

A Developmentally Regulated Membrane Protein Gene in *Dictyostelium discoideum* Is Also Induced by Heat Shock and Cold Shock

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Received 23 June 1987/Accepted 2 October 1987

We have analyzed the expression of the *Dictyostelium* gene P8A7 which had been isolated as a cDNA clone from an early developmentally regulated gene. The single genomic copy generated two mRNAs which were subject to different control mechanisms: while one mRNA (P8A7S) was regulated like the cell-type-nonspecific late genes, the other one (P8A7L) was induced during development, when cells were allowed to attach to a substrate, and when cells were subjected to stress, such as heat shock and cadmium. Interestingly the same induction was also observed with cold shock. RNA processing was inhibited by heat and cold shock, leading to nuclear accumulation of a precursor. The translated region of the cDNA was common to both mRNAs and encoded an unusually hydrophobic peptide with the characteristics of a membrane protein.

One of the key questions in molecular biology concerns the mechanisms of differential gene regulation. Triggered by external signals and possibly by a cascade of second messengers, sets of genes are induced while others are inactivated. Basically, two approaches are being used to understand the means by which gene expression is controlled: one focuses on the external signals, the receptors which recognize them, and the way the signal is transduced to the gene level; in the other, regulatory DNA segments are being identified and analyzed. Both approaches have been used to investigate the genetic control of *Dictyostelium* development (4, 6, 15, 19, 21, 22, 28). *trans*-Acting factors binding to control regions are currently under investigation in several laboratories.

Different control mechanisms can regulate the expression of a single gene, and in some cases transcripts distinguishable in size are produced under the respective conditions of gene induction. The *Dictyostelium ras* gene, for example, gives rise to a 1.2-kilobase (kb) mRNA in vegetative cells, while a 900 nucleotide (nt) mRNA is transcribed in development (23). Transcription of the Tdd-1 transposon (DIRS 1) can be induced by heat shock, while the opposite strand of the DNA encodes a family of developmentally regulated transcripts (24, 31).

The differential accumulation of mRNAs may be due to transcriptional regulation or posttranscriptional regulation via stabilization or destabilization of the RNA. Both mechanisms have been demonstrated for *Dictyostelium* (1, 14, 29; J. Amara and H. F. Lodish, manuscript in preparation), and "mixed" regulation is also possible.

Good candidates to investigate these more complex control mechanisms are developmentally controlled genes which generate more than one class of mRNA. The cDNA clone P8A7 was chosen for that reason, and the expression of the different gene transcripts was extensively studied as a basis for further work on its regulation. pP8A7 was isolated from a cDNA library constructed from membrane-bound polysomal RNA of cells developed for 6 h (9). By differential screening, the P8A7 gene was characterized as being induced in development.

In this paper we show the nucleotide sequence of the cDNA clone and present strong evidence that it encodes a membrane protein. We further demonstrate that one of the two mRNAs is strictly regulated during development, while the other one is induced by heat shock, cold shock, and cadmium and by cell-cell contacts in development and cell substrate contacts in vegetative amoebae.

MATERIALS AND METHODS

Strains and culture conditions. The axenic strain AX2 of *Dictyostelium discoideum* was used for these studies. Vegetative growth in axenic medium and development in suspension culture and on filters were basically done as described (16) except that cells were plated for development on black Millipore filters for better visual inspection of development. Suspension cultures were usually shaken at 150 rpm, except for slow and fast shaking experiments (80 and 220 rpm, respectively).

For vegetative growth on petri dishes, up to 5×10^7 cells were plated on an 8.5-cm dish in 10 ml of AX2 medium. Cells can be continuously grown under these conditions when medium and detached cells are removed every 2 to 3 days and fresh medium is applied.

For growth on a bacterial lawn, 10^7 cells were plated with 0.5 ml of a stationary-phase culture of *Klebsiella aerogenes* on normal-agar plates (0.1% glucose, 0.1% Bacto-peptone, 2% Bacto-agar in 17 mM phosphate buffer, pH 6.0), grown for 24 h, and harvested as described (18).

For heat shock experiments, vegetative cells in AX2 medium were heated in a 30°C (13) waterbath. During heat shock and during the recovery period at 22°C, temperature was monitored by a thermometer in the culture.

For the attachment kinetics, cells from a log-phase suspension culture were transferred to a 250-ml Falcon cell culture flask. Cells were allowed to settle for various periods of time, and incubation was stopped by adding an equal volume of phenol saturated with 0.5 M Tris, pH 8.4, followed by vigorous shaking. RNA was then purified by the standard procedure.

Molecular biology. Molecular biology methods were done as described (18). Enzyme reactions were performed as specified by the manufacturer. As hybridization probes, in

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vitro transcripts of the *DraI* fragment (Fig. 1) were synthesized by using the Gemini system (Promega Biotech). Northern (RNA) blot hybridization was carried out at 55°C in 50% formamide.

hsp70 transcripts were detected by using the nick-translated *Dictyostelium* hsp70 gene fragment (25). Hybridization and washing were done at 37°C.

For Northern blotting, 20 µg of total RNA was size fractionated on 1.2% agarose gels containing formaldehyde and then transferred to nitrocellulose membranes.

DNA fragments of the cDNA clone were subcloned into M13 vectors and sequenced by the chain termination method (26). Both strands were sequenced at least once. In some cases elevated reaction temperatures (55°C) or increased amounts of [³⁵S]dATP had to be used to determine the nucleotide sequence unambiguously.

For RNase H cleavage, 1 µg of a 26-mer oligonucleotide was annealed to 20 µg of total RNA in a buffer containing 40 mM Tris hydrochloride, pH 7.9, 10 mM MgCl₂, 60 mM KCl, and 1 mM dithiothreitol and simultaneously digested with 1 U of RNase H (W. Hill and M. Firpo, Promega Notes no. 6, 1986) in the presence of 40 U of RNasin for 20 h at 4°C. Digestion products were analyzed by Northern blots.

RESULTS

P8A7 is a single-copy gene. In a *HindIII* restriction digest of chromosomal *Dictyostelium* DNA, a single fragment hybrid-

ized with the cDNA clone (Fig. 1, lane 2), indicating a single genomic P8A7 copy. An *EcoRI* digest (lane 1) yielded one major band and an additional weak hybridization (indicated by an arrowhead). Since no *EcoRI* site was present in the cDNA clone, this could be due either to a cognate gene of weak homology or to an *EcoRI* site located in an intron which separates parts of the cDNA clone. *HinfI* cut once in the cDNA and resulted, as expected, in two hybridizing fragments (lane 5). The same was true for a *HindII* digest (lane 3), but we saw only one band, which we assumed was due to comigration of two fragments. This was also supported by the relative strength of the signal. *SspI* generated another unexpected pattern. The *DraI* probe used for hybridization extended only 4 nt 5' to the *SspI* site and therefore should recognize only the downstream *SspI* fragment. However, two bands were seen in the genomic digest. Together, the *HindIII*, *HindII*, and *HinfI* digests showed that the P8A7 gene was present in one colinear copy in the genome. The *EcoRI* and *SspI* digests suggested that the coding region was interrupted by an intron containing these two sites.

P8A7 encodes an extremely hydrophobic membrane protein. The DNA sequence of the cDNA clone and the amino acid sequence derived from the only open reading frame are shown in Fig. 2b. The nucleotide sequence has been determined from the 5' *EcoRI* to the 3'-proximal *DraI* site. An open reading frame beginning after the vector tailing extended to nt 450, encoding 139 amino acids. The stop codon was followed by 23 nontranslated nt, and the poly(A) tail which followed (data not shown) was about 120 nt in length and seemed to occasionally contain nucleotides other than A. The codon usage corresponded well with the preferences listed by Kimmel and Firtel (11), resulting in an A+T content of 70%, while the 3' noncoding region consisted of about 90% A+T and included two homopolymeric T regions.

The derived amino acid sequence was analyzed by a computer program capable of predicting secondary structure features (7). Figure 2a shows the hydrophobicity profile of the peptide. Four possible transmembrane domains were proposed, based on alpha-helix/β-sheet minima, hydrophobicity, and location of the clustered charged residues. One of these domains contained a lysine residue; it should, however, be taken into account that the charge of this amino acid can be displaced over six residues and can thus be located outside the membrane. The occurrence of several histidine residues within the proposed transmembrane regions was not contradictory, for Michel et al. (17) have found a high incidence of this amino acid in the membrane passages of the photosynthetic reaction center of *Rhodospirillum*. The hydrophilic C terminus was suggestive of an anchor domain, presumably on the inside of the membrane. Another unusual feature was six stretches of six amino acids each, which were flanked by glycine residues and which resided within the proposed transmembrane regions.

A search performed with the University of Wisconsin Genetics Computer Group program in several libraries revealed no significant homology to other known proteins.

P8A7 encodes two mRNAs which are differentially regulated. When RNA from different stages of development was examined, two differentially regulated mRNAs hybridizing to the P8A7 probe were detected. One of approximately 1 kb (P8A7L) accumulated only transiently, immediately after the cells were plated on filters. This was in contrast to most of the early cyclic AMP (cAMP)-induced mRNAs, which appeared at about 6 h, reached a peak at approximately 12 h, and slowly disappeared during further development (e.g.,

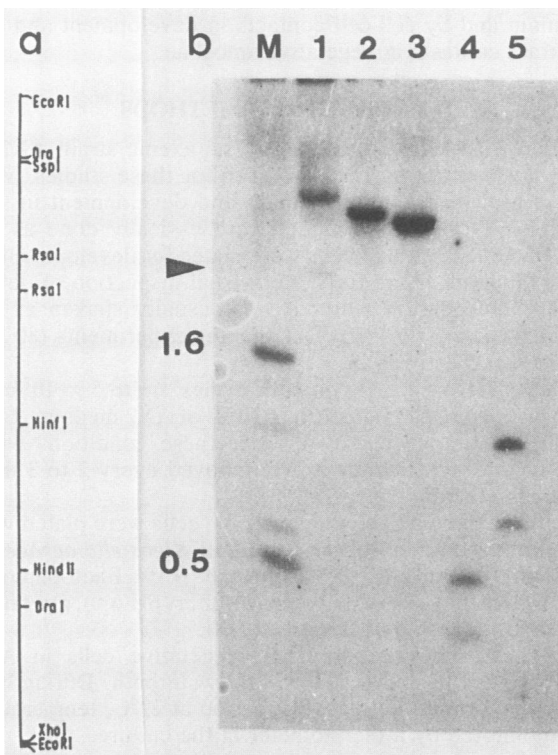


FIG. 1. Analysis of the P8A7 gene in the genome of *Dictyostelium*. (a) Restriction map of the 600-bp cDNA insert, with the 5' end at the top. Restriction sites used for further experiments are shown; *EcoRI* and *XhoI* are located in the polylinker of the vector. (b) Southern blot of chromosomal DNA digested with *EcoRI* (lane 1), *HindIII* (lane 2), *HindII* (lane 3), *SspI* (lane 4), or *HinfI* (lane 5), hybridized with a transcript from the internal *DraI* fragment in panel a. Lane M, Molecular weight markers; sizes are indicated in kilobases. The arrowhead points to a weakly hybridizing band, which is explained in the text.

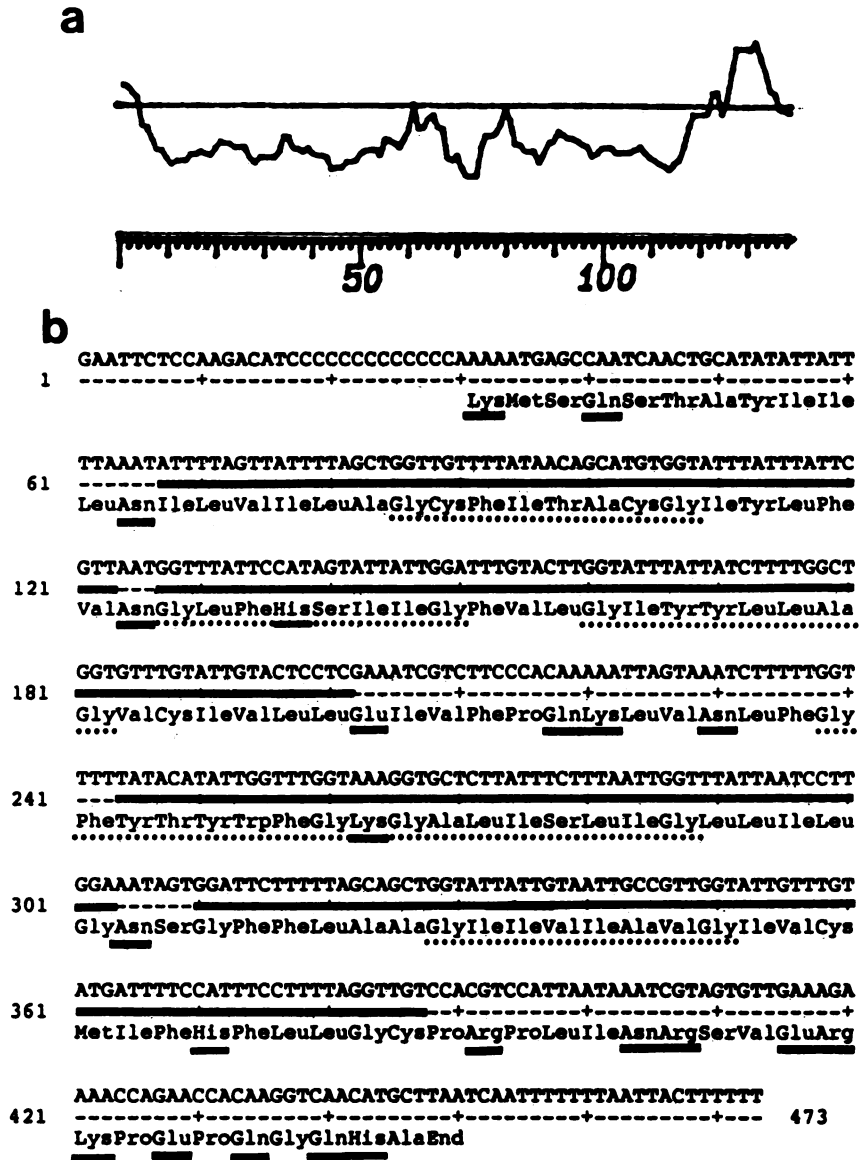


FIG. 2. DNA sequence of the P8A7 cDNA clone and the derived amino acid sequence. (a) Hydrophobicity plot; hydrophobic amino acids are below and hydrophilic residues are above the zero line. Values assigned were between +1.9 and -1.9. (b) Nucleotide and amino acid sequences. Suggested transmembrane regions are denoted by black bars above the peptide sequence. Black bars below the sequence indicate the location of charged residues, and the dotted lines mark stretches of six residues flanked by Gly. The explanation for including some charged residues in the proposed transmembrane regions is given in the text.

the contact site A gene transcript [20]). The other mRNA of approximately 700 nt (P8A7S) was expressed at low levels in vegetative cells and was significantly induced by the onset of starvation. Expression reached a distinct peak between 10 and 14 h of development (see Fig. 4a).

The expression pattern of the P8A7S mRNA resembled that of the prestalk-specific and cell-type-nonspecific late genes described by Mehdy et al. (16). Comparison of RNA from isolated prestalk and prespore cells showed that the mRNA was expressed in approximately equal amounts in both cell types (data not shown). When cells were disaggregated at 12 to 15 h of development, prestalk and prespore mRNAs were rapidly degraded and reaccumulated only when cAMP was added to the suspension (16). In contrast, P8A7S mRNA was not specifically degraded by this treatment and remained at a high level, similar to the cell-type-nonspecific RNAs (data not shown).

To examine the difference between the two transcripts, oligonucleotide-directed RNase H cleavage (see also Materials and Methods) was performed on RNA from cells accumulating either P8A7S (filter-developed cells, t_{10} ; see also Fig. 4a) or P8A7L (cells recovered after heat shock for 30 min, see also Fig. 6a). The oligonucleotide was complementary to the sequence from nt 82 to 107 (Fig. 2).

Both RNAs generated a cleavage product of the same size (approximately 500 nt), which hybridized to a probe specific to sequences downstream to the oligonucleotide annealing site (Fig. 3). This demonstrates that the size difference between P8A7L and P8A7S is exclusively due to sequences upstream of the cleavage site.

Since both transcripts were associated with polysomes (data not shown), they represented two functional mRNAs which might encode proteins with different N termini.

P8A7L expression can be induced by different means. (i)

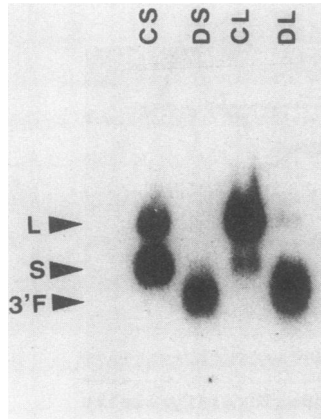


FIG. 3. Site-directed cleavage of P8A7 mRNAs. RNA from heat shock-recovered cells (lanes CL and DL) and from filter-developed cells (lanes CS and DS) were separated on an agarose gel before (lanes CL and CS) and after (lanes DL and DS) site-directed RNase H cleavage. The blot was probed with an SP6 transcript covering sequences downstream of the cleavage site. S and L mark the P8A7S and P8A7L mRNAs, respectively. 3'F indicates the position of the 500-nt 3' fragment.

Formation of cell-cell contacts in development. P8A7L was expressed immediately after cells were plated for development, but 2 h later the RNA was no longer detectable, indicating a rapid turnover, inactivation of transcription, or

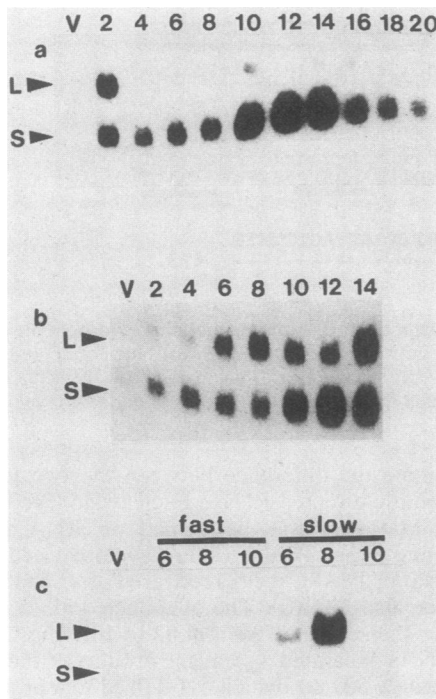


FIG. 4. mRNA accumulation in development and the role of cell-cell contacts. Total cellular RNA was extracted from cells plated for development (a) or from cells developing in suspension (b and c). Cells were shaken in phosphate buffer at 150 rpm (b), 80 rpm (c, slow), or 220 rpm (c, fast). Numbers indicate hours after the onset of development. Vegetative cells were included as a control (lanes V). S and L indicate the locations of P8A7S and P8A7L mRNA, respectively.

both (Fig. 4a). Later, at 10 h after the initiation of development, the stage of tight aggregate formation, expression was observed in parallel to the high accumulation of P8A7S.

When cells were developing in suspension culture (Fig. 4b), P8A7L was continuously accumulated in increasing amounts throughout development, starting at about 6 h. It should be noted that this time coincided with the formation of small, probably loose aggregates. When cells were shaken at low speed, aggregate formation was more pronounced; clumps of several hundred cells were observed and development appeared to proceed further. This was indicated by the expression of late genes which are not induced under fast-shaking conditions (16). Under these conditions, a high but transient induction of P8A7L was found to be correlated with the formation of large aggregates (Fig. 4c).

(ii) **Cell substrate contact in vegetative cells.** The unusually rapid induction of P8A7L immediately upon starvation made it somewhat unlikely that expression was triggered by the onset of development. Therefore, we were interested to see whether expression of the mRNA was possibly due to culture conditions or the change of culture conditions.

When axenically grown cells were plated for development, this not only triggered the onset of development, but also resulted in a rapid change in cell shape; spherical cells from a suspension culture adhered to the filter, spread, and flattened. This change was fast and could account for the immediate induction of P8A7L upon plating. In an attempt to test this possibility, we isolated RNA from cells grown in suspension culture and then plated for different periods of time in axenic medium on plastic dishes. Since no treatment of the cells except careful pipetting was involved, a shock response could be excluded.

P8A7L was induced 30 min after the cells were allowed to adhere to the plastic surface (Fig. 5a). It should be noted that the cells needed 3 to 5 min for settling and another 30 to 60 s to spread on the surface. With induction by cell-substrate contact, expression of the large mRNA became higher than expression of the small one. About 2 h after plating, P8A7L accumulation was reduced to approximately the same level as P8A7S. This reflects the situation found after continuous growth on petri dishes. In contrast, cells grown in suspension culture only accumulated minor amounts of P8A7L compared with P8A7S mRNA.

To exclude the possibility that this expression was cell density dependent, plating was done at different cell titers ranging from 10^6 to $10^4/\text{cm}^2$. At least in the last case, all cells were single, eliminating all putative cell-cell interactions.

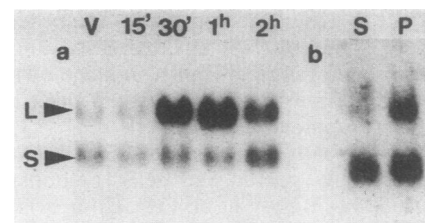


FIG. 5. Influence of cell-substrate interactions on P8A7 expression in vegetative amoebae. Axenically grown cells from a suspension culture were allowed to settle onto a plastic surface for the times indicated (minutes or hours). RNA was extracted and analyzed by Northern blot (a). RNA from cells prior to attachment is shown in lane V. (b) RNA from cells grown on *Klebsiella* in suspension culture (lane S) or grown on a bacterial lawn on an agar plate (lane P). P8A7S and P8A7L transcripts are indicated by S and L, respectively.

RNA analysis clearly showed that expression of the large mRNA was high and at about the same level as P8A7S RNA at all cell densities (data not shown).

If the induction of P8A7L was only due to cell substrate contacts, similar results should be obtained with cells grown in a bacterial suspension and on a bacterial lawn. This was indeed the case, and the large mRNA was expressed only when cells were allowed to adhere to a substrate (Fig. 5b).

(iii) Factors causing cell stress. In some cases we have seen stronger expression of P8A7L in vegetative cells from axenic suspension cultures, especially when cells were extensively washed or left on ice for a prolonged period of time. These observations indicate that the mRNA might also be induced under stress. P8A7L was strongly induced within 15 min after heat shock, while the small mRNA disappeared, as would be expected for non-heat shock mRNAs (25) (Fig. 6a). During recovery from the stress, the large mRNA was rapidly downregulated and decayed with an estimated half-life of about 10 min. The small one reappeared after several hours. This strongly suggests two different kinds of heat shock regulation on the same gene.

Surprisingly, a third transcript of about 2 kb (P8A7P) accumulated after heat shock. We assume that this RNA was an unspliced precursor molecule (see below) since heat shock can inhibit RNA processing (30).

In contrast to P8A7L, accumulation of two hsp70 mRNAs was slower and reached its maximum, under the conditions used here, only in the recovery period (Fig. 6b).

Interestingly, in cells grown at low temperature (4°C) for a prolonged time, the heat shock pattern of P8A7 RNA accumulation was also observed. Figure 6c shows RNA isolated after 5 h of growth in axenic suspension culture at 4°C.

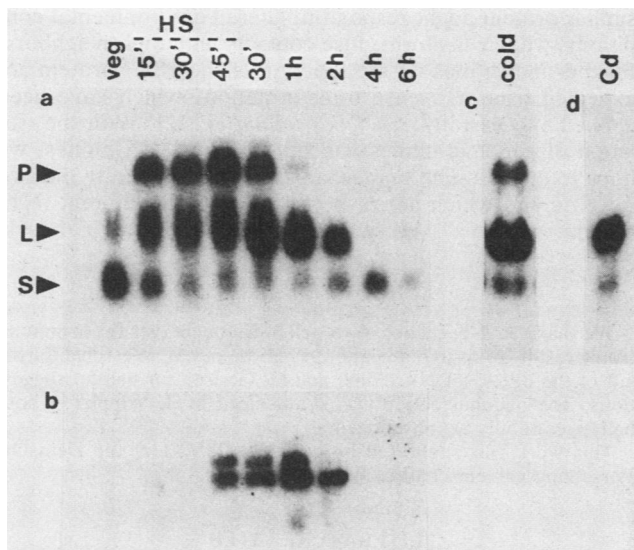


FIG. 6. Various stress conditions affect the accumulation of P8A7-specific RNAs. Vegetative amoebae in suspension culture were heat-shocked at 30°C for 15, 30, or 45 min and then allowed to recover for 30 min or 1, 2, 4, or 6 h at 22°C. RNA was extracted and analyzed by Northern blot (a). The same filter was stripped and then rehybridized with a nick-translated *Dictyostelium* hsp70 probe (b). In lane cold, RNA was isolated from cells grown in axenic medium for 5 h at 4°C (c). In lane Cd, RNA was extracted from vegetative cells treated for 30 min with 100 μ M Cd(NO₃)₂ (d). Lane veg shows RNA extracted from an untreated culture. S and L indicate the small and large P8A7 mRNAs, respectively. P marks the precursor, which is only accumulated under heat and cold shock conditions.

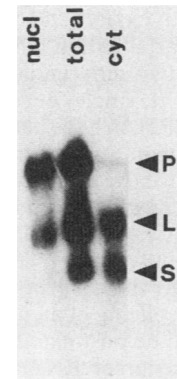


FIG. 7. Northern analysis of RNA from nuclei and cytoplasm. Cells were heat-shocked for 30 min and separated into nuclear (nucl) and cytoplasmic (cyt) fractions. RNA extracted from these fractions and whole-cell RNA as a control (total) was run on a Northern gel and hybridized with a transcript of the cloned *Dra*I fragment (Fig. 1a). Weak hybridization to the P8A7P transcript in the cytoplasmic fraction and at least part of the P8A7L mRNA in the nuclear fraction is probably due to some cross-contamination of nuclei and cytoplasm. Symbols are as described in the legend to Fig. 6.

Similar to the heat shock response, P8A7L accumulated to high levels and the additional 2-kb RNA was observed. However, no decrease or only a minor loss of the small mRNA took place. Compared with heat shock, differences were also found in the kinetics of the cold shock response: P8A7L accumulated more slowly, starting at 2 h and reaching a maximum at 5 to 6 h. On the other hand, the amount of the 2-kb transcript slowly decreased between 2 and 6 h after the culture was transferred to the low temperature (data not shown).

Since heat shock genes can be induced by heavy metals, we tested whether P8A7L would also accumulate when cells were treated with cadmium. This was the case (Fig. 6d). However, the 2-kb transcript did not appear and, similar to the cold shock experiment, no reduction of P8A7S was observed.

The 2-kb transcript is a precursor RNA. To test whether the 2-kb transcript, appearing under stress in heat and cold shock, was really a non-spliced precursor, we isolated RNA from a cytoplasmic- and from a nuclear-enriched fraction of heat-shocked cells and analyzed it by Northern blots (Fig. 7). As expected, the 2-kb transcript was predominantly found in the nuclear fraction, with only trace amounts in the cytoplasm, which were probably due to some nuclear contamination. This confirmed our assumption that P8A7P was an immature nuclear transcript of the gene.

DISCUSSION

We have analyzed the regulation of the *Dictyostelium* P8A7 gene during development, under different growth conditions, and under stress. Sequencing data show that the gene product is highly hydrophobic. Further analysis suggests a peptide structure comprising four transmembrane regions and a carboxy-terminal anchor domain. Since the cDNA clone is incomplete at the 5' end, we did not find the expected amino-terminal leader sequence.

Up to now, this gene has showed the most complex pattern of differential regulation observed in *Dictyostelium*. In addition, different controlled processing mechanisms or different promoters regulating expression of the same gene

have to be postulated to explain the appearance of the three sizes of RNA. A similar situation is found in the *Dictyostelium ras* gene, where two differentially regulated messages are transcribed from a single genomic copy (23). Two differently regulated mRNAs have also been detected for the EB4 gene (2; W. Nellen, unpublished observations), but nothing is known about how they are generated.

To this end, several mechanisms can be discussed for the origin of different P8A7 mRNAs. Two promoters may be used to control the different types of expression, or a common precursor may be subject to differential splicing events. In both cases, splicing of an unusually large intron of more than 300 nt has to be postulated. The appearance of an even larger nuclear precursor RNA during heat shock further obscures the structure of this gene. The 2-kb RNA, P8A7P, may be the primary transcript for either of the two mRNAs, which accumulates because processing is impaired during stress (30). If this is the case, about 1 kb of intron sequence have to be spliced out to generate the P8A7 mRNAs. The mean intron size in *Dictyostelium* is 100 to 200 nt (11), and to date there is no precedent for larger intervening sequences. Our mapping data strongly suggest that in contrast to some other *Dictyostelium* genes (e.g., actin [8]), splicing is involved in the generation of both of the P8A7 mRNAs.

Both mRNAs are found associated with polysomes and both have the coding part of the cDNA in common. RNase H experiments show that they also share the entire region downstream of the oligonucleotide annealing site, indicating that the size difference is due to sequences in the 5' region of the transcript. It is not known, however, whether the additional sequences are located in the translated or untranslated region. We assume that both mRNAs encode membrane proteins; the translation product of P8A7L might be larger by approximately 100 N-terminal amino acids.

The regulation of P8A7S is very similar to the cell-type-nonspecific genes described by Mehdy et al. (16). This is confirmed by roughly equal expression in isolated prespore and prestalk cells and the largely cAMP-independent expression in disaggregated slugs (data not shown).

In addition, the differential expression of the two mRNAs during development and under different growth conditions appears to be correlated with cell contacts: the large mRNA is expressed when cells can adhere to a substrate in vegetative growth and at the time when large aggregates are formed in suspension culture. Similarly, the small mRNA accumulates strongly when tight aggregates are formed during development on filters. Together, these observations indicate an involvement of the P8A7 membrane protein in cell contacts.

A striking difference between the two mRNAs is the fact that P8A7L under most conditions accumulated only transiently. This was observed when cells from suspension culture were plated for development and also when cells were starved in a slow shaking suspension. Even when vegetative cells out of a suspension culture were plated on plastic, the accumulation of the mRNA overshoot and then soon decreased again, though to a higher level than before plating. This might be due to a feedback mechanism or an intrinsic feature of the promoter, allowing only strong transient expression.

The induction of the large and rapid loss of the small mRNA during heat shock clearly shows the different control mechanisms regulating expression of this gene under conditions entirely unrelated to development and cell contact. The P8A7L mRNA behaved like a heat shock mRNA and was

accumulated even faster than the *Dictyostelium hsp70* mRNA. In contrast, the P8A7S mRNA had characteristics of a non-heat shock transcript and was rapidly degraded under stress conditions.

Since the disappearance of non-heat shock mRNAs during stress is at least partially due to differential destabilization, P8A7L and P8A7S most likely are different also in posttranscriptional regulation. Another unusual feature of this gene is the induction of P8A7L by cold shock. Although a response to low temperature has recently been shown for some *Escherichia coli* proteins (10), cold shock has not yet been described as an inducer of stress genes. At least for *Dictyostelium*, low-temperature induction of heat shock genes seems to be a more general phenomenon. Mueller-Taubenberger et al. (manuscript in preparation) have also seen cold shock induction for the *Dictyostelium* ubiquitin mRNAs (27) which are known to be heat shock inducible. On the other hand, hsp70 expression is not affected by low temperatures (M. Maniak and W. Nellen, unpublished observations).

While the heat shock response was rapidly induced, maximal expression of P8A7L after cold shock was only observed after 2 h. Though we cannot exclude that a different mechanism is responsible for cold shock induction of the gene, the different kinetics could easily be explained by decreased rates of biochemical processes at low temperature.

Assuming that this strict regulation by stress is not just coincidence, one has to propose multiple functions for the P8A7 gene product(s), which is to our knowledge the first heat-shock-induced membrane protein gene described. It is, for example, possible that alterations in the membrane could serve as a protection against external insults and the same reaction might be caused by contact with a substrate. A similar protein might respond to altered environmental conditions, when cells form close contacts with their neighbors. Further indications on the function of the P8A7 protein are expected from antisense transformations which have been applied successfully in *Dictyostelium* (5, 12). With the isolation of genomic clones and full-length cDNA clones, we hope to understand the mechanisms which generate the two mRNAs and which are responsible for their different regulation.

ACKNOWLEDGMENTS

We thank A. Noegel and A. Mueller-Taubenberger for communicating results prior to publication, P. Hirth and M. Westphal for the gift of the pP8A7 cDNA clone, and G. Gerisch for helpful suggestions. The oligonucleotide was synthesized by J. Wippler, whose help is gratefully acknowledged.

This work was supported by grant Ne 285/2-1 of the Deutsche Forschungsgemeinschaft to W.N.

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