MOLECULAR AND CELLULAR BIOLOGY, Jan. 1988, p. 10–16
0270-7306/88/010010-07$02.00/0

Vol. 8, No. 1

Effect of Protein Synthesis Inhibition on Gene Expression during Early Development of *Dictyostelium discoideum*

C. K. SINGLETON,* S. S. MANNING, AND Y. FENG

Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235

Received 13 July 1987/Accepted 28 September 1987

Several genes which are deactivated on the initiation of development of *Dictyostelium discoideum* were identified by differential screening of various cDNA libraries. These genes have in common a decrease in the steady-state levels of their corresponding mRNAs on the onset of development and as development proceeds. When development was carried out in the absence of protein synthesis by inhibition with cycloheximide, the decrease in mRNA levels for most genes (V genes) was normal or slightly accelerated. For about 5% of the genes (H genes), however, cycloheximide caused an apparent induction of expression, as revealed by a slight or dramatic increase in mRNA levels, instead of the normal decrease. This effect was due to inhibition of protein synthesis and not to cycloheximide per se. The induction was found to be due to an enhancement of the transcription rate; normal rates of transcription for the H genes were dependent on continued protein synthesis during vegetative growth and development. Thus, two general regulatory classes exist for deactivation of gene expression on initiation of development, one of which is dependent on and one of which is independent of protein synthesis. Analysis of expression of these genes in mutant strains which are aggregation deficient allowed the classes to be subdivided further. Taken together, these characterizations allow several distinct regulatory mechanisms to be identified that are involved in the deactivation of gene expression on the onset of development in *D. discoideum*.

Dictyostelium discoideum has been used as a model system for investigating gene expression in a developmental setting for, among other reasons, its apparent simplicity (22, 23). A 24-h developmental program is initiated by removal of nutrients and results in the differentiation of initially equipotential amoebae primarily into two cell types, spore and stalk cells. Morphological and biochemical differentiation is brought about by a series of environmental and morphogenic signals which mediate differential gene expression in a spatial and temporal fashion (6, 14, 35). Results of early studies indicated that there are a few major times for large-scale changes in gene expression by the coordinate control of large groups of genes (1–3, 26). A closer and more comprehensive examination of the details of the expression of a number of genes has led to an appreciation of a greater complexity (5, 12). For example, the initial grouping of either prespore- or prestalk-specific genes into a coordinately regulated prespore and prestalk class was revised into several subclasses when the expression of these genes was analyzed in greater detail (27).

We recently identified (31) and characterized several genes which were deactivated on the onset of development, an event which might a priori be thought to be rather simple and straightforward. The characterizations which were carried out, however, suggested that several mechanisms for gene deactivation were operative when development was initiated. Herein, we extend these studies by reporting the identification and characterization of several more genes that are also deactivated but that fall into a different major regulatory class. When development was carried out in the absence of protein synthesis, the levels of the corresponding mRNAs for the newly identified genes either remained constant or increased dramatically, instead of undergoing their normal decrease with time of development. This effect was shown to be due to the requirement of continued protein synthesis for maintenance of proper transcription rates; in its absence, transcription rates for these genes increased. Analysis of the expression of the protein synthesis-dependent and -independent genes in mutant strains which are blocked early in the developmental program revealed further complexity in gene deactivation. From the results of this study, when taken together, at least four different mechanisms can be identified for shutting down the expression of vegetative growth specific genes at the onset of development.

* Corresponding author.

MATERIALS AND METHODS

D. discoideum strains were grown and cultured as described previously (31); KAx3 (28) was used as the fruiting wild-type strain. Development was initiated by removing bacteria or nutrient broth, followed by plating the cells as described previously (31). Cycloheximide, when used, was present at 400 μg/ml, and pactamycin (gift of The Upjohn Co., Kalamazoo, Mich.) was used at 300 μg/ml (17). Inhibition of protein synthesis was determined as outlined by Finney et al. (11).

cDNA synthesis and cloning. mRNA isolated from cells which had developed for 4 h in the presence of cycloheximide was used in the synthesis of double-stranded cDNA (25). Prior to S1 nuclease cleavage of the hairpin loop used to prime second-strand synthesis, *Pst*I linkers were ligated to the nonhairpin terminus. After hairpin loop cleavage, *Bam*HI linkers were added. After size selection on a low-melting-point agarose gel, the linker-terminated, double-stranded cDNA was ligated into *Pst*I-*Bam*HI-digested pGEM-1 (Promega-Biotec), and the ligation mixture was used to transform (18) competent C600SF8 cells. Screening of the resulting library was carried out as described previously (31). Radioactive cDNA generated from mRNA isolated from vegetatively growing cells and cells developed for 4 h in the presence of cycloheximide was used in the initial screenings. Colonies which showed enhanced hybridization

to the latter cDNA sample relative to that of the former were clonally isolated, and a final screening was carried out (31).

RNA isolation and analysis. Cytoplasmic mRNA and total cellular RNA were isolated as described previously (31), with slight modifications for quick-prep RNA. Quick-prep RNA facilitated the isolation of numerous RNA samples in a given experiment and was performed on 1.5×10^7 cells. Normal lysis, with the addition of 0.2% sodium dodecyl sulfate, was carried out in a 1.5-ml Eppendorf tube. After an initial phenol extraction, samples were frozen at −70°C for at least 2 h and thawed, and the aqueous phase was transferred to a new tube. A subsequent phenol extraction was followed by a chloroform extraction and then ethanol precipitation. The pellet was suspended in 0.15 ml of water, and RNA was quantitated by optical density measurements. Procedures for analysis of RNA samples by dot blots, for probe generation, and for hybridizations were performed as described previously (31).

Finally, in vitro nuclear transcription reactions were performed essentially as outlined by Crowley et al. (7). Equal counts per minute of the resulting radioactive RNA samples were hybridized as described above, but for 3 to 4 days to 5 μg of linearized and denatured plasmid DNAs bound to nylon filters.

RESULTS

Identification of cycloheximide-inducible genes. We have identified and isolated several genes which are induced during the preaggregative or initial phase of development of *D. discoideum* (P. Gregoli, C. McPherson, and C. Singleton, abstract, Fed. Proc. **46**:2134, 1987). Expression for most of these genes was first detectable between 2 and 4 h into development; none were found that showed transient expression during the first 2 h after the removal of nutrients. We reasoned that induction of any such transiently expressed genes may be independent of protein synthesis and may represent primary events of development initiation. Since in studies in other systems it has been found that protein synthesis inhibitors can often bring about a superinduction of transiently expressed genes or genes whose induction is the primary response to some inductive signal (see reference 19, and references therein), a cDNA library was set up with mRNA isolated from cells which had developed for 4 h in the presence of the protein synthesis inhibitor cycloheximide. This library was screened for clones containing cDNA inserts which preferentially hybridized to mRNA isolated from cells that were developed for 4 h and treated with cycloheximide relative to mRNA isolated from vegetatively growing cells. Fifteen clones with the desired hybridization properties were identified and named pLM-H1 to pLM-H15 (LM is a laboratory identification and H represents cycloheximide induction.) On subsequent analysis, several of these proved to correspond to the same gene(s). The characterization of the expression of eight different genes that were identified here, as well as one gene with similar properties which was previously identified (31), is presented below.

Kinetics of expression. In vegetatively growing cells, the H genes were expressed at moderate to low levels. In contrast to the expected results, the mRNA levels for the H genes all decreased either dramatically or only slightly on the initiation of development under normal conditions; no evidence was obtained for the transient induction of any of these genes. The relative mRNA levels for several H genes at various times during development in the absence and presence of cycloheximide are illustrated in Fig. 1. The mRNA levels corresponding to the H genes and V21 differed dramatically when protein synthesis was inhibited when compared with mRNA levels during development in the presence of protein synthesis (Fig. 1). Instead of the normal decrease in corresponding mRNA levels, a slight or dramatic increase over levels during vegetative growth was observed in the presence of cycloheximide. Quantitation of these and similar expression profiles gave rise to plots (Fig. 2) of relative mRNA levels versus time of development. An apparent induction of expression of the H genes and V21 can be seen in these plots. In the absence of protein synthesis, the observed increases in mRNA levels peaked between 4 and 8 h (12 hours for H4) and were followed by a decline. The significance of the decline in steady-state levels after 8 h of development is unclear, since the cells began to show signs of cell death and lysis after this period of time in the absence of protein synthesis.

In contrast, mRNA levels corresponding to the gene designated V1 showed a similar decrease in the presence and absence of cycloheximide. These results, along with a listing of mRNA and insert sizes of the identified genes, are presented in Table 1. On examination of earlier times in development, it was found that the normal decreases in the absence of and the apparent induction in the presence of cycloheximide were observable within the first 30 to 45 min of development. This is shown in Fig. 3 for H5 and H6; similar results were observed with all of the H genes and V21.

In a previous study (31), we identified and characterized several genes which showed decreases in their mRNA levels on the onset of development that were similar to those observed here for the H genes. These genes were designated the V genes and include V21 and V1 described above. Of 15 V genes examined, only one (V21) showed the phenotype described here for the H genes: an apparent induction of expression in the absence of protein synthesis. All of the other V genes were similar in their expression to V1 (Fig. 2). The decrease in the levels of the corresponding mRNAs on

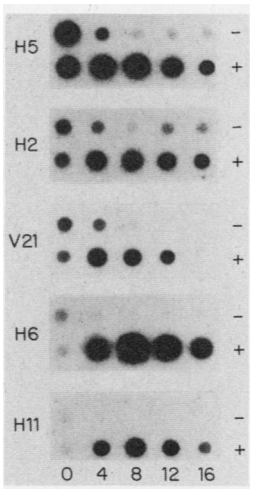

FIG. 1. Dot blot analysis of the mRNA levels corresponding to several H genes. Total cellular RNA was isolated at the indicated times (in hours, indicated at the bottom of the figure) before and after the onset of development in the presence (+) and absence (−) of cycloheximide. Equal amounts of each sample were spotted onto nylon and hybridized with pLM-H-specific probes generated with SP6 polymerase.

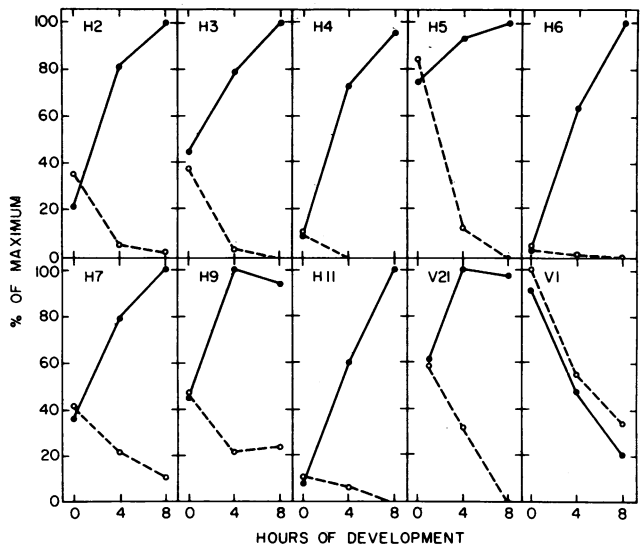

FIG. 2. Percentage of maximum expression levels versus time of development for the H genes, V21, and V1. Quantitation of data similar to that in Fig. 1 was done by densitometric scanning. Symbols: ●, Levels in the presence of cycloheximide; ○ and – – –, levels in the absence of cycloheximide. The maximal level (usually 8 h with cycloheximide) was set at 100%, and all other values are relative to this one.

TABLE 1. mRNA levels of H genes and V1 in the presence and absence of cycloheximide

cDNA	Approximate size of[a]:		Relative abundance at:		Cycloheximide
	mRNA (nucleotides)	Insert (base pairs)	0 h	8 h	
pLM-H2	1,540	1,150	0.36	0.02	–
			0.21	1.00	+
pLM-H3	900	1,150[b]	0.38	<0.02	–
			0.45	1.00	+
pLM-H4	2,700	1,090	0.10	<0.01	–
			0.09	0.90	+
pLM-H5	930	920	0.87	0.01	–
			0.70	1.00	+
pLM-H6	2,370	1,030	0.04	<0.01	–
			0.03	1.00	+
pLM-H7	590	500	0.43	0.20	–
			0.36	1.00	+
pLM-H9	1,450	1,220	0.47	0.26	–
			0.45	0.94	+
pLM-H11	1,100	1,000	0.12	<0.01	–
			0.08	1.00	+
pLM-V21	1,400	275	0.69	<0.01	–
			0.62	0.97	+
pLM-V1	920	790	1.00	0.34	–
			0.93	0.20	+

[a] Northern analysis was used to determine the transcript size, and comparisons between total RNA and oligo(dT) cellulose-selected RNA confirmed each species to be poly(A)$^+$ RNA.

[b] Whether the larger size of the insert was due to a cloning artifact or the insert derived from a larger precursor RNA was not determined.

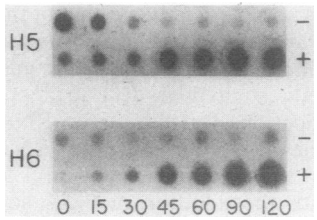

FIG. 3. Rapidity of cycloheximide induction for H-gene expression. Dot blots similar to those shown in Fig. 1 were carried out with total cellular RNA that was isolated at early times of development (in minutes) from cells that were developed in the presence (+) or absence (−) of cycloheximide.

the initiation of development were independent of continued protein synthesis. Actually, in the presence of cycloheximide, the observed decreases were slightly, but reproducibly, accelerated. Thus, at least two major mechanisms must be functioning in the deactivation of gene expression once development is initiated, one of which is dependent on and one of which is independent of protein synthesis.

Cause of induction. The primary effect of cycloheximide is thought to be the inhibition of protein synthesis. However, this drug can bring about other effects, both specific and nonspecific, such as mRNA stabilization and inhibition of transcription (13, 17). To determine if the observed induction of the expression of the H genes was due to protein synthesis inhibition or another effect of cycloheximide, we performed similar experiments with other inhibitors of protein synthesis, puromycin and pactamycin. Both of these drugs possess a different mechanism of action than cycloheximide. Puromycin also inhibits elongation but does not bring about the stabilization of polysomes as does cycloheximide, while pactamycin inhibits the initiation of protein synthesis. Induction of the H genes was observed with both drugs (data not shown); with puromycin the induction was qualitatively similar but not as dramatic. This could be due to the fact that we were only able to achieve about 90% protein synthesis inhibition with puromycin. Pactamycin, however, gave identical results, both qualitative and quantitative, as were obtained with cycloheximide. Thus, it is the lack of protein synthesis which results in the misregulation of H-gene expression. This is consistent with the fact that induction was observable after 30 min (Fig. 3), the time that is required for complete inhibition of protein synthesis by cycloheximide (11).

Since the inhibition of protein synthesis is a stressful event for developing cells, we examined if other stresses might also bring about an apparent induction of the H genes. Cells growing exponentially in axenic medium were allowed to grow at the normal temperature of 21°C, while a portion of the culture was shifted to 30°C, which is the temperature needed to induce the heat shock response in *D. discoideum* (24). RNA was isolated at intervals from 1 to 4 h, and the levels of the H and V gene mRNAs were determined by dot blot analysis. The results (data not shown) indicated that for both series of genes, the corresponding mRNA levels declined slowly as the heat shock response progressed. Thus, the induction of stress in the cells by at least one other method, namely, heat shock, did not bring about an induction in expression of the H genes.

Dependence on continuous protein synthesis. Since the H genes normally undergo a small to drastic drop in expression on the onset of development and this was the time that we

began protein synthesis inhibition, it was of interest to see if induction occurred at other times. Cells were grown on bacteria, harvested, and plated for normal development. After 0, 1, 2, 4, and 8 h, the filters on which the cells were supported were transferred to pads soaked with starvation buffer plus cycloheximide. A control set of cells was left on pads without drug. Development was allowed to proceed for an additional 4 h, after which the cells were lysed and RNA was isolated and probed. The results are presented in Fig. 4 for H5 and H6. Induction for these two genes, as revealed by an increase in steady-state mRNA levels, occurred for the H genes when protein synthesis was inhibited at any time during the first 8 h of development. The level of induction when inhibition was begun at 8 h was only about 60% of the level when inhibition occurred at other times. Whether this apparent downward trend continued was not examined by inhibiting protein synthesis at later times. The results of this experiment demonstrate that continued protein synthesis is necessary for the proper expression of the H genes. Indeed, protein synthesis inhibition for growing cells, as well as for developing cells, brought about a similar induction. This was shown by examining H-gene expression in cells that grew axenically in cultures to which cycloheximide was subsequently added directly. Before cycloheximide addition, H-gene expression was similar to that in cells that grew on bacteria (somewhat greater levels for H6). On inhibition of protein synthesis, however, H-gene expression showed the characteristic increase in mRNA levels (data not shown), which was found when protein synthesis was inhibited in developing cells.

Level of regulation. It is of interest to determine the mechanism(s) by which these genes are normally regulated in growing and developing cells and how the inhibition of protein synthesis alters this regulation. In particular, do the changes in expression levels occur because of changes in transcription rates, changes in mRNA stability, or both? We addressed this question by performing both in vitro nuclear transcription reactions (runons) and in vivo pulse-labeling experiments. Nuclei were isolated from axenically growing cells or cells developed for various times with and without the inhibition of protein synthesis. Relative rates of transcription in the isolated nuclei were revealed after in vitro transcription reactions, and the results of one such experiment in which nuclei from cells developed for 0 or 1 h were used are shown in Fig. 5. For those H genes which gave significant signals above that of the background (H5, H2, H3, H4, and H9), transcription rates decreased when development proceeded in the presence of protein synthesis. Coupled to in vivo pulse-labeling results (unpublished data), the decreases in relative transcription rates for the H genes accounted reasonably well for the decreases in steady-state

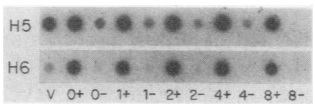

FIG. 4. Induction of H-gene expression by inhibiting protein synthesis for 4 h after normal development for various times. Cells were plated down for development under normal conditions. At the indicated times (in hours) cells on one of two filters per time point were transferred to a pad that contained cycloheximide (+). The other filter was placed on a pad that lacked cycloheximide (−), and both were allowed to develop for an additional 4 h. The cells were then lysed, and total RNA was isolated. V represents vegetative RNA (no development). Equal amounts of each sample were spotted onto nylon and hybridized with H5- and H6-specific probes.

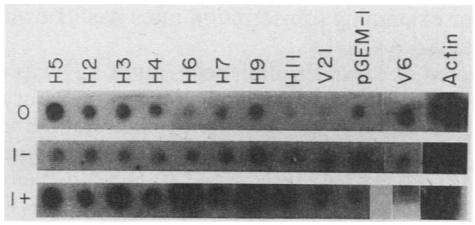

FIG. 5. Autoradiogram revealing relative transcription rates for the H genes and V21 and V6 under various conditions. Nuclei were isolated from axenically growing cells or cells developed for 1 h in the presence (1+) or absence (1−) of cycloheximide. Runons were performed in the presence of [α-^{32}P]UTP, and radioactive RNA was isolated. Linearized plasmids (indicated above the figure) were spotted onto nylon and hybridized with the runon RNA samples. pGEM-1 is the parental plasmid for all other plasmids, and thus represents background hybridization; actin indicates the spotting of a plasmid that possessed an insert corresponding to a *D. discoideum* actin gene.

mRNA levels found on the initiation of development. H9 showed no or a very little decrease in transcription rate, which is consistent with its small (ca. twofold) change in steady-state mRNA levels.

In contrast, when protein synthesis was inhibited, all of the H genes and V21 showed increased transcription rates (Fig. 5). Enhancement of transcription rates of these genes occurred when protein synthesis was inhibited in axenically growing cells or cells developed for 1 or 4 h. For each gene except H4, the increase in transcription was sufficient to account for the increase in steady-state mRNA levels found when protein synthesis was inhibited. This was seen when the fold increases in relative transcription rates or steady-state mRNA levels brought about by the inhibition of protein synthesis were compared (Table 2). The only large discrepancy occurred with H4, suggesting that enhanced mRNA stability may also be involved in the induction observed for this gene. The conclusion, which was derived from the in vitro nuclear transcription reactions, that inhibition of protein synthesis results in an enhancement of the relative transcription rate of the H genes is also consistent with the results of in vivo pulse-labeling experiments (unpublished data). This strengthens the conclusion, since each of these

TABLE 2. Increases in relative transcription rates or mRNA steady-state levels brought about by protein synthesis inhibition

Gene (mRNA)	Cycloheximide-induced approximate fold increased in[a]:	
	Transcription rate	Steady-state levels
H2	10	16
H3	14	26
H4	7	36
H5	27	11
H6	43	49
H7	6	4
H9	7	5
H11	13	9
V21	3	4

[a] Densitometric scannings of blots similar to those shown in Fig. 1 and 5 were used to quantitate the relative levels for each gene (mRNA) for nuclear transcription reactions and steady-state mRNA analysis. The fold increase was determined by dividing the value for cultures with cycloheximide by the value for cultures without cycloheximide. The experiment involved nuclei and RNA from cells that were developed for 4 h.

methods for examining transcription rates has its own set of problems and drawbacks.

As expected, the transcription behavior of V21 was similar to that of the H genes. For the other V genes (31), our results were most consistent with a similar decline in the transcription rates that occurred on initiation of development, as shown in Fig. 5 for V6. In contrast to the H genes, when protein synthesis was inhibited, transcription of the V genes declined, as occurs during normal conditions of development.

Regulation in aggregation-deficient mutants. The results presented above delineate two major mechanisms for gene deactivation once development is initiated. Although the timing of the changes in expression for a gene in one or the other class is similar to that of the other genes of the same class, differences do exist (Fig. 2) (31). These differences suggest the possibility that there are subclasses within the two major groups. To examine this possibility, we examined the expression of the H and V genes in several mutant strains which were unable to properly aggregate and thus did not complete development. Over 20 aggregation-deficient mutants were used, and these possessed a range of phenotypes, as revealed by the fact that they had terminal morphologies of smooth lawns to faint ripples to extended ripples to loose mounds. Thus, the strains employed probably possessed blocks at various times throughout the preaggregative period. The developmental profiles of the levels of H5 mRNA in five of the mutants are shown in Fig. 6. The mutant strains were HMW404, HMW426, and HMW452, which were originally isolated as being unable to induce the early developmental marker α-mannosidase (21), and HNT101 and HNT102, which were originally isolated based on their aggregationless morphology.

An unexpected expression profile was found in these mutants for several H genes, V21, and V4. Instead of the moderate levels of the corresponding transcripts normally found in vegetatively growing cells, the complete absence of expression or a much reduced level of expression was found

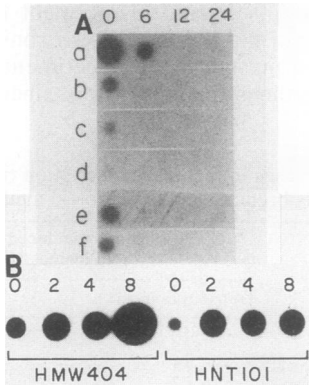

FIG. 6. Expression of H5 in various aggregation-deficient strains. (A) Total RNA was isolated at the indicated times (in hours) from the following strains plated down for development under normal conditions: KAx3 (row a), HMW452 (row b), HMW404 (row c), HNT101 (row d), HMW426 (row e), HNT102 (row f). Equal amounts of each sample were spotted onto nylon and hybridized with an H5-specific probe. (B) The same procedure described above for panel A was used, except that development was carried out in the presence of cycloheximide for the two mutants indicated. The somewhat higher level of H5 transcript detected at 0 h in panel B relative to the same time point in panel A is probably a consequence of the fact that cycloheximide was present in the buffer that was used during cell harvesting and removal of bacteria.

TABLE 3. Quantitation of vegetative levels for V4, V21, and H5 mRNA

Strain	Relative levels of mRNAs of the following genes in vegetatively growing cells[a]:		
	V4	V21	H5
KAx3	100	100	100
HMW404	0	11	2
HMW452	0	79	11
HNT101	1	5	<1
HMW426	1	14	37
HNT102	100	1	15

[a] Densitometric scanning of blots similar to those shown in Fig. 6 was used to quantitate the relative levels of each mRNA. The wild-type (KAx3) level was set at 100%, and all other values are relative to this.

for these genes in vegetative cells of these five mutant strains. For those cases in which expression was low, initiation of development was followed by qualitatively normal decreases in mRNA levels. This pattern of apparent misregulation was the only one observed in more than 20 mutants examined, including the timing mutant FM-1 (32). The expression profiles for H2, H3, H5, and H9 were similar in the mutants shown in Fig. 6, with the relative level of mRNA present in vegetatively growing cells being as follows: HNT101 < HMW404 < HMW452 < HNT102 < HMW426 < KAx3. V21 gave a similar relative order with only minor differences. In contrast, H7 was expressed normally in all mutant strains examined; and the low levels of H4, H6, and H11 precluded analysis. Expression of V4 was distinct from that of the H genes and V21, with the order being as follows: HMW404 = HMW452 < HNT101 = HNT426 <<< D5 = KAx3. There was no evidence of expression in vegetatively growing HMW404 and HMW452. All other V genes were regulated normally in these and the mutants not shown. The quantitation of the vegetative levels for V4, V21, and H5 mRNA are given in Table 3.

Since the inhibition of protein synthesis brought about induction of the H genes and V21 in the wild-type strain and since the misregulation that was found was one of decreased expression, it was of interest to examine the effect of cycloheximide on the levels of H-gene expression in the mutant strains. Growing HMW404 and HNT101 cells were harvested and plated down for development in the presence of cycloheximide. Cells were lysed at various times at up to 8 h of development, and the RNA was isolated and analyzed by probing for the presence of H mRNAs. Interestingly, for both mutants inhibition of protein synthesis resulted in an induction of expression of the H genes and V21, which had maximal levels comparable to those of induction in wild-type cells, as shown in Fig. 6B for H5.

DISCUSSION

The findings presented herein in conjunction with results of our previous study (31) allow two major classes of genes which are deactivated on the initiation of development in *D. discoideum* to be delineated. Expression of the V genes (excluding V21) occurs during vegetative growth and declines during development; proper regulation of this class of genes is independent of protein synthesis. Similarly, the H genes and V21 are also expressed during vegetative growth and are deactivated during early development. In contrast, however, proper regulation of the H genes requires continuous protein synthesis. When deactivated genes are directly screened for, independent of the effect of protein synthesis

on their expression (31), 19 of 20 (95%) of the identified genes were found to be in the independent class, while 1 (5%) deactivated gene fell into the dependent class. For the genes of both classes, we examined the effect of inhibition of protein synthesis only after the establishment of their expression. Thus, it is not known what role, if any, newly synthesized proteins have in the initial establishment of the expression of these genes. This perhaps could be examined by studying the effect of protein synthesis inhibitors during spore germination, dedifferentiation, or both (10). Since these genes are inactivated in cells (spores) late in development yet are expressed in vegetatively growing cells, activation or establishment of their expression must occur during these two processes that lead to the growth phase.

The examination of the expression of the H and V genes in aggregation-deficient strains allowed differentiation between members in a single class. V4 had an aberrant expression pattern in several mutants that were blocked very early in development, while all other V genes were regulated normally in these and other mutant strains. Most but not all of the H genes and V21 also demonstrated atypical expression profiles in these same strains plus one other strain which gave normal V4 expression. The true significance of the data from the mutant strains is unclear, primarily because of a lack of information on the detailed nature of the lesion in each strain, and must await the more rigorous characterization of each mutation. The form of apparent misregulation was of an unexpected type: the lack of or very low expression in vegetatively growing mutant cells instead of the more understandable form of a lack of decrease in expression with development. The significance of such a pattern of misregulation is unclear, but it suggests that mutants supposedly blocked only in early development may also have subtle alterations in normal vegetative growth. This would be consistent with other observations that vegetative growth and early development are not mutually exclusive events (4), and that these mutants may be expressing an early portion of the developmental program before starvation.

Nonetheless, the different behaviors of the various V and H genes in the mutant strains suggest that, while they belong to the same class and perhaps share common control mechanisms, the genes within each class can be subdivided. This may reflect the use by the different subclasses of factors (see below) which are distinct but which act by the same overall mechanism or other, and as yet unknown, subtle differences in the detailed mechanism of the regulation of each gene. Finally, the other major point derived from study of the mutants is that inhibition of protein synthesis in two of the mutants results in induction of H-gene expression, suggesting that their lack of expression in these mutants under normal conditions of growth and development can be overcome and is not irreversible.

Induction of H-gene expression by the inhibition of protein synthesis was shown to occur at the level of transcription for all of the H genes and V21, with perhaps a significant contribution to H4 induction by mRNA stabilization. The relative transcription rates of the H genes were significantly increased when protein synthesis was inhibited. This effect was observed when inhibition occurred in vegetatively growing cells or cells that had developed for anywhere from 0 to 8 h. Several models are consistent with the properties of H- and V-gene expression and their developmental regulation. One such scenario would be that proper control of H-gene expression requires a balance of negative and positive factors. Inhibition of protein synthesis could result in the loss of a labile negative factor (8, 29), resulting in the observed induction or maintenance of expression even during development. Our observation of induction after 8 h of development, when H genes have been shut down, implies that positive factors are still present but are normally overpowered by the developmentally related negative factor(s) which are removed by protein synthesis inhibition. The results also suggest that the negative factors play some role throughout the life cycle. The induction in the mutant strains demonstrates that expression is possible in these strains (thus positive factors are still present), and that perhaps the mutant phenotype with respect to the H genes is due to a higher negative-factor activity in vegetative mutant cells compared with that in wild-type cells. In comparison, the regulation of V genes appears to be more simple. Positive factors may bring about expression in vegetatively growing cells, and these are perhaps inactivated or lost on initiation of development. The inactivation must be independent of protein synthesis. Other models are also feasible (for example, see reference 30), and delineation of the molecular details of the regulation of these genes will require further work.

It is unclear what role any of the H genes play during the life cycle of *D. discoideum*. They are expressed in moderate to very low levels during vegetative growth and decline to even lower or undetectable levels during development. In other systems, investigators have previously demonstrated effects that are similar to those we observed in this study for the H genes of protein synthesis inhibitors on the expression of other genes; the most prominent of these are cell cycle-dependent genes of mammalian systems and growth factor-inducible genes. These include inductive and repressive effects on particular genes which result from both changes in transcription rates (8, 15, 16, 19, 20, 29) and changes in mRNA stability (9, 17, 33). A common feature of several of these genes is the transient nature of their expression on induction under normal conditions and usually a short half-life for the corresponding mRNAs. The H genes possess a similar dependency on protein synthesis, and H mRNAs also have very short half-lifes, as is revealed when transcription inhibitors are used (Y. Feng and C. McPherson, unpublished data). It is possible that one or more of these genes is cell cycle dependent and functions at a specific time in the cycle. Since our vegetative cells were derived from asynchronous cultures, the vegetative levels observed reflect an average over the entire cell cycle. Thus, it would be of interest to investigate H-gene expression, especially those H genes that are expressed at very low levels even in vegetatively growing cells (H4, H6), in synchronized cultures (34) to examine this possibility and shed light on the function(s) of the H-gene products.

ACKNOWLEDGMENTS

We thank the other members of our laboratory for stimulating discussions and critical assessment of the results.

This study was supported in part by grant DCB-8506117 from the National Science Foundation and by institutional research grant IN-25Z from the American Cancer Society.

LITERATURE CITED

1. **Alton, T. H., and H. F. Lodish.** 1977. Developmental changes in messenger RNAs and protein synthesis in *Dictyostelium discoideum*. Dev. Biol. **60**:180–206.
2. **Barklis, E., and H. F. Lodish.** 1983. Regulation of *Dictyostelium discoideum* mRNAs specific for prespore or prestalk cells. Cell **32**:1139–1148.
3. **Blumberg, D. D., and H. F. Lodish.** 1980. Complexity of nuclear and polysomal RNAs in growing *Dictyostelium discoideum*

cells. Dev. Biol. **78**:268–284.
4. **Burns, R. A., G. P. Livi, and R. L. Dimond.** 1981. Regulation and secretion of early developmentally controlled enzymes during axenic growth in *Dictyostelium discoideum*. Dev. Biol. **83**:407–416.
5. **Cardelli, J. A., D. A. Knecht, R. Wunderlich, and R. L. Dimond.** 1985. Major changes in gene expression occur during at least four stages of development of *Dictyostelium discoideum*. Dev. Biol. **110**:147–156.
6. **Chisholm, R. L., E. Barklis, and H. F. Lodish.** 1984. Mechanism of sequential induction of cell-type specific mRNAs in *Dictyostelium* differentiation. Nature (London) **310**:67–69.
7. **Crowley, T. E., W. Nellen, R. H. Gomer, and R.A. Firtel.** 1985. Phenocopy of discoidin 1-minus mutants by antisense transformation in *Dictyostelium*. Cell **43**:633–641.
8. **Elder, P. K., L. J. Schmidt, T. Ono, and M. J. Getz.** 1984. Specific stimulation of actin gene transcription by epidermal growth factor and cycloheximide. Proc. Natl. Acad. Sci. USA **81**:7476–7480.
9. **Endo, T., and B. Nadal-Ginard.** 1986. Transcriptional and posttranscriptional control of c-*myc* during myogenesis: its mRNA remains inducible in differentiated cells and does not suppress the differentiated phenotype. Mol. Cell. Biol. **6**:1412–1421.
10. **Finney, R., B. Varnum, and D. R. Soll.** 1979. Erasure in *Dictyostelium discoideum*, a dedifferentiation involving the programmed loss of chemotactic functions. Dev. Biol. **73**:290–303.
11. **Finney, R. E., C. J. Langtimm, and D. R. Soll.** 1985. A characterization of the preaggregative period of *Dictyostelium discoideum*. Dev. Biol. **110**:157–170.
12. **Finney, R. E., C. J. Langtimm, and D. R. Soll.** 1985. Regulation of protein synthesis during the preaggregative period of *Dictyostelium discoideum* development: involvement of close cell associations and cAMP. Dev. Biol. **110**:171–191.
13. **Gale, E. F., E. Cundliffe, P. E. Reynolds, M. H. Richmond, and M. J. Waring.** 1972. The molecular basis of antibiotic action. John Wiley & Sons, Inc., New York.
14. **Gomer, R. H., S. Datta, and R. A. Firtel.** 1986. Cellular and subcellular distribution of a cAMP-regulated prestalk protein and prespore protein in *Dictyostelium discoideum*: a study on the ontogeny of prestalk and prespore cells. J. Cell Biol. **103**:1999–2015.
15. **Greenberg, M. E., A. L. Hermanowski, and E. B. Ziff.** 1986. Effect of protein synthesis inhibitors on growth factor activation of c-*fos*, c-*myc*, and actin gene transcription. Mol. Cell. Biol. **6**:1050–1057.
16. **Jacoby, D. B., J. A. Engle, and H. C. Towle.** 1987. Induction of a rapidly responsive hepatic gene product by thyroid hormone requires ongoing protein synthesis. Mol. Cell. Biol. **7**:1352–1357.
17. **Kelly, R., D. R. Shaw, and H. L. Ennis.** 1987. Role of protein synthesis in decay and accumulation of mRNA during spore germination in the cellular slime mold *Dictyostelium discoideum*. Mol. Cell. Biol. **7**:799–805.
18. **Kushner, S. R.** 1978. An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids, p. 17–21. *In* H. B. Boyer and S. Nicosia (ed.), Genetic engineering. Elsevier/North-Holland Publishing Co., Amsterdam.
19. **Lau, F. L., and D. Nathans.** 1985. Identification of a set of genes expressed during the G0/G1 transition of cultured mouse cells. EMBO J. **4**:3145–3151.
20. **Linial, M., N. Gunderson, and M. Groundine.** 1985. Enhanced transcription of c-myc in bursal lymphoma cells requires continuous protein synthesis. Science **230**:1126–1132.
21. **Livi, G. P., J. A. Cardelli, and R. L. Dimond.** 1986. α-Mannosidase mutants of *Dictyostelium discoideum*: early aggregation essential genes regulate enzyme precursor synthesis, modification, and processing. Differentiation (Berlin) **29**:207–215.
22. **Loomis, W. F.** 1975. *Dictyostelium discoideum*, a developmental system. Academic Press, Inc., New York.
23. **Loomis, W. F. (ed.)** 1982. The development of *Dictyostelium discoideum*. Academic Press, Inc., New York.
24. **Loomis, W. F., and S. Wheeler.** 1980. Heat shock response of *Dictyostelium*. Dev. Biol. **79**:399–408.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Mehdy, M. C., D. Ratner, and R. A. Firtel.** 1983. Induction and modulation of cell type specific gene expression in *Dictyostelium*. Cell **32**:763–771.
27. **Oyama, M., and D. D. Blumberg.** 1986. Changes during differentiation in requirements for cAMP for expression of cell type specific mRNAs in the cellular slime mold, *Dictyostelium discoideum*. Dev. Biol. **117**:550–556.
28. **Poole, S. J., and R. A. Firtel.** 1984. Genomic instability and mobile genetic elements in regions surrounding two discoidin I genes of *Dictyostelium discoideum*. Mol. Cell. Biol. **4**:671–680.
29. **Ringold, G. M., B. Dieckmann, J. L. Vannice, M. Trahey, and F. McCormick.** 1984. Inhibition of protein synthesis stimulates the transcription of human β-interferon genes in Chinese hamster ovary cells. Proc. Natl. Acad. Sci. USA **81**:3964–3968.
30. **Sen, S., and D. Baltimore.** 1986. Inducibility of K immunoglobulin enhancer-binding protein NF-KB by a posttranslational mechanism. Cell **47**:921–928.
31. **Singleton, C. K., R. L. Delude, and C. E. McPherson.** 1987. Characterization of genes which are deactivated upon the onset of development in *Dictyostelium discoideum*. Dev. Biol. **119**:433–441.
32. **Soll, D. R., L. Mitchell, B. Kraft, S. Alexander, R. Finney, and B. Varnum-Finney.** 1987. Characterization of a timing mutant of *Dictyostelium discoideum* which exhibits high frequency switching. Dev. Biol. **120**:25–37.
33. **Stiles, C. D., K. L. Lee, and F. T. Kenney.** 1976. Differential degradation of mRNAs in mammalian cells. Proc. Natl. Acad. Sci. USA **73**:2634–2638.
34. **Weijer, C. J., G. Duschl, and C. N. David.** 1984. A revision of the *Dictyostelium discoideum* cell cycle. J. Cell Sci. **70**:111–131.
35. **Williams, J. G., A. Ceccarelli, S. McRobbie, H. Mahbubani, R. R. Kay, A. Early, M. Berks, and K. A. Jermyn.** 1987. Direct induction of *Dictyostelium* prestalk gene expression by DIF provides evidence that DIF is a morphogen. Cell **49**:185–192.