-shocked for 1 hr and incubated an additional 30 min at 30°C in the presence of cycloheximide (50 μg/ml). Aliquots of these supernatants were layered onto 15–50% linear sucrose gradients and centrifuged as described in ref. 9. (*A*) Normal cell supernatant. (*B*) Heat-shocked cell supernatant. (*C*) Supernatant from heat-shocked cells incubated with cycloheximide. The direction of sedimentation and the positions of the 80S monosome peaks are designated.

similar in size to the one observed in early developing cells (6–8).

Newly Synthesized p31 and p31.5 Do Not Accumulate During Heat Shock. A comparison of the polypeptides made in heat-shocked cells with those made in normal cells reveals that hsp70 and a larger heat shock protein (82,000 M_r, hsp82) accumulate to high levels during heat shock (ref. 17; Fig. 3, lanes a and g). Such a comparison also reveals that many of the polypeptides synthesized at 22°C continue to be made at 30°C (ref. 17; compare lanes a and g, Fig. 3). This result is consistent with the idea that normal mRNAs (i.e., non-heat shock mRNAs) are not inactivated or destabilized during heat shock, although they probably are less efficiently translated (see also ref. 17).

In the experiment shown in Fig. 3, *Dictyostelium* cells were incubated for 1 hr either at 22°C or 30°C and then labeled with [^{35}S]methionine for 2 hr at the same temperatures. The relative abundance of newly synthesized p31 and p31.5 in postmitochondrial supernatants and whole cell lysates prepared from these cells was measured by immunoprecipitation to determine whether p31 and p31.5 accumulate to higher levels during heat shock. Immunoprecipitation of the protein in whole cell lysates was performed in consideration of the possibility that some p31 and p31.5 might be localized in a cellular compartment other than the soluble cytoplasm during heat shock. The immunoprecipitates from whole cell lysates were washed with and without 0.4% NaDodSO$_4$ (see *Materials and Methods*) because we were concerned that chromatin fragments or other macromolecular complexes in these lysates might be nonspecifically precipitated. Lane b of Fig. 3 shows that p31 and p31.5 are the major polypeptide species precipitated from a 22°C postmitochondrial supernatant using anti-p31/p31.5 and *S. aureus*. The proteins not precipitated from this extract are shown in lane c. Fig. 3, lanes d–f, show that similar results are obtained by using a whole cell lysate and demonstrate that the immunoprecipitates from such lysates must be washed with 0.4% NaDodSO$_4$ to remove nonspecifically bound proteins (compare lanes d and e). The major contaminant in whole cell immunoprecipitates not washed with NaDodSO$_4$ is actin. Lanes h and i of Fig. 3 show the proteins precipitated and not precipitated, respectively, from the 30°C postmitochondrial supernatant. Only trace amounts of labeled p31 and p31.5 were precipitated from this extract (faint bands in lane h). Identical results were obtained by using the 30°C whole cell lysate (see Fig. 3, lanes j–l). Thus, newly synthesized p31 and p31.5 do not accumulate during heat shock (i.e., they are not heat shock proteins).

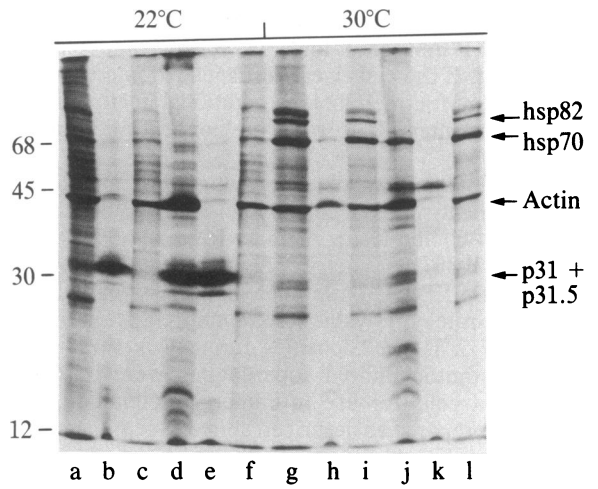

FIG. 3. Accumulation of newly synthesized p31 and p31.5 in normal and heat-shocked cells. Exponentially growing *Dictyostelium* cells were incubated for 1 hr at either 22°C or 30°C and then labeled for 2 hr, at the same temperatures, with [^{35}S]methionine (50 μCi/ml). After labeling, the cultures were divided into two equal parts to prepare a postmitochondrial supernatant and a whole cell lysate from each. Immunoprecipitation reactions, using anti-p31/p31.5, were performed on 200-μl aliquots of these lysates and supernatants, and the immunoprecipitated and nonprecipitated proteins were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis and fluorography. Lanes: a, total cellular protein, 22°C; b, immunoprecipitated protein, 22°C postmitochondrial supernatant; c, nonprecipitated protein, 22°C postmitochondrial supernatant; d, immunoprecipitated protein, 22°C whole cell lysate, precipitate not washed with 0.4% NaDodSO$_4$; e, immunoprecipitated protein, 22°C whole cell lysate, precipitate washed with 0.4% NaDodSO$_4$; f, nonprecipitated protein, 22°C whole cell lysate; g, total cellular protein, 30°C; h, immunoprecipitated protein, 30°C postmitochondrial supernatant; i, nonprecipitated protein, 30°C postmitochondrial supernatant; j, immunoprecipitated protein, 30°C whole cell lysate, precipitate not washed with 0.4% NaDodSO$_4$; k, immunoprecipitated protein, 30°C whole cell lysate, precipitate washed with 0.4% NaDodSO$_4$; l, nonprecipitated protein, 30°C whole cell lysate. The positions of molecular weight markers in this gel are designated: 68, bovine serum albumin (68,000 M_r); 45, ovalbumin (45,000 M_r); 30, carbonic anhydrase (30,000 M_r); 12, cytochrome *c* (12,000 M_r). The positions of hsp82, hsp70, actin, and p31 and p31.5 are also designated.

Selective Degradation of p31 and p31.5 During Heat Shock. In the preceding sections, we have shown that the rate of translational initiation in heat-shocked *Dictyostelium* cells is reduced by 4- to 5-fold and that only trace amounts of newly synthesized p31 and p31.5 accumulate at elevated temperatures. In addition, we have shown that newly synthesized p31 and p31.5 do not accumulate during early development, a time when translational initiation is similarly reduced up to 5-fold. Since most of the preexisting p31 and p31.5 in early developing cells is rapidly and selectively degraded (9), we were interested in determining whether preexisting p31 and p31.5 suffered a similar fate during heat shock. Therefore, cells were labeled with [^{35}S]methionine at 22°C for three generations and the decay of labeled p31 and p31.5 and total protein was monitored during a 4-hr chase at 30°C. The chase period was limited to 4 hr because *Dictyostelium* cells maintained at 30°C die with a half-life ($t_{1/2}$) of 18 hr (17). At 4 hr, ≈85–90% of the cells remain viable, and the surviving cells begin to grow again at 22°C with no appreciable lag (17).

Fig. 4*C* shows that preexisting p31 and p31.5 are rapidly degraded during heat shock. We estimate that these polypeptides decay with initial $t_{1/2}$ values of ≈25 min in heat-shocked cells. Even though [^{35}S]methionine continued to be incorporated into total protein during the first 30 min at 30°C, we believe that the chase of prelabeled p31 and p31.5 was

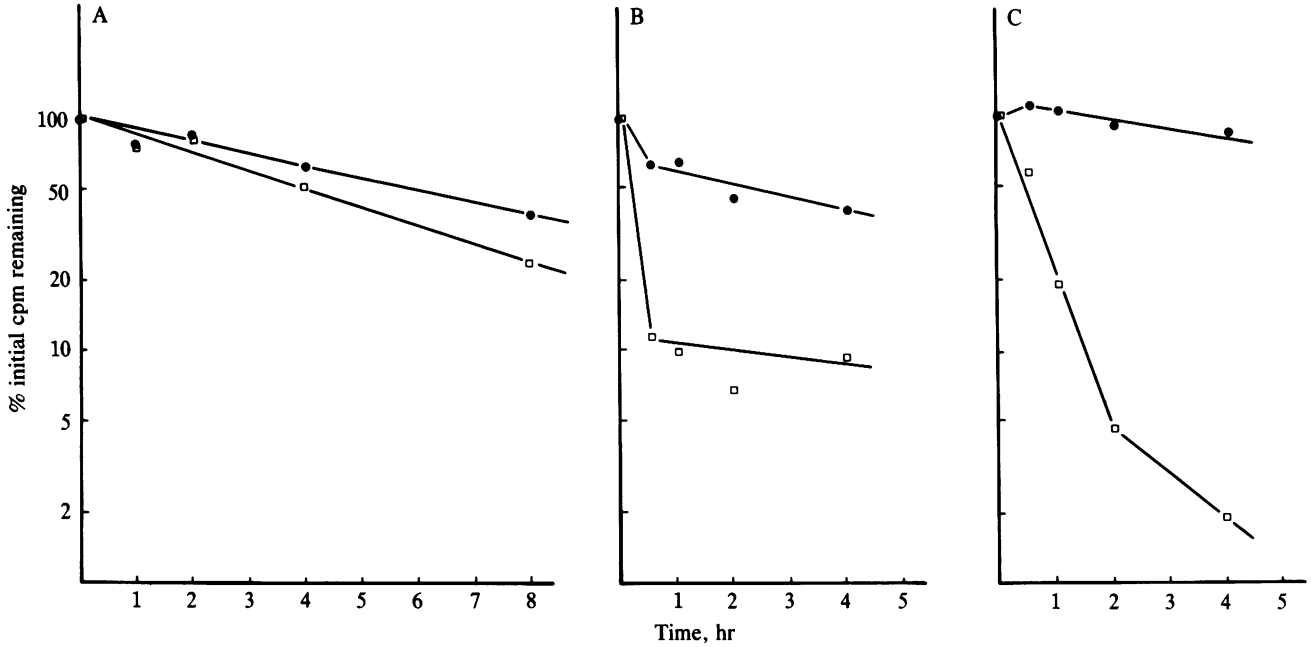

FIG. 4. Decay of prelabeled p31 and p31.5 in growing, developing, and heat-shocked cells. (*A*) Growing cells. Cells grown for 4 hr in the presence of [^{35}S]methionine (50 μCi/ml) were pelleted, resuspended in fresh growth medium containing additional unlabeled methionine, and then incubated further at 22°C. Postmitochondrial supernatants were prepared from cells withdrawn at the designated times, and the amounts of residual p31 and p31.5 were measured using immunoprecipitation, $NaDodSO_4$/polyacrylamide gel electrophoresis, fluorography, and densitometry. The samples used in the precipitation reaction mixtures contained equal amounts of protein. The amount of total labeled protein at each time point was determined by using hot Cl_3CCOOH precipitation. (*B*) Developing cells. Cells grown for 24 hr in the presence of [^{35}S]methionine (50 μCi/ml) were washed and plated for development as described (9). Postmitochondrial supernatants were prepared from cells harvested at the indicated times, and the residual amounts of labeled p31 and p31.5 were measured as described for *A* except that scintillation spectrometry of excised, solubilized gel slices was used instead of densitometry. The samples used in the precipitation reaction mixtures were derived from equal numbers of cells. The amount of total labeled protein was measured as for *A*. (*C*) Heat-shocked cells. Cells were grown for 24 hr in the presence of [^{35}S]methionine (50 μCi/ml), pelleted, resuspended in fresh growth medium containing additional unlabeled methionine, and then heat shocked at 30°C. Whole cell lysates were prepared from cells withdrawn at the indicated times, and the amounts of residual labeled protein and p31 and p31.5 were measured as described for *A*. The samples used in the precipitation reaction mixtures contained equal numbers of Cl_3CCOOH-precipitable cpm, and the data were corrected to compensate for the decay of total labeled protein. ●, Total labeled protein; □, p31 and p31.5.

effective much earlier and that the calculated $t_{1/2}$ values for these proteins are not overestimated. This conclusion is based on the observation that the heat-shock response in *Dictyostelium* is induced within 10 min after shifting the temperature to 30°C (18). In growing cells (22°C), prelabeled p31 and p31.5 decay with $t_{1/2}$ values of ≈4 hr (ref. 9; see Fig. 4*A*); in early developing cells, these proteins decay with estimated $t_{1/2}$ values of 8–10 min until 90% of the molecules have been degraded, and then the remaining p31 and p31.5 appear to be stabilized (ref. 9; see Fig. 4*B*). Interestingly, except for the first 30 min of development, the total labeled protein in growing, developing, and heat-shocked cells seems to decay at about the same rate ($t_{1/2}$ = 6 hr). We conclude, therefore, that a significant change in the decay kinetics of total cellular protein does not occur at 30°C and that p31 and p31.5 are selectively degraded during heat shock. This selective degradation appears to occur at a slightly slower rate than that observed in early developing cells, and smaller residual amounts of protein are obtained. The more rapid decay kinetics of prelabeled p31 and p31.5 during the first 30 min of development may be the result of both specific and increased generalized proteolysis since total protein is also degraded more quickly at this time. The lower residual amounts of p31 and p31.5 found in heat-shocked cells may reflect the increasing inviability of these cells.

Selective Degradation of p31 and p31.5 Is Not Observed in Cycloheximide-Treated Cells. The results presented thus far have established that the major poly(A)-associated proteins of *Dictyostelium* are selectively degraded in two instances when translational initation is restricted. To determine whether these polypeptides are similarly degraded when translational elongation is inhibited, we labeled cells with [^{35}S]methionine and chased the labeled protein in the presence or absence of cycloheximide. At a cycloheximide concentration of 10 μg/ml, the overall rate of protein synthesis is reduced to ≈50% of that observed in control cells (i.e., the same reduced rate of synthesis observed in heat-shocked and developing cells; data not shown). When chased under these conditions, prelabeled p31 and p31.5 are not rapidly degraded (see Fig. 5). In fact, these polypeptides appear to be slightly more stable in drug-treated cells. Similar results were obtained when cycloheximide was used at a concentration of 50 μg/ml (data not shown). It should be noted that the decay of total labeled protein is not significantly affected by cycloheximide. During these experiments, total labeled protein decayed with a $t_{1/2}$ of ≈6.5 hr (data not shown).

DISCUSSION

In this study, we have measured the levels of accumulation and the rates of decay of the two major poly(A)-associated proteins of *Dictyostelium* (p31 and p31.5) during heat shock and early development. We find that significant amounts of newly synthesized p31 and p31.5 do not accumulate in either heat-shocked or developing cells and that preexisting p31 and p31.5 are rapidly and selectively degraded in both cell types. Although we have not described the accumulation and decay of the minor poly(A)-associated species, p29.5, during heat shock and development, it appears that the abundance of this polypeptide, which is closely related to p31 and p31.5, is regulated in a similar manner (ref. 9; R.E.M., unpublished

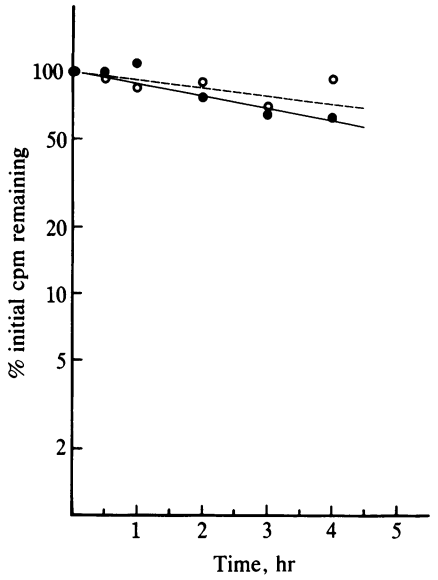

FIG. 5. Decay of p31 and p31.5 in the presence or absence of cycloheximide. Exponentially growing cells were labeled with [^{35}S]methionine for 24 hr (50 μCi/ml), and the labeled protein was chased in the presence or absence of cycloheximide (10 μg/ml). Postmitochondrial supernatants were prepared from cells removed at the indicated times, and the amounts of residual labeled protein and p31 and p31.5 were measured as described for Fig. 4*C*. ●, No cycloheximide; ○, with cycloheximide.

observations). Thus, the poly(A)-associated proteins of *Dictyostelium* are not heat shock proteins. The polyclonal antibodies used in these experiments did not precipitate any of the previously characterized *Dictyostelium* heat shock proteins (17, 18, 20), indicating further that p31 and p31.5 are not related to any known heat shock protein. Considering the highly conserved nature of *hsp70* genes and polypeptides (16, 19, 20, 23, 24) and the probable conservation of poly(A)-binding protein function, our results do not support the conclusion of Schönfelder *et al.* (13) that the major poly(A)-binding protein and the hsp70 of HeLa cells are identical.

We have also shown that the rate of translational initation in heat-shocked and early developing *Dictyostelium* cells is reduced to about the same extent. Significantly, when translational elongation is inhibited with cycloheximide, preexisting p31 and p31.5 remain stable. Therefore, a correlation seems to exist between the abundance and stability of these poly(A)-associated proteins and the rate of translational initiation. Since a 5- to 20-fold reduction in the cellular levels of p31 and p31.5 (from Fig. 4 *B* and *C*) occurs in parallel with only a 2-fold reduction in the overall rate of protein synthesis, we conclude that these polypeptides are not essential for translation *per se*. They probably are, however, necessary for the *efficient* translation of poly(A)$^+$ mRNAs. Such a role for poly(A)-associated proteins has been suggested (4). It is likely that the *Dictyostelium* heat shock mRNAs, like those synthesized by other eukaryotes, are polyadenylylated (20, 25–28). Nonetheless, these mRNAs are translated very efficiently at elevated temperatures (ref. 17; Fig. 3). Recent work by McGarry and Linquist (29) and Hultmark *et al.* (30) suggests, however, that the translational efficiency of heat shock mRNAs is dependent on sequence(s) and factor(s) other than poly(A). It is interesting to note that histone mRNAs, which are poly(A)$^-$, are also efficiently translated during heat shock (25, 26). This provides additional evidence that the regulatory mechanisms ensuring the efficient translation of poly(A)$^+$ and poly(A)$^-$ mRNAs may be quite different (4).

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