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Translational Control of Discoidin Lectin Expression in *drsA* Suppressor Mutants of *Dictyostelium discoideum*

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Genetic analysis in *Dictyostelium discoideum* has identified regulatory genes which control the developmental expression of the discoidin lectin multigene family. Among these, the *drsA* mutation is a dominant second-site suppressor of another mutation, *disB*, which has the discoidinless phenotype. We now demonstrate a novel mechanism by which the *drsA* allele exerts its suppressive effect on the *disB* mutation. Interestingly, *drsA* does not merely bypass the *disB* mutation and restore the wild-type pattern of lectin expression. Rather, *drsA* mutant cells have high levels of discoidin lectin synthesis during growth but do not express lectins during aggregation. In contrast, wild-type cells only express lectin protein during the aggregation period of development. Phenocopies of the *drsA* mutation show a pattern of discoidin expression similar to that seen in the bona fide mutant. These data suggest that there may be a mechanism of negative feedback, resulting from the high levels of discoidin lectin made during growth, which inhibits further discoidin lectin expression during development. Northern (RNA) analysis of developing *drsA* mutant cells shows that these cells contain high levels of discoidin mRNA, although no discoidin lectin protein is being translated from these messages. Therefore, expression of the discoidin gene family can be controlled at the level of translation as well as transcription.

The program of gene expression underlying morphogenesis in *Dictyostelium discoideum* has been extensively studied (16). Although an elegant description exists of the quantitative, spatial, and temporal expression of a large number of genes and their products, less is known regarding the molecular mechanisms regulating these genes. One of the most intensively studied systems in this organism is the gene family that encodes the discoidin lectins (22, 30, 33). These genes are coordinately transcribed during the aggregation phase of bacterially grown cells (17). Vegetatively growing cells do not express the discoidin lectins (23). There are three homologous discoidin I genes encoding proteins of approximately 28 kDa and a single discoidin II gene which produces a protein of 26 kDa. All of the gene products bind to galactose (23). The binding of the discoidin I molecules is Ca^{2+} dependent (2).

In previous studies we have isolated mutant strains defective in the developmentally regulated expression of these genes (6). The defects in these strains were shown to be at the level of transcription and specific to the discoidin genes (5). Genetic studies indicate that the mutations define a network of *trans*-acting regulatory genes (1). Each mutation maps to a different chromosome. Two of the mutations—*disA* and *disB*—are null mutations and block the expression of all the lectin genes at the transcriptional level. A third mutation—*drsA*—is a dominant second-site, or phenotypic, suppressor of one of the null mutations, *disB*. Thus, double mutants carrying both *drsA* and *disB* express the discoidin lectin proteins. In contrast, *drsA* does not suppress the *disA* mutation, and cells containing both of these mutations do not express the discoidin lectins. The epistatic relationship of these genes allowed us to propose a model for the gene action sequence: $disB^+ \rightarrow drsA^+ \rightarrow disA^+$ (1, 4).

The *drsA* mutation is not a simple phenotypic suppressor of *disB*. That is, it does not simply reverse the pattern of discoidin lectin expression to that observed in bacterially grown wild-type cells. Our genetic analyses indicated that strains carrying *drsA* also overexpressed the lectins and, more significantly, expressed the lectins during vegetative growth. Thus, in *drsA* mutants there is a loss of the strict developmental regulation of the discoidin lectin genes that is observed in the wild-type. On the basis of these mutant phenotypes, we hypothesized that *drsA* strains were constitutive for discoidin expression (1).

We now have examined further the mechanism of second-site suppression by the *drsA* mutation. The overproduction and misregulation of the discoidin lectin genes in these mutant strains are not due to alterations in the genome such as translocations or duplications. Our data confirm that *drsA* mutant strains synthesize high levels of discoidin lectin protein during growth. However, unlike the wild type, these mutants do not express the lectin proteins during the aggregation phase of development. Phenocopies of the *drsA* mutants display the same behavior. These observations suggest that the expression of the discoidin lectins during growth may inhibit later expression during development, perhaps through an autoregulatory mechanism. Surprisingly, although *drsA* mutant cells do not synthesize discoidin lectin protein during development, they do contain high levels of specific discoidin mRNA during this period. Taken together, these results demonstrate that in *drsA* mutant strains, developmentally regulated lectin expression is down-regulated at the level of translation. This represents a new level of regulation for the expression of these molecules.

MATERIALS AND METHODS

Strains, conditions for growth and development, and genetic techniques. The strains used in this study and their genotypes and origins are listed in Table 1. Cells were generally grown on SM plates (29) in association with *Klebsiella aerogenes* at 22°C. In some experiments (as noted in Results) the cells were grown in a suspension of *K. aerogenes* in 17 mM

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TABLE 1. Haploid strains of *D. discoideum* used in this study

Haploid strain	Diploid parent	Genotype[a]											Reference
		acr	*alp*	*axe*	*bsg*	*bwn*	*cyc*	*dis*	*drs*	*man*	*nag*	*tsg*	*whi*	
NC4	Wild type	+	+	+, +	+, *B500*	+	+	+, +	+	+	+	+	+	20
HL101	DL45	+	*A1*	+, *B1*	+, +	+	*A1*	+, *B50*	*A50*	*A2*	*A211*	*D12*	*A1*	18
SA102	DSA7	+	+	+, +	*A1*,[b] +	*A1*	*A1*	+, +	*A50*	*A2*	*A211*	*D12*	*A1*	1
SA298	DSA21	+	+	+, +	+, *B500*	+	+	+, +	+	+	+	*D12*	*A1*	1
SA310	DSA21	+	*A1*	+, *B1*	+, *B500*	+	+	+, *B50*	*A50*	+	*A211*	[c]	+	1
SA311	DSA21	+	*A1*	+, *B1*	+, *B500*	+	+	+, *B50*	+	+	+	*D12*	*A1*	1
SA313	DSA21	+	+	+, +	+, *B500*	+	+	+, +	*A50*	+	*A211*	[c]	+	1
SA391	DSA163	*A50*	+	+, +	*A1*, *B500*	+	+	*A50*, +	*A50*	*A2*	*A211*	[c]	+	1

[a] Phenotypes of the mutations at these loci are as follows: *acr*, resistance to 2% methanol; *alp*, alkaline phosphatase deficient; *axe*, capable of growth in axenic medium; *bsg*, inability to grow with *Bacillus subtilis* as a food source; *bwn*, produces brown pigment; *cyc*, resistance to 500 mg of cycloheximide per ml; *dis*, does not express discoidin lectins during development; *drs*, phenotypic suppressor of *disB* and overproducer of the discoidin lectins (this mutation is fully characterized in this paper); *man*, α-mannosidase-1 deficient; *nag*, *N*-acetylglucosaminidase deficient; *tsg*, temperature sensitive to growth at 27°C; *whi*, white spored; +, wild-type alleles. The linkage relationships of these alleles are given in the indicated references.

[b] SA102 was incorrectly reported as $bsgA^+$ in reference 1.

[c] Strains have unmapped *tsg* mutations.

phosphate buffer (pH 6.5) while being shaken at 200 rpm. In either case, cells were harvested at the end of log-phase growth, washed in sterile LPS buffer (20 mM KCl, 2.5 mM $MgCl_2$, 40 mM potassium phosphate (pH 6.5) containing 0.5 mg of streptomycin sulfate per ml), and allowed to develop at 22°C in a moist chamber on black filter paper disks (Thomas Scientific) at a density of 10^8 cells per 42-mm-diameter filter (29). The genetic techniques and nomenclature used in this study were discussed in detail in the previous papers in this series (1, 4).

Isotopic labeling during development. Sterile filters with developing cells were transferred to a 70-μl drop of LPS buffer containing 10 mCi of [^{35}S]methionine (ICN Radiochemicals). The labeling period was 30 min. The labeling conditions have no adverse effect, and pulse-labeled cells which are allowed to continue development do so normally compared with undisturbed controls. The developing cells or cell aggregates were then washed from the filters in sterile water, centrifuged, drained, and stored as a frozen pellet.

Immunoprecipitation and Western blotting. Frozen cell pellets were lysed in 10 mM Tris-HCl (pH 8.0)–0.5% Nonidet P-40 for 30 min at 4°C. After removal of cell debris by centrifugation, the sample was divided for both immunoprecipitation and Western blotting (immunoblotting) with anti-discoidin antibodies, thus allowing these analyses to be performed on sister cells. The Western analyses were performed with peroxidase-conjugated goat anti-rabbit antibody. Details of these methods have been presented elsewhere (5, 6). Western analyses and immunoprecipitations were performed on extracts from equal numbers of cells.

Southern blotting. Southern analysis of the discoidin lectin gene family has been described (6). Genomic DNA was isolated from purified nuclei. The pcDd Disc-IV_{32} discoidin I cDNA probe (24) was used to detect DNA restriction fragments containing the discoidin genes. General recombinant DNA methods were as previously described (25).

Northern (RNA) analysis. Total RNA was purified from cells, and 2.5 μg of RNA from each time point was separated in agarose gels containing formaldehyde and transferred to nitrocellulose as previously described (25). Staining the rRNA bands confirmed equal loading. After blotting, the gels were restained to ensure complete transfer. The blots were probed with nick-translated pcDd Disc-IV_{32} cDNA to detect the presence of specific discoidin I mRNA. The blots for all of the strains examined were probed together so that the results would be directly comparable.

RESULTS

Discoidin lectin genes are not duplicated or rearranged in *drsA* mutants. One of the phenotypes associated with the *drsA* mutation is the overproduction of the discoidin lectins. This phenotype could be due to a variety of factors, including an increase by duplication in the number of lectin genes in these mutant strains or the rearrangement of the lectin genes so that they are under the control of more active promoters. We now have shown by Southern analysis that these possibilities are not the cause of this phenotype. Figure 1 shows Southern blots of genomic DNA cut with the restriction endonucleases *Eco*RI, *Hin*dIII, and *Kpn*I and probed with the pcDd Disc-IV_{32} discoidin cDNA. We have analyzed DNA from a number of strains carrying the mutant *drsA* allele or its wild-type counterpart. These recombinant strains come from crosses between *drsA* and $drsA^+$ strains and have a variety of genetic backgrounds (see Table 1), so we can eliminate genetic background effects in our analysis. None of the digests show an increase in the number or relative density of fragments containing any of the discoidin genes. Differences in hybridization density between samples are directly correlated with differences in the amount of DNA loaded on the gel (data not shown).

The *Kpn*I digests show that one of the major fragments hybridizing to the discoidin I cDNA probe is larger in some strains than in others. That is, some strains have a band at ~20 kb, whereas others have a band at ~12 kb. However, this restriction fragment length polymorphism (RFLP) is not associated with the presence of the mutant *drsA* allele or its associated phenotypes. That is, the RFLP and the *drsA* mutation do not cosegregate. Rather, this discoidin gene RFLP is directly correlated to the presence of linkage group II (chromosome II) from parent strain HL101, which was used to generate these recombinant strains. These data support the conclusion of Welker (32) that the discoidin I genes map to linkage group II. Moreover, these experiments do not suggest that the discoidin lectin genes in *drsA* mutant strains have been translocated to the control of another promoter but rather that the observed increase in lectin expression is due to an alteration in the *drsA* regulatory gene.

***drsA* mutant strains express discoidin lectin protein almost exclusively during vegetative growth.** We had previously shown by Western analyses of developmental time courses that strains carrying the *drsA* mutation had discoidin lectin in

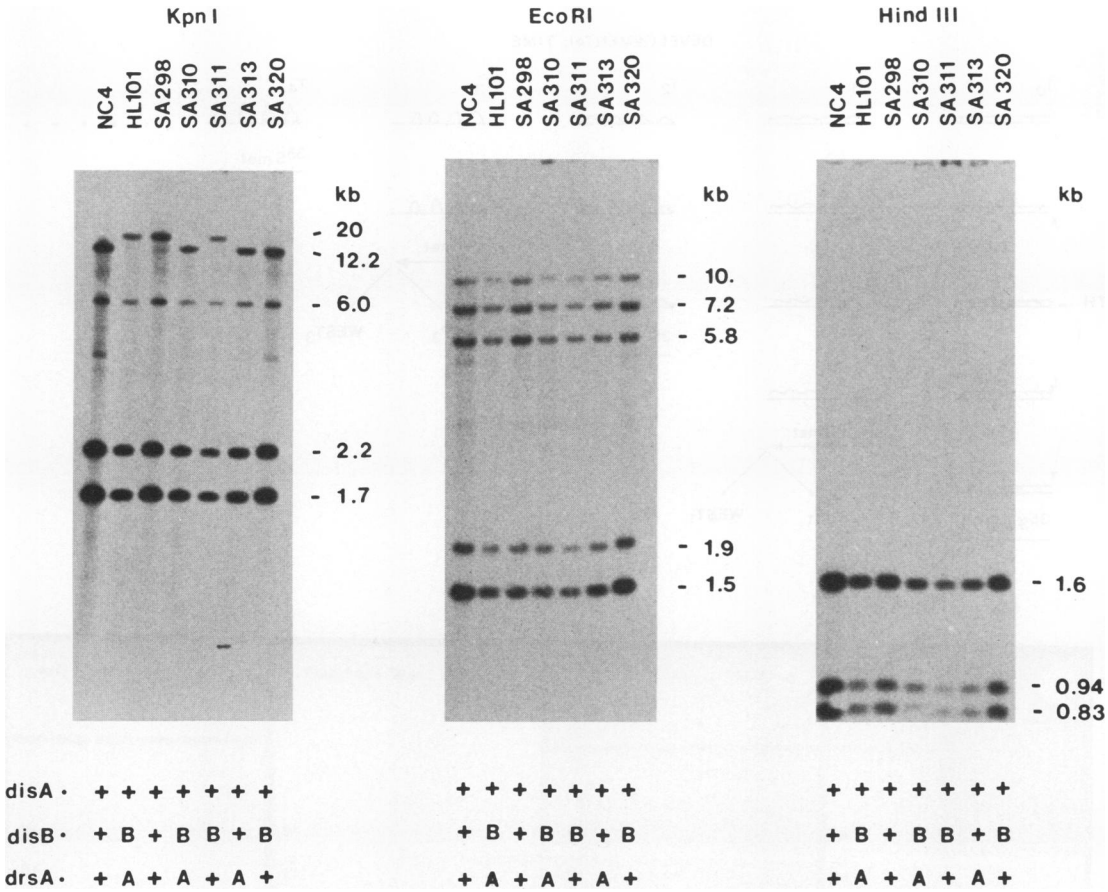

FIG. 1. Southern analysis of the discoidin genes from strains carrying the wild-type and mutant *drsA* alleles. Nuclear DNA was prepared from parental and recombinant strains. Full genotypes of the mutants are given in Table 1. The figure shows whether the strain is mutant or wild type at the *disA*, *disB*, and *drsA* loci, which are the regulatory genes controlling discoidin expression. The DNA was cut with the indicated restriction endonucleases, run on 1% agarose gels, and blotted to nylon membranes. The discoidin I gene-specific cDNA probe pcDd Disc-IV$_{32}$ was labeled by nick translation and used for hybridization. As described in the text, the data indicate that the organization and number of the discoidin genes in *drsA* mutants are unaltered compared with those of the wild type. The RFLP seen in the *Kpn*I digests is not associated with the *drsA* mutation and is discussed in the text. Differences in hybridization density between samples are directly correlated with differences in the amount of DNA that was loaded on the gels. The light band at ~4 to 5 kb in NC4 and SA320 was seen in all samples in the original autoradiograms.

both growing and developing cells (1, 4). This is in contrast to wild-type cells which first begin to accumulate discoidin during the aggregation phase of development. We hypothesized that discoidin expression in these strains was constitutive as a result of the mutant *drsA* regulatory gene. Thus, we expected that discoidin would be synthesized at all times during growth and development rather than the limited time during the aggregation phase of development in wild-type cells. We performed pulse-labeling studies on these strains and found that the results differ dramatically from what we had expected. Strains carrying the mutant *drsA* allele, irrespective of genetic background, synthesize discoidin lectin protein during growth and for a very short period after the initiation of development. No discoidin lectin is synthesized during either aggregation or the later stages of development.

Figure 2 shows the experimental design (Fig. 2A) and the results (Fig. 2B) of these studies. Each panel in Fig. 2B represents the analysis of a different recombinant strain. The bottom half of each panel is the autoradiogram of the anti-discoidin immunoprecipitate of extracts from cells pulse-labeled with [^{35}S]methionine for 30-min periods throughout development. The top half of each panel is a Western analysis, with anti-discoidin antibody, of the identical extract used for the immunoprecipitation. The Western analysis shows the total level of discoidin lectin at each hour of development, whereas the immunoprecipitation shows the level of de novo discoidin lectin biosynthesis at corresponding times (numbers at the bottom of each panel indicate the hour at which the 30-min pulse-labeling period was begun). In all cases, the cells aggregated and developed synchronously (>95%) throughout the entire developmental program. The cells always completed development normally and formed fruiting bodies. The developmental synchrony was reproducible between experiments.

The experiment (Fig. 2B) takes advantage of recombinant strains resulting from a cross between HL101 (*drsA*) and NC4 (*drsA*$^+$) so that effects resulting from genetic background can be eliminated. Wild-type strain NC4 (panel a) shows the pattern of discoidin lectin synthesis and accumulation previously reported. Growing cells (time zero of development) neither contain nor synthesize lectin. This is in agreement with previous work which showed that wild-type

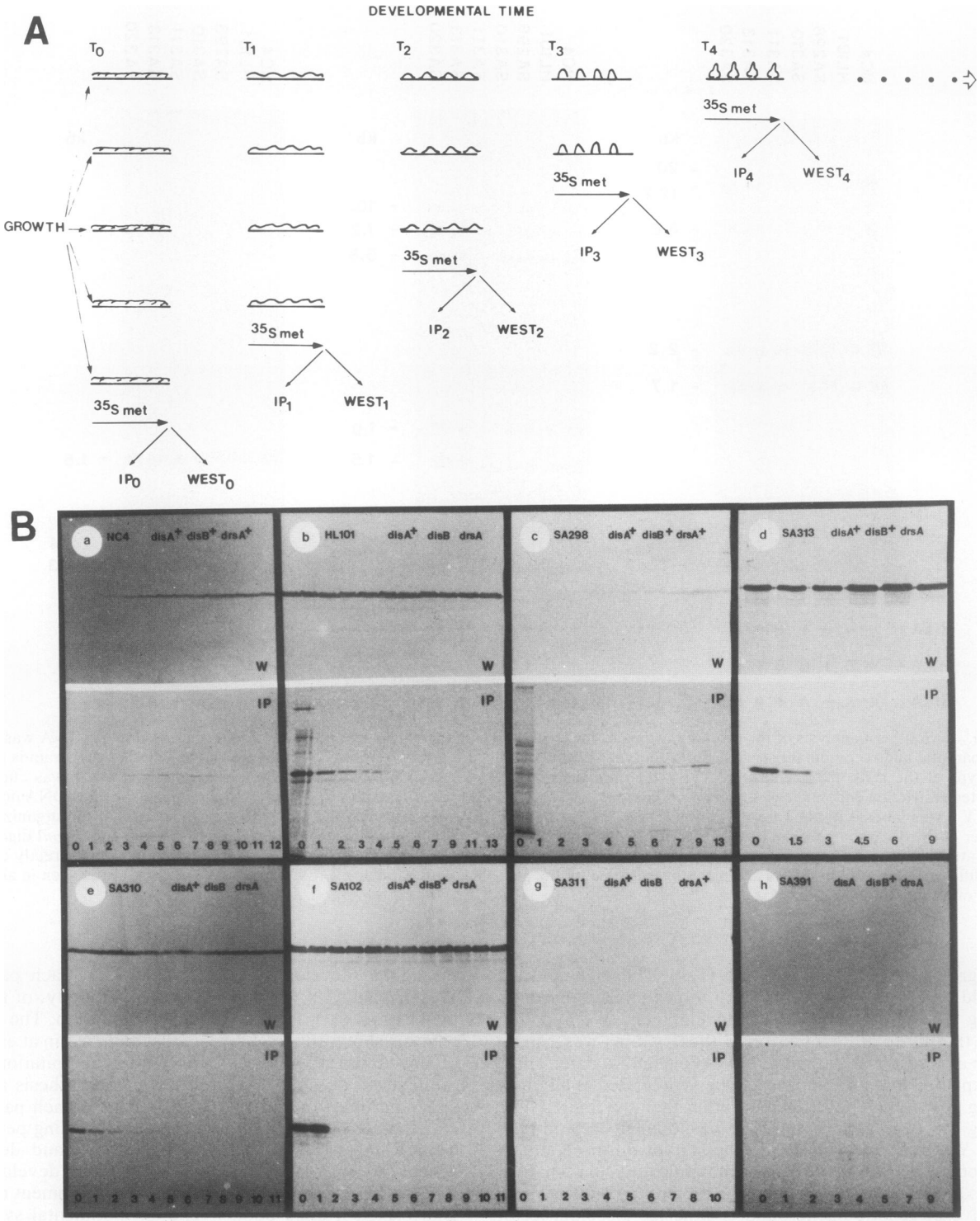

FIG. 2. Expression and accumulation of the discoidin lectins in wild-type and *drsA* mutant strains. (A) The strategy for these experiments is as follows. Growing cells are harvested and set on filters for development. Filters with developing cells are transferred hourly to drops of [^{35}S]methionine (100 μCi) for pulse-labeling. The cells are harvested after 30 min, washed, and frozen. The morphologies shown do not correspond to any particular strain and are presented only to illustrate that synchronous development proceeds with time. Extracts are prepared and the sample is split for immunoprecipitation (IP) and Western blotting (WEST). (B) Each panel represents the data from a different strain. Only the genotypes of the discoidin regulatory genes are shown. Full genotypes are given in Table 1. The upper half of each panel is the Western analysis (W), and the lower half is the IP. The numbers at the bottom of each panel refer to the hour of development at the beginning of the 30-min labeling period for that sample. Occasionally, the immunoprecipitates of zero time samples show background bands, as seen in panels b and c. Detailed discussion of the experimental results is presented in the text.

growing cells do not transcribe the lectin genes (17). Immunoprecipitation of pulse-labeled NC4 cells shows that lectin synthesis begins during h 3 of development and continues until h 9. Western analysis of the same cells indicates that lectin begins to accumulate at h 3, concomitant with the onset of synthesis and remains at a steady level at least 3 h after synthesis has stopped (see Discussion). Strain SA298 (panel c) is a recombinant strain carrying the wild-type $drsA^+$ allele as well as the $disA^+$ and $disB^+$ alleles. As expected, the pattern of discoidin lectin expression and accumulation is the same as in wild-type strain NC4. In contrast, strain HL101 (panel b) and recombinants SA313 (panel d) and SA310 (panel e), all carrying the *drsA* mutant allele, show a very different pattern of synthesis and accumulation of the discoidin lectins. Western analysis shows that all of these strains have high levels of lectin in freshly harvested growing cells (time zero of development) and retain the lectin throughout the entire period of development examined. Synthesis of lectin as reflected by the pulse-labeling is largely confined to the first 1 or 2 h after the initiation of development, which is well before the period of expression during aggregation in wild-type cells (cf. panels a and c). *drsA* mutant strain SA102 (panel f) is the product of a different genetic cross in a significantly different genetic background (Table 1) and shows the same altered pattern of discoidin synthesis and accumulation. This supports the specific nature of the phenotype associated with the *drsA* mutation. As expected, strains carrying *disB* (SA311, panel g) or the nonsuppressible *disA* mutant allele (SA391, panel h) neither synthesize nor accumulate discoidin during either growth or development (1, 5).

A *drsA* phenocopy also misregulates discoidin lectin expression. The preceding analysis indicated that the expression of discoidin lectin during growth may be responsible for the down-regulation of its normal expression during development through some type of negative autoregulatory mechanism. Wild-type NC4 cells can be grown in liquid on a suspension of bacteria. Previous work from our laboratory has shown that the accumulation of early developmentally controlled enzymes is normal in cells grown in this manner (3). Interestingly, the discoidin lectins accumulate to high levels in vegetative cells grown in this manner and remain in the cells during development (9, 10; unpublished data). Thus, NC4 cells grown in bacterial suspensions are *drsA* phenocopies, because they both overexpress and misregulate the expression of the discoidin lectins.

We wished to know whether the pattern of discoidin lectin synthesis in these cells is the same as that seen in bona fide *drsA* mutants (Fig. 2). Thus, wild-type NC4 cells were grown in association with bacteria either on plates or in liquid suspension. In either case, the generation time was approximately 4 h. Cells from both growth conditions were harvested, allowed to develop, and pulse-labeled hourly with [^{35}S]methionine (Fig. 2A). Figure 3 shows the pattern in wild-type cells of discoidin lectin synthesis resulting from the altered growth conditions. As shown in Fig. 3a, strain NC4 grown on plates synthesizes lectin during aggregation (3 to 9 h of development). Western analysis shows a parallel accumulation of lectin protein. In contrast, Western analysis shows that NC4 cells grown in a liquid suspension of bacteria have high levels of lectin in vegetatively growing cells and that this level is maintained throughout development. The immunoprecipitations from these phenocopy cells show that virtually no lectin synthesis takes place during development. Only very low levels of synthesis are seen in h 1 and 2 of development. These results are very similar to

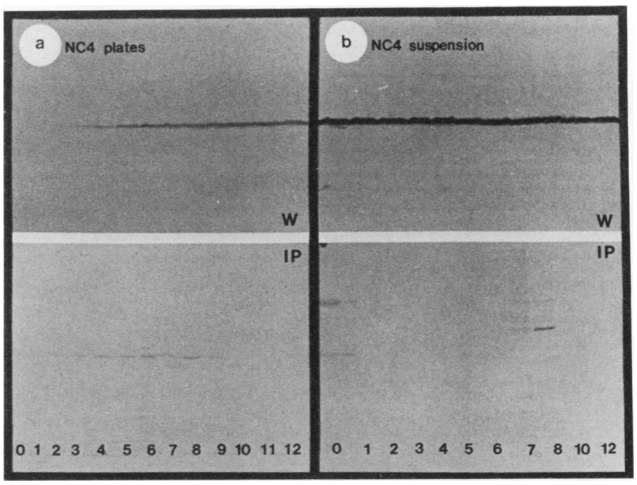

FIG. 3. Expression and accumulation of the discoidin lectins in a *drsA* phenocopy. The analyses are essentially as described in the legend to Fig. 2. However, we compared discoidin expression in wild-type cells under two conditions of growth, one of which produces a phenocopy of the *drsA* mutation. The numbers at the bottom of the panels refer to the hour of development at the beginning of the 30-min labeling period for that sample. (a) Strain NC4 grown under standard conditions on SM agar plates with *K. aerogenes*. (b) Strain NC4 grown on *K. aerogenes* suspended in 17 mM phosphate buffer, pH 6.5. Note that the Western analysis (W) of NC4 grown in suspension (b) is a phenocopy of the pattern of discoidin lectin accumulation seen in bona fide *drsA* mutants (Fig. 2B, panels b, d, e, and f). IP, immunoprecipitation.

what is observed in *drsA* mutant strains; discoidin synthesis occurs during growth, is at very low levels at the onset of development, and is absent during aggregation.

Translation of discoidin mRNA is inhibited in *drsA* mutant strains. The demonstration that *drsA* mutant strains do not synthesize discoidin lectin during development raised the question of whether regulation was at the transcriptional or translational level. To answer this question we isolated total RNA hourly from developing cells of the *drsA* mutant strains SA313, SA102, HL101, and SA310 and wild-type NC4. Equal amounts of the RNAs were separated on formaldehyde gels and transferred to nitrocellulose. The blots from all five strains were probed together with nick-translated specific discoidin cDNA. Thus, the results are directly comparable between strains. Figure 4A to D demonstrate that *drsA* mutant cells have high levels of discoidin lectin mRNA during growth (time zero in the figure). In addition, the levels of discoidin mRNA remain high through the first 4 to 6 h of development, depending on the strain examined. These results, when compared with those in Fig. 2B (panels d, f, b, and e, respectively), clearly show that the discoidin lectin mRNA in these mutant strains is not being translated into new discoidin lectin protein during the aggregation stage of development. Although high levels of discoidin mRNA are present, virtually no corresponding lectin protein synthesis is observed at these times.

Figure 4E shows that wild-type strain NC4 does not contain lectin mRNA during growth but accumulates it during development, as reported previously (17). Comparison of this Northern analysis to results in Fig. 2B (panel a) indicates that the patterns of lectin mRNA accumulation and lectin protein synthesis are essentially identical. However, even here translation appears to be turned off later in

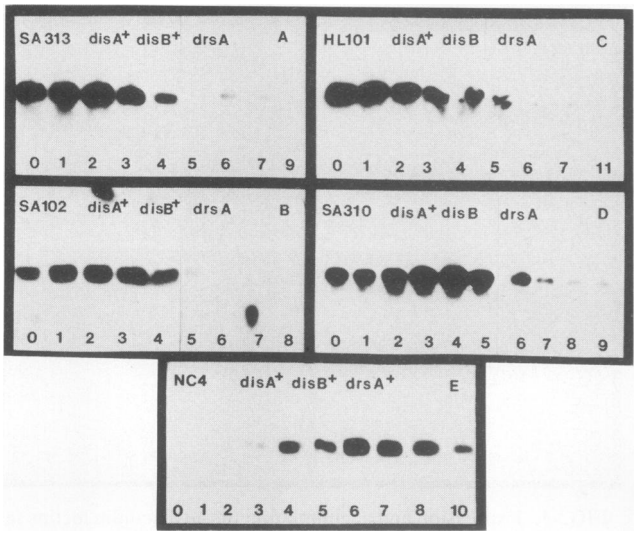

FIG. 4. Northern analysis of mRNA levels in *drsA* mutant strains. Cells of the *drsA* mutant strains SA313, SA102, HL101, and SA310 and wild-type NC4 were collected at hourly intervals. Total RNA was prepared, and 2.5 μg of each sample was separated on formaldehyde gels and blotted to nitrocellulose. Equal loading was confirmed by staining the rRNA bands. The blots from all five experiments were probed together with nick-translated discoidin-specific cDNA pcDd Disc-IV$_{32}$. Each panel is a composite of the two gels used for each experiment. Gels were blotted side by side, and the blots for all experiments were hybridized together. The numbers at the bottom of the figure refer to the hour of development at which the samples were harvested.

development, when there is still substantial mRNA in the cells. Wild-type cells also appear to contain discoidin lectin mRNA later in development than the *drsA* mutant strains. This is due primarily to the somewhat more rapid aggregation of *drsA* strains which results in a correspondingly more rapid accumulation of cyclic AMP (cAMP) which is correlated with the down-regulation of discoidin mRNA transcription (see Discussion). Overall, these analyses indicate that the expression of discoidin lectins can be controlled at the level of translation.

DISCUSSION

Previous genetic and biochemical studies identified a network of regulatory genes which act together to regulate the developmental expression of the discoidin multigene family. One of the most interesting findings to emerge from these studies was the identification of the *drsA* mutation as a dominant phenotypic suppressor of the *disB* null mutation. We now have demonstrated an unusual mechanism of this suppression. Because of the unique phenotype associated with the *drsA* mutation, including high-level discoidin lectin expression during growth, we suspected that the mechanism of suppression was not simply a bypass of the *disB* mutation. However, our original hypothesis that *drsA* resulted in constitutive discoidin expression (1) has been shown to be incorrect. Indeed, our pulse-labeling studies of *drsA* mutant strains show that synthesis of lectin protein is essentially restricted to growing cells in these strains. It was the lectin synthesized during vegetative growth that we were assaying by Western analysis in developing cells in our original characterization of the suppressive effect of *drsA* (1).

The observation that *drsA* mutant strains express discoidin at high levels during growth but do not synthesize substantial amounts of additional lectin during development is particularly intriguing. One possibility is that there may be some mechanism of negative feedback, perhaps by the discoidin proteins themselves, which inhibits further discoidin lectin expression during development. This hypothesis is consistent with our experiment showing that a *drsA* phenocopy made by growing the wild-type strain on bacteria in suspension also synthesizes discoidin during growth but not during development.

We have shown that this inhibition of discoidin lectin synthesis during development is at the level of translation. *drsA* mutant strains have high levels of discoidin gene transcripts during development but fail to synthesize the lectin protein at the corresponding time. Much of the *Dictyostelium* developmental program is regulated transcriptionally (8). In some cases cAMP levels appear to regulate stability of mRNAs, although it is not clear whether these stabilized messages continue to be translated (19). The situation we have characterized here appears to be different, because the stability of the mRNA does not appear to be affected, but translation is blocked. This block is most likely at the level of the initiation of translation of newly transcribed mRNA. A similar situation has been described for the *Dictyostelium* ribosomal protein mRNAs in which translation is coordinately reduced at the onset of development and control is at the initiation step (27, 28). Yeasts have been shown to be capable of translationally regulating the over abundance of ribosomal protein messages in cells which have been transformed with extra copies of ribosomal protein genes (21). We suggest that *D. discoideum* may use a similar strategy to regulate the abnormally high level of discoidin lectin expression in *drsA* mutant strains (Fig. 5). Lectin expression continues during growth because the increase in cell volume maintains lectin concentration below the threshold necessary for total inhibition of translation. Expression of lectin does continue at a substantial rate during h 1 of development in *drsA* strains and, coupled to the cessation of cell division, may bring the concentration of lectin above the threshold. It will be interesting to determine whether discoidin itself or another factor can inhibit the in vitro translation of discoidin mRNA. Clearly, this is not the only possibility, and the *drsA* mutant allele may have a more direct effect on translation.

The Western analyses shown in Fig. 2 and 3 indicate another striking feature of this system. Once expressed, the discoidin lectins are remarkably stable molecules which remain unchanged in the cells at least through the maxifinger stage seen at 14 h under these conditions of development. This is particularly remarkable in *drsA* mutant strains in which there is no synthesis of lectin protein after h 2 of development. Indeed, we have now shown that the lectin protein remains intact in the cells throughout development, neither substantially decreasing in amount nor degrading. Ultimately, a large proportion of the lectin protein becomes localized in the spores (4a). These observations raise questions regarding the function, intracellular localization, and remarkable stability of the discoidin lectin protein during development. Springer et al. (26) have presented evidence that the discoidin lectin, which contains an RGD sequence, may act as an extracellular matrix molecule which promotes cell-substrate adhesion and directs cell migration during aggregation. They also showed that *disA* and *disB* mutant strains fail to form streams during aggregation. This phenotype was supported by experiments using antisense transfor-

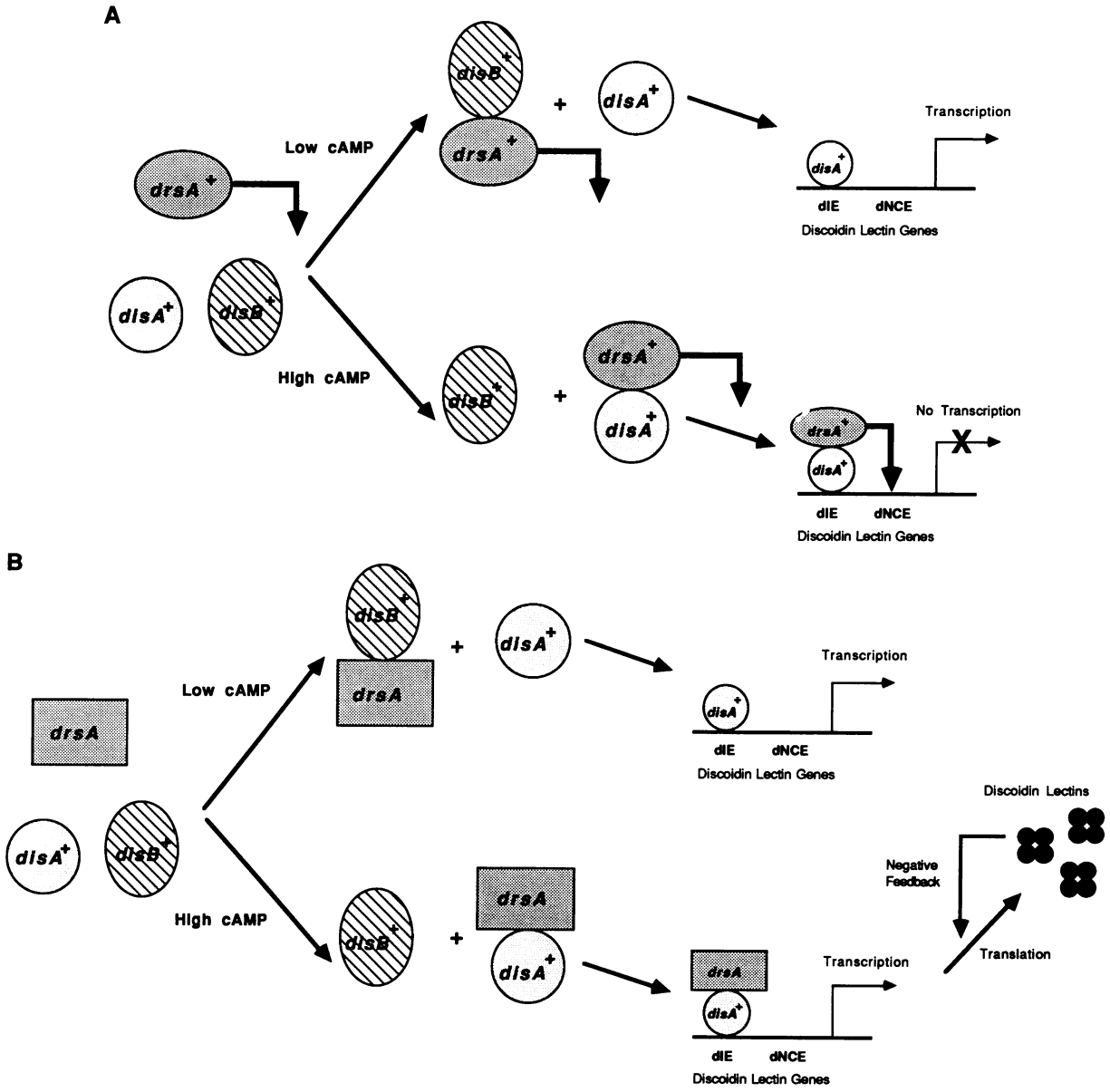

FIG. 5. Model for the regulation of the expression of the discoidin multigene family. The *drsA*$^+$ protein can bind to either the *disA*$^+$ or the *disB*$^+$ gene products, and these interactions are modulated by the level of cAMP in the cells. (A) Under conditions of low cAMP, when *drsA*$^+$ is bound to *disB*$^+$, the *disA*$^+$ protein acts to positively regulate transcription of the discoidin lectin gene family by binding to the discoidin induction element (dIE). Under conditions of high cAMP, when *drsA*$^+$ protein binds to *disA*$^+$ protein, the *drsA*$^+$ protein is positioned on the promoter so that it can bind (↴) to the dNCE and thereby block transcription. (B) Mutant *drsA* protein (now shown as a rectangle) has lost its dNCE binding domain and thus cannot block transcription. The present study has shown that the accumulation of discoidin lectins during growth results in negative feedback, perhaps by the lectins themselves, which inhibits the translation of discoidin lectin mRNA during development. A more detailed description of the model is presented in Discussion.

mation to block discoidin expression during aggregation (12). Nevertheless, the fact that most of the lectin remains in the cells throughout development and becomes localized in spores suggests that these ubiquitous proteins may have additional functions later in development. Indeed, the discoidin lectins have substantial homology to human coagulation factor V (14), which raises additional questions about its in vivo function.

Previous work has shown that the transcription of the discoidin genes appears to be under both positive and negative regulation. Clarke et al. (9, 10) have shown that the accumulation of a prestarvation factor in axenically growing cultures is correlated with the initiation of transcription of the discoidin genes. In contrast, cAMP has been shown to be a negative regulator of discoidin gene transcription (34), since cAMP accumulation during development results in cessation of transcription of these genes. Recently, we have shown that cAMP, as well as folic acid, also negatively regulates discoidin lectin expression during growth of axenic cells (4). At the molecular level, the discoidin I γ gene promoter has now been shown to have distinct elements essential for developmental induction (discoidin induction

element [dIE]) and down-regulation by extracellular cAMP (discoidin-negative cAMP element [dNCE]) (31).

We have attempted to graphically summarize this information in the model presented in Fig. 5. The model takes into account the evidence regarding effector molecules and the mechanism of suppression and genetic dominance of the mutant *drsA* allele. The model is based on positive regulation of gene expression, although other models, including those with negative regulators, cannot be excluded. In this model (Fig. 5A), the wild-type $disA^+$ gene product is a transcriptional activator of the discoidin lectin genes, because *disA* mutants (regardless of the *disB* and *drsA* alleles) are always unable to express lectin. The $drsA^+$ gene product can form heterodimers with either the $disA^+$ or the $disB^+$ product. Under conditions of low-cAMP concentrations, such as at the onset of development, the $drsA^+$-$disB^+$ protein interaction is favored. This allows free $disA^+$ gene product to promote transcription of the discoidin lectin genes by binding to the dIE promoter. When the cAMP level increases during aggregation, the $drsA^+$-$disA^+$ interaction is favored. The binding of this protein complex to the promoter, via the $disA^+$ protein, positions the $drsA^+$ protein to interact with the dNCE through its dNCE binding domain (Fig. 5, ↴) and therefore block transcription of the discoidin genes. These competing heterodimer interactions are reminiscent of those proposed between the helix-loop-helix domains of the Id, MyoD, and E12/E47 proteins which regulate transcription during myogenesis (7, 13). The model also accounts for the null phenotype of mutant cells with the *disB* mutation. In this case, the mutant *disB* product is unable to bind the $drsA^+$ protein which is then always able to complex with the $disA^+$ protein and block transcription.

Figure 5B presents the model in terms of the mutant *drsA* gene. In this case, the mutant *drsA* protein is hypothesized to have lost its dNCE binding domain and concomitantly gained a higher affinity for the $disA^+$ protein. Thus, under high-cAMP conditions the mutant *drsA* protein binds to the $disA^+$ protein but cannot block transcription because of the loss of its dNCE binding domain. Therefore, the suppression of *disB* mutants by *drsA* is indirect and results from the inability of the *drsA* mutant protein to block transcriptional activation. It has previously been shown that the *drsA* mutation is dominant in heterozygous diploids (1). The model accounts for this observation because the higher affinity of the mutant *drsA* protein for the $disA^+$ protein outcompetes the $drsA^+$ molecules encoded by the wild-type allele. Therefore, in the heterozygous diploid most of the heterodimers have a mutant *drsA* protein which is defective in blocking transcription because of the loss of its dNCE binding domain. Overall, this model accounts for many of the diverse molecular and genetic observations that have been made on the expression of the discoidin lectin gene family.

One of the remaining open questions is how the effector molecules (cAMP, folic acid, and prestarvation factor) are transduced and act to regulate expression of the discoidin genes. In this regard, we have shown (unpublished data) that discoidin lectin expression appears normal (lectin synthesis occurs during aggregation) in strain HC85, which has been shown to have a deletion in the Gα2 protein gene and does not respond chemotactically to cAMP (11, 15). Thus, the regulation of the transcription of the discoidin lectin genes may not be mediated through the cAMP receptor-G protein complex. The details of this regulatory mechanism await cloning of the regulatory genes and analysis of both their transcription and the activities of their products.

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