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Multiple Regulatory Genes Control Expression of a Gene Family during Development of *Dictyostelium discoideum*†

STEPHEN ALEXANDER,* ANN MARIE CIBULSKY, AND SUSAN D. CUNEO

Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California 92037

Received 4 June 1986/Accepted 5 September 1986

Mutant strains of *Dictyostelium discoideum* carrying *dis* mutations fail to transcribe specifically the family of developmentally regulated discoidin lectin genes during morphogenesis. The phenotypes of these mutants strongly suggested that the mutations reside in regulatory genes. Using these mutant strains, we showed that multiple regulatory genes are required for the expression of the lectin structural genes and that these regulatory genes (the *dis*$^+$ alleles) act in *trans* to regulate this gene family. These regulatory genes fall into two complementation groups (*disA* and *disB*) and map to linkage groups II and III, respectively. A further regulatory locus was defined by the identification of an unlinked supressor gene, *drsA* (discoidin restoring), which is epistatic to *disB*, but not *disA*, and results in the restoration of lectin expression in cells carrying the *disB* mutation. Mutant cells carrying the *drsA* allele express the discoidin lectin gene family during growth and development, in contrast to wild-type cells which express it only during development. Therefore, the suppressor activity of the *drsA* allele appears to function by making the expression of the discoidin lectins constitutive and no longer strictly developmentally regulated. The data indicate that normal expression of the discoidin lectins is dependent on the sequential action of the *disB*$^+$, *drsA*$^+$, and *disA*$^+$ gene products. Thus, we described an interacting network of regulatory genes which in turn controls the developmental expression of a family of genes during the morphogenesis of *D. discoideum*.

An extremely detailed description exists of the qualitative and quantitative biochemical changes which accompany and underlie the multicellular development of the cellular slime mold *Dictyostelium discoideum* (16, 30). Many of these changes in biochemical phenotype have been shown to be the direct result of differential gene transcription during development (4, 6, 10, 16). However, virtually nothing is known about the mechanisms and molecules controlling this differential transcription, and no specific regulatory genes or their products have been identified.

One of the most intensively studied developmentally controlled events in this organism is the synthesis, during the early stages of morphogenesis of bacterially grown cells, of a family of proteins with lectin activity, the discoidin lectins (10, 25). Accumulation of the lectin proteins requires prior mRNA synthesis (18). Transcription occurs from three separate homologous discoidin I genes and a single nonhomologous discoidin II gene (10, 26, 32). We have previously isolated two mutant strains (SA31 and SA219) which aggregate and complete development but do not express the discoidin lectins (3). Subsequently, we have shown that the mutations in these strains act at the level of transcription since isolated nuclei from the mutant strains carrying these mutations (termed *dis*) do not contain nascent discoidin-specific RNA, in contrast to nuclei from developing wild-type (*dis*$^+$) strains (2). In addition, we presented evidence to show that the *dis* mutations were specific in their effect on the expression of the discoidin lectins. Taken together, the data indicated that strains SA31 and SA219 contained mutations in regulatory genes which are involved in specifically controlling discoidin expression. Using the *dis* mutations in SA31 and SA219 as a starting point, this regulatory system has now been examined further and additional elements have been defined. The present report describes a *trans*-acting multigene regulatory network which specifically controls the developmental expression of the family of discoidin lectin genes. In addition, a newly defined mutation (*drsA*) in one of these regulatory genes results in the abnormal expression of the discoidin lectins during growth on bacteria as well as development. Thus, this mutation is responsible for a breakdown in the normally strict developmental regulation of these genes.

MATERIALS AND METHODS

Strains and growth conditions. The primary strains used in this study are listed with their corresponding genotypes in Table 1. All cultures were started monthly from cloned silica gel storage cultures. The cultures were grown on SM agar in association with *Klebsiella aerogenes* (29). Cells were allowed to develop on black paper filters as described previously (27, 29).

Genetic analysis. Diploids were formed by the fusion of two parental haploid strains and selected by the complementation of different mutations for sensitivity to growth either at the restrictive temperature (27°C) (13, 22) or using *Bacillus subtilis* as a food source (23, 33). The system allows the selection of the heterozygous diploids which are formed at a low frequency (10^{-5}). Ploidy of the putative diploid strains was determined by examining the spore size and shape after cloning and confirmed by their ability to segregate recombinant strains representing both parental genotypes. Random segregation of diploids was achieved by growth on SM agar containing either benlate or thiabendazole (a gift from Keith Williams) (34, 37). Haploid segregants were cloned a minimum of two times on SM agar, and their phenotypes were examined at each cloning step. The presence or absence of discoidin in the segregants was assayed by scraping a loopful (containing 2×10^7 to 4×10^7 cells) of aggregating cells from the border of a clone of each strain and suspending the cells in 0.5 ml of 1% sodium dodecyl sulfate (SDS). The extract

* Corresponding author.

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TABLE 1. Haploid strains of *D. discoideum*

Strain	Genotype[a]										Reference
	dis	*cyc*	*acr*	*whi*	*tsg*	*bwn*	*bsg*	*alp*	*nag*	*man*	
NC4	+	+	+	+	+	+	*B500*	+	+	+	21
SA31	*A50*	+	+	+	+	+	*B500*	+	+	+	3
SA32	*A50*	+	*A50*	+	+	+	*B500*	+	+	+	This study
SA219[b]	*A51*	+	+	+	+	+	*B500*	+	+	+	3
SA220	*A51*	+	*A51*	+	+	+	*B500*	+	+	+	This study
X9	+	*A1*	*A1*	*A1*	*D12*	*A1*	+	+	+	+	38
X11	+	+	*A1*	*A1*	*A1*	*A1*	+	+	+	+	38
XP55	+	*A5*	+	+	+	+	*A5*	+	+	+	24
SA132	*A51*	+	*A51*	+	+	+	*A5*	+	+	+	This study
HL101	*B50*	*A1*	+	*A1*	*D12*	+	+	*A1*	*A211*	*A2*	19
SA38	*B50*	+	+	*A1*	*D12*	+	+	*A1*	+	+	2
SA102	+	*A1*	+	*A1*	*D12*	*A1*	+	+	*A211*	*A2*	This study

[a] Phenotypes of the mutations at these loci are: *dis*, absence of expression of the discoidin lectins during development; *cyc*, resistance to 500 μg of cycloheximide per ml; *acr*, resistance to 2% methanol; *whi*, white spored; *tsg*, temperature sensitive to growth at 27°C; *bwn*, produces brown pigment; *bsg*, inability to grow with *B. subtilis* as a food source; *alp*, alkaline phosphatase deficient; *nag*, *N*-acetylglucosaminidase deficient; *man*, α-mannosidase-1 deficient; +, wild-type allele. The linkage relationships of these alleles are shown in subsequent tables.

[b] Strain SA219 had been reported to fall into the *disB* complementation group (2). This was based on the ability of strain SA38 (a segregant from a cross between SA219 and HL101) to complement SA31 (*disA*). At the time we were unaware of the suppressed *disB* mutation in strain HL101. This mutation and its suppressor *drsA* are characterized in this paper.

was boiled, and after centrifugation for 5 min in an Eppendorf centrifuge, 2 μl was applied to nitrocellulose. Discoidin was then determined by the immunochemical assay described previously (3). Resistance to cycloheximide (*cycA*) and methanol (*acrA*) were tested on SM plates containing 500 μg of cycloheximide per ml and 2% methanol, respectively. White spores (*whiA* and *whiB*) were scored visually. The production of brown pigment can be scored visually or by suspending 10^7 cells in a buffered salts medium containing phenylalanine and tyrosine (20). Temperature sensitivity (*tsg*) was determined by comparing growth at 22°C (permissive temperature) with that at 27°C (restrictive temperature). Sensitivity to growth on *B. subtilis* was assayed by comparing growth on *K. aerogenes* with that on *B. subtilis*. The *bsgA* and *bsgB* genotypes of haploid segregants from diploid strains having both these mutant loci were determined by complementation analysis to the standard strains XP55 and NC4 carrying the *bsgA* and *bsgB* mutations, respectively. Strains were assayed for the absence of α-mannosidase (*manA*), *N*-acetylglucosaminidase (*nagA*), and alkaline phosphatase (*alpA*) in multiwell tissue culture plates as described previously (12, 17, 19). Aggregating cells were used for α-mannosidase and *N*-acetylglucosaminidase assays. Preculminates were harvested for the assay of alkaline phosphatase. Once genotypes were established, segregants of interest were stored on silica gel.

Gel electrophoresis and Western blotting. SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli (15). The separated proteins were electrophoretically transferred from the gels to nitrocellulose sheets as described previously (31). The discoidin lectins were detected immunochemically as previously described (3).

Genetic nomenclature. Haploid and diploid strains are denoted by the prefixes SA and DSA, respectively. Nomenclature for mutant alleles conforms to the conventions of Demerec et al. (9), e.g., *disA* and *disB* for mutations at different loci, either of which results in the absence of discoidin expression during development. The wild-type alleles of these loci are denoted as $disA^+$ and $disB^+$. Phenotypes are denoted Dis^+ and Dis^- for strains producing and lacking discoidin during development. Independent mutations from this laboratory have been given allele numbers increasing from 50, e.g., *disA50*, *disA51*, etc.

RESULTS

Strains SA31 and SA219 both carry mutations in the *disA* complementation group. Mutant strains SA31 and SA219 were isolated independently. Both fail to express any of the discoidin lectin gene products (3). Biochemical analysis of these strains suggested that these mutations reside in *trans* regulatory genes and express their effect at the level of transcription of the lectin genes (2). We have now been able to obtain evidence to support this suggestion using parasexual genetic analysis to determine both complementation and linkage of genetic markers.

Both strains SA31 and SA219 arose in a genetic background which contained the *bsgB* mutation (sensitivity for growth on *B. subtilis* [21]). Thus, these haploid strains were suitable for parasexual genetic analysis by fusing to other haploid strains carrying either *tsg* (sensitive to growth at 27°C) or other complementing *bsg* mutations. The genotypes of the strains used in this study are presented in Table 1. It should be noted that only six of the seven chromosomes are genetically marked. For historical reasons the unmarked linkage group is designated V and thus is absent from the crosses.

Both strains SA31 and SA219 were fused to strains X9 and X11. The resultant diploids (DSA) are shown in Tables 2 and 3. After haploidization of the heterozygous diploids in the presence of benlate or thiabendazole (which induce random haploidization), segregants were clonally isolated, and their phenotypes were determined. Strains representing the classes of haploid segregants obtained in these crosses are shown in Tables 2 and 3. For both strains SA31 and SA219 the inability to express discoidin cosegregated with linkage group II, i.e., in opposition to the *whiA1*, *tsgD12*, and *acrA1* mutations in strains X9 and X11. Selection of haploid segregants by their ability to grow on methanol by virtue of their *acrA1* mutation on linkage group II always resulted in the cosegregation of the dis^+ allele in every segregant recovered by this method. Not every possible class of segregant was recovered in each cross (e.g., no haploid segregants containing the *cycA1* allele on linkage group I were recovered from the cross of SA219 and X9). However, there was clearly no linkage of the *dis* mutations from either SA31 or SA219 to linkage group I (*cycA1*), linkage group III

TABLE 2. Genotypes of parental haploids, diploids, and selected haploid segregants of crosses with SA31

Diploid[a]	Haploids	*dis*/*dis*$^+$ genotype	Linkage group					
			I	II	III	IV	VI	VII
DSA25	Parents							
	SA31	*disA50*	+	+ + +		+		*bsgB500*
	X9	+	*cycA1*	*whiA1 tsgD12 acrA1*		*bwnA1*		+
	Segregants							
Dis$^+$	SA43	+	+	*whiA1 tsgD12 acrA1*		+		*bsgB500*
	SA49	+	+	*whiA1 tsgD12 acrA1*		*bwnA1*		+
	SA74	+	+	*whiA1 tsgD12 acrA1*		*bwnA1*		*bsgB500*
Dis$^-$	SA51	*disA50*	+	+ + +		*bwnA1*		+
	SA23	*disA50*	*cycA1*	+ + +		*bwnA1*		+
DSA73	Parents							
	SA31	*disA50*		+ +	+	+		*bsgB500*
	X11	+		*whiA1 acrA1*	*tsgA1*	*bwnA1*		+
	Segregants							
Dis$^+$	SA330	+		*whiA1 acrA1*	*tsgA1*	+		+
	SA331	+		*whiA1 acrA1*	*tsgA1*	+		*bsgB500*
Dis$^-$	SA327	*disA50*		+ +	*tsgA1*	*bwnA1*		+
	SA339	*disA50*		+ +	*tsgA1*	+		*bsgB500*
	SA340	*disA50*		+ +	+	*bwnA1*		+
	SA341	*disA50*		+ +	+	+		+
	SA342	*disA50*		+ +	*tsgA1*	+		+
DSA62	Parents							
	SA32	*disA50*	+	*acrA50*	+			*bsgB500*
	XP55	+	*cycA5*	+	*bsgA5*			+
	Segregants							
Dis$^+$	SA124	+	+	+	+			+
	SA127	+	+	+	*bsgA5*			+
	SA128	+	+	+	+			*bsgB500*
	SA131	+	*cycA5*	+	*bsgA5*			*bsgB500*
Dis$^-$	SA122	*disA50*	+	*acrA50*	+			+

[a] Diploids DSA25 and DSA73 were made from strains SA31 and X9 or X11 by complementation of the *bsgB500* mutation of SA31 with the *tsgD12* or *tsgA1* mutations in strains X9 and X11, respectively. SA32 is a methanol-resistant (*acrA50*) derivative of SA31. Diploid DSA62 was formed by complementation of the *bsgB500* mutation from SA32 and the *bsgA5* mutation of strain XP55. Random haploidization is accomplished by growth of the diploids on plates in the presence of benlate or thiabendazole. A detailed discussion of these crosses is in the Results section. Phenotype: Dis$^+$, discoidin-producing strains; Dis$^-$, discoidinless strains.

(*bsgA1* or *tsgA1*), linkage group IV (*bwnA1*), or linkage group VII (*bsgB500*). The *dis* mutations were also shown to be unlinked to linkage group VI by crossing SA31 and SA219 to strain HL101 which is marked on linkage group VI by *manA*. Both *manA* and *manA*$^+$ discoidinless strains were recovered (data not shown). The data strongly suggest that the *dis* mutations in both strains SA31 and SA219 map to linkage group II, and this was tested directly.

Direct mapping of the *dis* mutations in both SA31 and SA219 to linkage group II was accomplished as follows. Resistance to methanol during growth is the result of mutations at a single locus (*acrA*) which maps on linkage group II (38). Strains SA31 and SA219 were plated on SM plates containing 2% methanol, and stable methanol-resistant variants were selected at a frequency of about 10^{-6}. One methanol-resistant derivative of each strain (SA32 [*acrA50*] from SA31 and SA220 [*acrA51*] from SA219) was clonally isolated. The *acrA50* and *acrA51* mutations were shown to be allelic to *acrA1* in strain X9 and thus map to linkage group II. Both derivative strains, like their parents, were unable to express the discoidin lectins. These strains were then crossed into strain XP55 which produces the discoidin lectins (*dis*$^+$) and which is unmarked on linkage group II. These crosses produced diploid strains DSA62 (SA32) and DSA63 (SA220). If the *dis* mutations in strains SA31 and SA219 are linked to linkage group II, then all segregants which are resistant to growth on methanol (*acrA50* or *acrA51*) should also be *dis*. As shown in Tables 2 and 3, random segregation of these diploid strains produced haploid strains of both *dis*$^+$ and *dis* genotypes. As expected, all *dis*$^+$ strains were sensitive to methanol and all *dis* strains were resistant. Direct selection of haploid segregants on methanol plates after initial haploidization on thiabendazole resulted in the exclusive isolation of *dis* strains (data not shown). This is in direct contrast to the results obtained above in which strains SA31 and SA219 were crossed with X9 and X11 in which the *acrA1* allele is in the *dis*$^+$ parent. No evidence of cosegregation with the other marked linkage groups was observed.

Interestingly, in the cross of SA32 to XP55 all segregants containing linkage group I (*cycA5*) from XP55 and linkage group II (*acrA50*) for SA32 were unable to aggregate. Apparently both strains have suppressed aggregationless mutations, and this specific combination of linkage groups results in their desuppression. Resolution of this interesting phenomenon requires closer examination. All these strains were

TABLE 3. Genotypes of parental haploids, diploids, and selected haploid segregants of crosses with SA219

Diploid[a]	Haploids	*dis*/*dis*$^+$ genotype	Linkage group					
			I	II	III	IV	VI	VII
DSA53	Parents							
	SA219	*disA51*	+	+ + +		+		*bsgB500*
	X9	+	*cycA1*	*whiA1 tsgD12 acrA1*		*bwnA1*		+
	Segregants							
Dis$^+$	SA106	+	+	*whiA1 tsgD12 acrA1*		*bwnA1*		+
	SA107	+	+	*whiA1 tsgD12 acrA1*		*bwnA1*		*bsgB500*
	SA108	+	+	*whiA1 tsgD12 acrA1*		+		+
	SA109	+	+	*whiA1 tsgD12 acrA1*		+		*bsgB500*
Dis$^-$	SA112	*disA51*	+	+ + +		*bwnA1*		*bsgB500*
	SA114	*disA51*	+	+ + +		+		+
	SA115	*disA51*	+	+ + +		*bwnA1*		+
DSA66	Parents							
	SA219	*disA51*		+ +	+	+		*bsgB500*
	X11	+		*whiA1 acrA1*	*tsgA1*	*bwnA1*		+
	Segregants							
Dis$^+$	SA141	+		*whiA1 acrA1*	*tsgA1*	+		*bsgB500*
	SA144	+		*whiA1 acrA1*	*tsgA1*	+		+
Dis$^-$	SA143	*disA51*		+ +	*tsgA1*	*bwnA1*		+
	SA146	*disA51*		+ +	*tsgA1*	+		+
DSA63	Parents							
	SA220	*disA51*	+	*acrA51*	+			*bsgB500*
	XP55	+	*cycA5*	+	*bsgA5*			+
	Segregants							
Dis$^+$	SA133	+	+	+	*bsgA5*			+
	SA136	+	*cycA5*	+	*bsgA5*			*bsgB500*
	SA137	+	*cycA5*	+	+			+
Dis$^-$	SA132	*disA51*	+	*acrA51*	*bsgA5*			+
	SA135	*disA51*	*cycA5*	*acrA51*	+			*bsgB500*

[a] Diploids DSA53 and DSA66 were made from strains SA219 and X9 or X11 by complementation of the *bsgB500* mutation of SA219 with the *tsgD12* or *tsgA1* mutations in strains X9 and X11, respectively. SA220 is a methanol-resistant (*acrA51*) derivative of SA219. Diploid DSA63 was formed by complementation of the *bsgB500* mutation from SA220 and the *bsgA5* mutation of strain XP55. Further details are in the Results section. Phenotype: Dis$^+$, discoidin-producing strains; Dis$^-$, discoidinless strains.

unable to express the discoidin proteins. These strains contain the *acrA50* mutation and are thus expected to carry the *dis* mutation as well. However, it is generally the case that aggregationless strains fail to express the discoidin lectins. Therefore, the genotype of this class of discoidinless segregant is ambiguous and is not included in Table 2. Presumably, aggregation-competent revertants would still be discoidinless owing to the *dis* mutation linked to *acrA50*.

Mapping of *dis* mutations of both strains SA31 and SA219 to linkage group II suggested that the mutations are allelic. This was determined by complementation. One haploid segregant strain (SA132; Table 3) resulting from the cross between SA220 and XP55 carried the *dis* mutation (from SA220) and the *bsgA5* mutation from XP55 instead of the *bsgB500* mutation in the original mutant strains. Thus, this strain was now suitable for backcrossing into the original parental *dis* mutant strains (carrying *bsgB500*) to determine complementation. Diploids were formed between strains SA31 and SA219 and strain SA132 carrying the *dis* mutation from strain SA219. The cloned diploids from these crosses did not express the discoidin lectins and thus do not complement. After haploidization, strains carrying linkage group II from either SA132 (identified by the presence of *acrA51*) or either SA31 or SA219 (*acrA*$^+$) were recovered (Table 4). None of the isolated segregant strains, irrespective of genotype, expressed discoidin, thus confirming linkage to linkage group II. Therefore, based on these analyses, the mutations in SA31 and SA219 were assigned to the single complementation group *disA*, which resides on linkage group II. SA31 and SA219 contain the *disA50* and *disA51* alleles, respectively. Mapping of these mutations with multiple tester strains ensured that the results were not affected by translocations that occur in some strains (35).

Identification of the *disB* complementation group and an unlinked phenotypic suppressor locus *drsA*. One of our crosses between strain SA219 and a multiply marked strain, HL101, produced an unexpected result. Strain HL101 is white spored owing to the presence of the *whiA1* allele on linkage group II (Table 1). As predicted from our previous results, we recovered yellow-spored (*whiA*$^+$) discoidinless strains because *disA51* resides on linkage group II. In addition, some white-spored haploid segregants, e.g., SA38 (Table 1), were recovered which were unable to produce discoidin although they could aggregate and form fruiting bodies normally. Because these strains carry linkage group II from discoidin-producing strain HL101, they are expected to be *disA*$^+$. Previous work showed that SA38, like SA31 and SA219, was blocked in discoidin expression at the level

of transcription (2). Therefore, we suspected that the *dis* mutation in SA38 was not in the *disA* complementation group. This was tested by crossing SA38 with either SA31 (*disA50*) or SA219 (*disA51*), in which both parents are unable to express discoidin. The diploid strains DSA18 and DSA58 from these crosses expressed discoidin and produced segregants capable of producing discoidin. Table 5 shows the genotypes of representative segregants which express the discoidin lectins from the crosses of SA38 with either SA31 or SA219.

The ability of the haploid segregants from this cross to express discoidin required that they be both white spored (*dis*$^+$ from HL101) and wild type for the production of alkaline phosphatase (*alpA*$^+$ from SA219 or SA31), indicating that they require the presence of two distinct *dis*$^+$ loci on both linkage groups II and III. The *bsgB*$^+$ allele in strain SA38 had no influence on discoidin production. These data show that the *dis* mutation recovered in strain SA38 is complementary and nonallelic to *disA* and appears to map to linkage group III, i.e., on the *alpA*-marked chromosome from strain HL101. This mutation (*disB50*) defines a second regulatory locus, *disB*$^+$, which is required together with the *disA*$^+$ allele for a cell to express the discoidin lectin gene family during development. In addition, these results strongly suggest that the *disB50* mutation is suppressed in strain HL101 by another mutant gene. This latter mutation, which we termed *drsA50* (discoidin restoring), is epistatic to the *disB* mutation and results in the expression of the discoidin genes in strains carrying both mutations. We suggest that the *disB50* and *drsA50* mutations arose independently during the mutagenesis which was used to produce the *alpA1* and *nagA211* mutations, respectively (11, 19). The two affected linkage groups were subsequently brought together in strain HL101 by a cross (19).

We confirmed the linkage assignment of *disB50* and its suppression by crossing HL101 to wild-type strain NC4

TABLE 4. Backcrossing of the *disA51* allele from strain SA219 with strains SA31 and SA219[a]

Diploid	Haploids	*dis*/*dis*$^+$ genotype	Linkage group		
			II	III	VII
DSA132	Parents				
	SA31	*disA50*	+	+	*bsgB500*
	SA132	*disA51*	*acrA51*	*bsgA5*	+
	Segregants				
Dis$^-$	SA199	*disA50*	+	*bsgA5*	*bsgB500*
	SA201	*disA50*	+	*bsgA5*	+
	SA209	*disA50*	+	+	+
	SA204	*disA51*	*acrA51*	*bsgA5*	*bsgB500*
	SA206	*disA51*	*acrA51*	+	+
	SA210	*disA51*	*acrA51*	+	*bsgB500*
DSA131	Parents				
	SA219	*disA51*	+	+	*bsgB500*
	SA132	*disA51*	*acrA51*	*bsgA5*	+
	Segregants				
Dis$^-$	SA285	*disA51*	+	*bsgA5*	+
	SA288	*disA51*	+	*bsgA5*	*bsgB500*
	SA283	*disA51*	*acrA51*	*bsgA5*	*bsgB500*

[a] Strain SA132 carries the *disA51* mutation from strain SA219 (see Table 3). Diploids DSA132 and DSA131 were formed from strains SA132 and SA31 or SA219 by complementation of the *bsgA5* mutation in SA132 with the *bsgB500* mutations in strains SA31 and SA219. Haploid segregants were isolated and analyzed. All segregants were found to be unable to produce discoidin during development (Dis$^-$).

TABLE 5. Discoidin-producing segregants resulting from crossing SA38 into SA31 and SA219[a]

Diploid	Haploids	*dis*/*dis*$^+$ genotype	Linkage group		
			II	III	VII
DSA18	Parents				
	SA38	*disB50*	*whiA1 tsgD12*	*alpA1*	+
	SA31	*disA50*	+	+	*bsgB500*
	Segregants				
Dis$^-$	SA86	*disA50*	+	+	+
	SA90	*disB50*	*whiA1 tsgD12*	*alpA1*	*bsgB500*
Dis$^+$	SA83	+	*whiA1 tsgD12*	+	*bsgB500*
	SA88	+	*whiA1 tsgD12*	+	+
DSA58	Parents				
	SA38	*disB50*	*whiA1 tsgD12*	*alpA1*	+
	SA219	*disA51*	+	+	*bsgB500*
	Segregants				
Dis$^-$	SA360	*disB50*	*whiA1 tsgD12*	*alpA1*	*bsgB500*
Dis$^+$	SA365	+	*whiA1 tsgD12*	+	*bsgB500*
	SA359	+	*whiA1 tsgD12*	+	+

[a] Diploids DSA18 and DSA58 were made by the complementation of the *bsgB500* mutation of SA31 and SA219 with the *tsgD12* mutation of SA38. All parents are unable to express discoidin during development. Haploid segregants from DSA18 and DSA58 were isolated and analyzed. Both discoidinless (Dis$^-$) and discoidin-producing (Dis$^+$) segregants were isolated from each cross. These data are discussed in the Results selection.

(Table 6). Although both parental strains express discoidin, segregants from the resulting diploid (DSA21) were found which did not express discoidin. The segregant strains containing the *disB50* mutation cosegregated only with the *alpA1* mutation from strain HL101 which is on linkage group III. No evidence of linkage of *disB* was seen with linkage group I, II, VI, or VII. A single strain, SA310, which carries the *disB50* allele (marked by *alpA1*) produces discoidin indicating that the mutation is suppressed in this segregant as it is in the parent HL101. This was the only strain recovered from the cross which carried linkage group IV marked by *nagA211* (a structural gene mutation in *N*-acetylglucosaminidase) from strain HL101. We suggest that *drsA50* (the suppressor of *disB50*) is located on linkage group IV.

Nature of *drsA50* suppression of the *disB50* allele. We wished to know the mechanism by which *drsA50* suppresses the *disB50* mutation. One possibility was that *drsA50* is simply the *disB*$^+$ allele translocated to the *nagA211*-marked linkage group IV. However, the following observation suggested another possibility. Strains HL101 and the haploid segregant SA310 (Table 6) both carry the *nagA211*-marked linkage group IV. Developing cells of these strains express dramatically higher levels of the discoidin proteins than segregants carrying the *nagA*$^+$ allele on linkage group IV. This was observed in *nagA211* carrying segregants from other crosses as well (e.g., SA102 described below). This observation suggested that these strains are constitutive for the production of discoidin and therefore accumulate it to higher levels. The prediction was that growing mutant cells would have high levels of discoidin in contrast to wild-type cells grown on bacteria which do not express discoidin during growth.

To test this, strains HL101 (*nagA211 drsA50*), wild-type NC4 (*nagA*$^+$ *drsA*$^+$), and the segregant SA310 (*nagA211 drsA50*) from the cross of NC4 and HL101 (Table 6) were grown in association with bacteria and harvested while in logarithmic growth. All strains had a generation time of 3.5 h. Samples of log-phase cells were immediately harvested

TABLE 6. Identification of the *disB50* mutation and its phenotypic suppressor *drsA50*

Diploid[a]	Haploids[b]	*dis*/*dis*$^+$ genotype	Linkage group					
			I	II	III	IV	VI	VII
DSA21	Parents							
	NC4	+	+	+	+	+	+	*bsgB500*
	HL101	*disB50*	*cycA1*	*whiA1*	*alpA1*	*nagA211*	*manA2*	+
	Segregants							
Dis$^+$	SA297	+	+	+	+	+	*manA2*	*bsgB500*
	SA298	+	+	*whiA1*	+	+	+	*bsgB500*
	SA299	+	+	*whiA1*	+	+	*manA2*	*bsgB500*
	SA312	+	+	+	+	+	+	+
	SA313	+	+	+	+	*nagA211*	+	*bsgB500*
Dis$^-$	SA304	*disB50*	+	*whiA1*	*alpA1*	+	*manA2*	*bsgB500*
	SA311	*disB50*	+	*whiA1*	*alpA1*	+	+	*bsgB500*
	SA319	*disB50*	+	+	*alpA1*	+	*manA2*	*bsgB500*
	SA320	*disB50*	+	+	*alpA1*	+	+	*bsgB500*
Suppressed Dis$^-$	SA310	*disB50*	+	+	*alpA1*	*nagA211*	+	*bsgB500*

[a] Diploid DSA21 was made from strains NC4 and HL101 by complementation of the *bsgB500* mutation of NC4 and the *tsgD12* mutation (linkage group II) of HL101. Diploids were selected at 27°C by growth on *B. subtilis*. Dis$^+$, Discoidin-producing strains; Dis$^-$, discoidinless strains.

[b] Strains SA297, SA298, SA299, SA312, and SA313 express discoidin because they carry the *disB*$^+$ allele linked to *alpA*$^+$ on linkage group III as well as the *disA*$^+$ allele on linkage group II. Strains SA304, SA311, SA319, and SA320 are discoidinless owing to the presence of the *disB50* allele linked to *alpA1* on linkage group III. Strain SA310 produces discoidin although it carries the *disB50* allele on linkage group III because it is suppressed by *drsA50* linked to the *nagA211* mutation on linkage group IV. A more detailed discussion of this cross as well as further analysis of the phenotypic suppressor mutation *drsA50* is in Results.

for testing, while others were dispersed on filters and allowed to proceed through development. Samples of each strain were taken at intervals during morphogenesis. The samples from the developmental time courses were extracted and run on SDS-polyacrylamide gels, and the separated proteins were subsequently transfered to nitrocellulose. The blots were developed with antibody to discoidin. Figure 1 shows the results from this experiment. The data clearly show that cells harvested from log-phase growth of wild-type strain NC4 contain no detectable discoidin protein. The level of discoidin in the cells rises as development proceeds starting at about 5 h of development and leveling off at approximately 10 h. In contrast, log-phase cells of strain HL101 contain high levels of the discoidin proteins. These levels appear to increase slightly during development in agreement with our early data showing that developing cells of strain HL101 transcribe the discoidin genes (2). As predicted, strain SA310 also shows the abnormal pattern of discoidin expression with growing cells expressing the lectins. Similar to strain HL101, SA310 cells also accumulate discoidin during development (although more gradually because of a more rapid onset of aggregation in HL101) owing to a combination of synthesis and the cessation of cell division at the onset of development. The heterozygous diploid strain DSA21 (*drsA50*/*drsA*$^+$) also has abnormal discoidin expression indicating that the *drsA50* allele is dominant over its wild-type counterpart.

These data demonstrate that strains HL101 and SA310 are unable to strictly regulate the developmental expression of the discoidin gene family owing to the constitutive effect of the *drsA50* mutation. In addition, the *drsA50* mutation also results in an increased level of discoidin in the mutant cells. The effect of the *drsA50* mutation appears specific for discoidin expression since the regulation of α-mannosidase

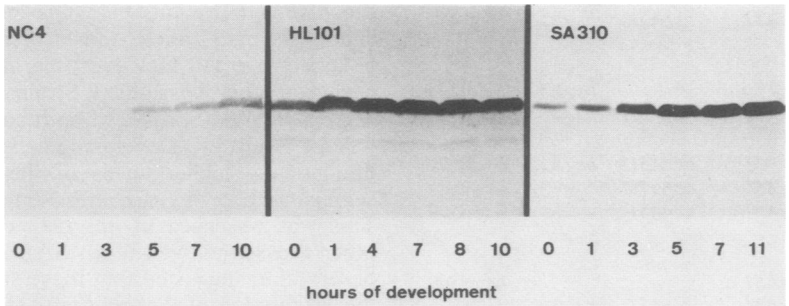

FIG. 1. Patterns of expression of the discoidin lectins during growth and development of cells carrying the *drsA* mutation. Cells of wild-type strain NC4 (*drsA*$^+$ *nagA*$^+$), strain HL101 *drsA50 nagA211*), and strain SA310 (*drsA50 nagA211*) were harvested from log-phase growth with *K. aerogenes*, washed, and deposited on filters (10^8 cells per filter) for development. Samples of growing cells yet to begin the program of development were taken just before deposition on filters. All strains had a generation time of 3.5 h. Developing cells were then harvested from filters at intervals during development as indicated in the figures. Cell samples were immediately frozen and lysed in SDS-polyacrylamide gel electrophoresis sample buffer. Protein samples equivalent to 5×10^6 cells were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose, and discoidin was detected immunochemically. The data show that discoidin is expressed during both bacterial growth and development in the *drsA50* mutant strains HL101 and SA310 but is developmentally controlled in wild-type strain NC4. Strains HL101 and SA310 also express discoidin at a higher level than NC4. The onset of aggregation in strain HL101 is at 5 h of development compared with 7.5 h for strains SA310 and NC4 consistent with the more rapid developmental accumulation of discoidin in HL101.

(another early product) remains unaffected (data not shown). Thus, the *drsA50* mutation defines a third locus (*drsA*$^+$) which is involved in the normal regulation of the expression of the discoidin gene family.

Lack of suppression of the *disA50* allele by *drsA50*. We wished to establish further the relationships of the regulatory genes controlling discoidin expression. This was accomplished by determining whether the *drsA50* allele could suppress the discoidinless phenotype of the *disA* mutations as well as that of *disB50*. Thus, we constructed strains which contained the *drsA50* and *disA50* alleles in the absence of *disB50*. This was accomplished by crossing strain SA32 (*disA50 acrA50* on linkage group II) to SA102 (*drsA50 nagA211* on linkage group IV) to form the diploid DSA163. The latter haploid strain is a segregant from a cross of XP99 (24) and HL101 which does not carry the *disB50* mutation as did the parent HL101. Strain SA102 had the typical phenotype associated with the *drsA50* allele and expressed the discoidin lectin during growth as well as development. Segregants were selected which were resistant to growth on methanol and thus *disA50* by virtue of its being linked to *acrA50*. These segregants were screened for the linkage group IV from SA102 which contains *drsA50* by their lack of *N*-acetylglucosaminidase (*nagA211*). Using this combination of selection and screening we isolated recombinant strains with the genotype *disA50 drsA50*. All these strains were discoidinless. Therefore, *drsA50* does not suppress *disA50*. Figure 2 presents a summary view of the interaction of these loci.

DISCUSSION

We examined the interactions of mutations affecting a specific developmentally controlled event during morphogenesis of *D. discoideum*. The present study indicates that the expression of the discoidin lectin gene family is regulated by the integrated action of three other genes. We showed that mutations at two loci result in the absence of the expression of the family of discoidin genes during development. These mutations define the *disA* and *disB* complementation groups and map to linkage groups II and III, respectively. The products of both the *disA*$^+$ and *disB*$^+$ loci are necessary for discoidin expression. A third mutation described here is a phenotypic suppressor of the *disB* mutation. This mutation, termed *drsA*, appears to map to linkage group IV from strain HL101 which is marked by the presence of another mutation in the structural gene for *N*-acetylglucosaminidase (*nagA211*). The *drsA* mutation restores discoidin production by making cells carrying this allele constitutive for discoidin expression. Cells containing the *nagA211*-marked linkage group IV express discoidin during growth as well as during development in contrast to wild-type cells which express discoidin only during development. Therefore, in these mutant cells there is a breakdown of the strict developmentally regulated expression of the discoidin genes by bacterially grown cells. This situation is reminiscent of the physiologically induced high-level expression of discoidin during growth of axenic strains in liquid medium (25), although the significance of this similarity remains obscure. The rate of transcription of the discoidin lectin genes in wild-type cells is specifically sensitive to the increasing levels of cyclic AMP in the developing multicellular aggregates. This leads to the eventual cessation of message expression (5, 36). It will be interesting to determine whether mutant cells containing the *drsA* allele are sensitive to cyclic AMP during either growth or development.

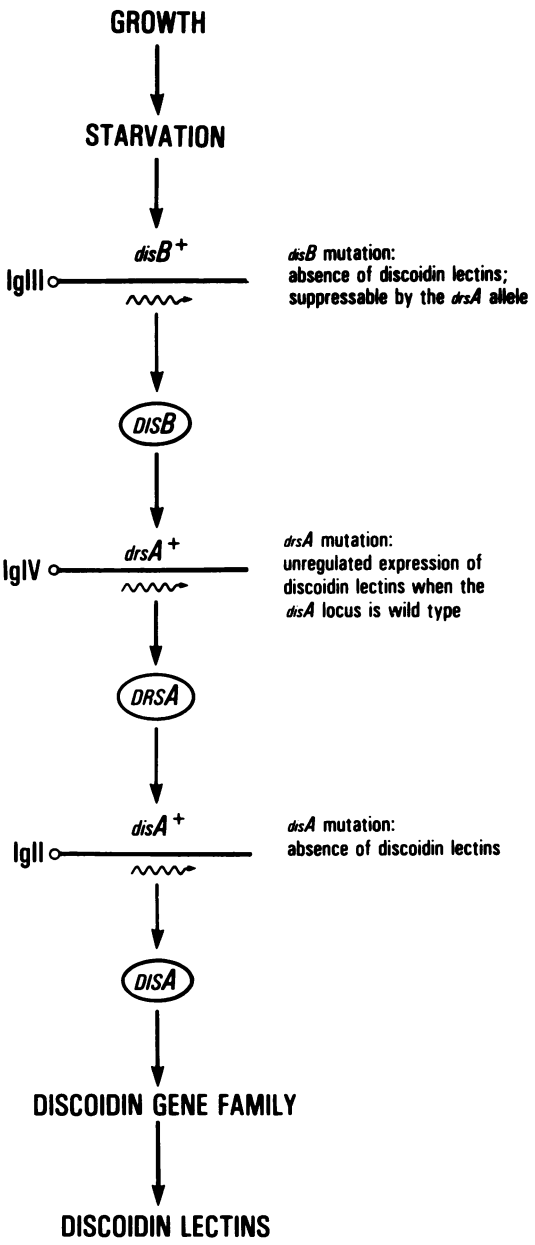

FIG. 2. Model for the developmentally regulated transcriptional control of the discoidin gene family in bacterially grown wild-type cells. Removal of the food source (starvation) after growth begins the program of development. The *disB*$^+$ locus is transcribed, and its gene product acts on the *drsA*$^+$ locus causing its transcription. The product of this locus in turn acts on the *disA*$^+$ locus resulting in transcription. The gene product from the *disA*$^+$ locus causes the expression of the discoidin lectin genes. The sites of action of the *disA*, *disB*, and *drsA* mutations are indicated in the figure. *disA* and *disB* result in the absence of discoidin gene expression during development, and *drsA* results in continual unregulated expression of the gene family during growth as well as development in the presence of the *disA*$^+$ allele. lg, Linkage group; wavy arrows, transcription of the indicated genes. DISA, DISB, and DRSA in ovals refer to the products of their respective wild-type alleles.

A schematic representation of the interaction of the *disB*$^+$, *drsA*$^+$, and *disA*$^+$ genes is shown in Fig. 2. We suggest that the *disB*$^+$ gene is expressed early in development and its gene product acts on the *drsA*$^+$ gene causing its transcrip-

tion. The product of this gene then acts on the *disA*$^+$ gene resulting in its expression. The *disA*$^+$ gene product in turn causes the expression of the genes encoding the discoidin lectin proteins. The model suggests a mechanism of minimum complexity, and further refinement awaits the isolation of additional mutations affecting discoidin expression.

It will be interesting to examine carefully the developmental behavior of mutant cells carrying the *drsA50* allele. Recent reports implicate discoidin in the cell-substratum interaction that occurs as cells begin to aggregate (8, 28). In this regard, we are developing congenic pairs of strains differing only in linkage group IV. With these strains, specific comparisons between the effect of the *drsA50* and *drsA*$^+$ alleles can be performed in an otherwise identical genetic background. Comparing the subtle developmental effects of mutant and wild-type alleles in different genetic backgrounds can lead to ambiguous interpretations owing to the action of other loci which differ in the strains being compared.

Although *D. discoideum* has been extensively studied from the viewpoint of differential gene expression during development, no other regulatory genes have been clearly demonstrated. This situation has to some extent limited the usefulness of an otherwise superbly manipulatable system for the study of multicellular morphogenesis. Our ability to isolate nonconditional mutants defective in discoidin transcription was the result of the combinations of the nonessential nature and specific regulation of discoidin expression. Their phenotypes suggested that the genetic defect was in a regulatory locus, and subsequent work has now confirmed this. We anticipate that the continued isolation of additional mutants and revertants affecting discoidin expression, including second-site suppressors (7), will result in the identification of other regulatory genes. We are particularly interested in the isolation of mutants which are blocked in the production of other early gene products as well. Several of these gene products (e.g., α-mannosidase and *N*-acetylglucosaminidase) have been shown to be unnecessary to complete morphogenesis (12, 17). We have been able to show genetically that the expression of *N*-acetylglucosaminidase can be dissociated from the expression during development of α-mannosidase and discoidin (1). Thus, there are different signals that start these two developmental pathways. Whether they both stem from an earlier common pathway remains to be seen, and we hope that the further dissection of the discoidin regulatory gene network will help shed light on this problem of the regulation and organization of early development. The transduction of signals for development (such as starvation in the case of *D. discoideum*) into the regulated expression of developmental genes is common to all developing systems, but remains poorly understood.

The mutations described in this report are allowing us to explore further the genetic and molecular control of specific developmental gene expression in *D. discoideum*. This should allow us to eventually achieve the level of understanding for this multicellular system that has been achieved for the genetic control of mating-type switching (14) in *Saccharomyces cerevisiae*. In the latter organism the genetic regulatory systems have proven to be complex, and we are not surprised to see an increasing level of complexity developing among the discoidin regulatory genes.

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