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The Discoidin I Gene Family of *Dictyostelium discoideum* Is Linked to Genes Regulating Its Expression

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ABSTRACT

The discoidin I protein has been studied extensively as a marker of early development in the cellular slime mold *Dictyostelium discoideum*. However, like most other developmentally regulated proteins in this system, no reliable information was available on the linkage of the discoidin genes to other known genes. Analysis of the linkage of the discoidin I genes by use of restriction fragment length polymorphisms revealed that all three discoidin I genes as well as a pseudogene are located on linkage group II. This evidence is consistent with the discoidin I genes forming a gene cluster that may be under the control of a single regulatory element. The discoidin I genes are linked to three genetic loci (*disA*, *motA*, *daxA*) that affect the expression of the discoidin I protein. Linkage of the gene family members to regulatory loci may be important in the coordinate maintenance of the gene family and regulatory loci. A duplication affecting the entire discoidin gene family is also linked to group II; this appears to be a small tandem duplication. This duplication was mapped using a DNA polymorphism generated by insertion of the Tdd-3 mobile genetic element into a Tdd-2 element flanking the γ gene. A probe for Tdd-2 identified a restriction fragment length polymorphism in strain AX3K that was consistent with generation by a previously proposed Tdd-3 insertion event. A putative duplication or rearrangement of a second Tdd-2 element on linkage group IV of strain AX3K was also identified. This is the first linkage information available for mobile genetic elements in *D. discoideum*.

THE microbial eukaryote *Dictyostelium discoideum* has received considerable attention as a model system in which to study gene expression, since during asexual fruiting body formation *D. discoideum* genes are regulated both temporally and in a cell-type-specific manner. The discoidin genes and gene products have been extensively characterized (CLARKE, KAYMAN and RILEY 1987; KAYMAN, BIRCHMAN and CLARKE 1988; ALEXANDER, CIBULSKY and CUNEO 1986; CROWLEY *et al.* 1985; POOLE and FIRTEL 1984; TSANG, DEVINE and WILLIAMS 1981; DEVINE, TSANG and WILLIAMS 1982; SPRINGER, COOPER and BARONDES 1984; COOPER and BARONDES 1984). Synthesis of discoidin I is induced in starving cells during early aggregation (MA and FIRTEL 1978; DEVINE, TSANG and WILLIAMS 1982) but also can be induced in vegetative cells under appropriate conditions (CLARKE, KAYMAN and RILEY 1987). This protein has sequence homology to fibronectin and is proposed to play roles in adhesion of cells to the substratum and in cell streaming during aggregation (CROWLEY *et al.* 1985; SPRINGER, COOPER and BARONDES 1984; ALEXANDER, SHINNICK and LERNER 1983). Mutations which affect the expression of discoidin have been identified (ALEXANDER, CIBULSKY and CUNEO 1986; ALEXANDER and SHINNICK 1985; ALEXANDER, SHINNICK and LERNER 1983; KAYMAN, BIRCHMAN and CLARKE 1988). In strains bearing these mutations, expression of discoidin I is disrupted; for example, discoidin may not be synthesized during aggregation (ALEXANDER, CIBULSKY and CUNEO 1986; ALEXANDER, SHINNICK and LERNER 1983) or discoidin synthesis occurs during vegetative growth in axenic media (KAYMAN, BIRCHMAN and CLARKE 1988). Some of these mutations are known to interfere with coordinate transcription of discoidin-specific RNA but do not affect the discoidin genes directly (ALEXANDER and SHINNICK 1985). Three of the loci in which mutations occur have been mapped to linkage group II: *disA* (ALEXANDER, CIBULSKY and CUNEO 1986), *motA* (KAYMAN, BIRCHMAN and CLARKE 1988) and *daxA* (KAYMAN, BIRCHMAN and CLARKE 1988). The product of the *disA* locus is thought to cause expression of the discoidin genes; *disA* acts last in a proposed regulatory pathway (ALEXANDER, CIBULSKY and CUNEO 1986).

The use of restriction fragment length polymorphisms (RFLPs) for linkage analysis of *D. discoideum* has recently been described (WELKER *et al.* 1986; DINGERMANN *et al.* 1987). DNA polymorphisms affecting the sequences in or near genes are common in wild isolates of *D. discoideum*. These isolates can be crossed via the parasexual cycle to well-marked tester strains. In segregant haploids derived from such crosses the segregation of the parental DNA fragments relative to classical genetic markers can be easily followed by

correlating their presence in specific segregants with the presence of established genetic markers (Welker *et al.* 1986; Dingermann *et al.* 1987). These experiments require only the availability of suitable DNA to serve as probe and of one or more strains in which DNA polymorphisms have been identified.

DNA probes for many genes whose expression changes during aggregation and later stages of fruiting body formation have been isolated, these include probes for the discoidin I gene family. This gene family in the type strain NC4 was shown to contain the three genes (α, β, γ) and one pseudogene (Poole and Firtel 1984; Tsang, Devine and Williams 1981). The pseudogene and two of the other genes (β and γ) are known to be closely linked (Poole and Firtel 1984). However, nothing was known of the location of the third gene family member or whether the discoidin genes might be located near genes involved in discoidin regulation. This work establishes that the entire discoidin I gene family maps to linkage group II and is therefore linked to at least three loci regulating its expression.

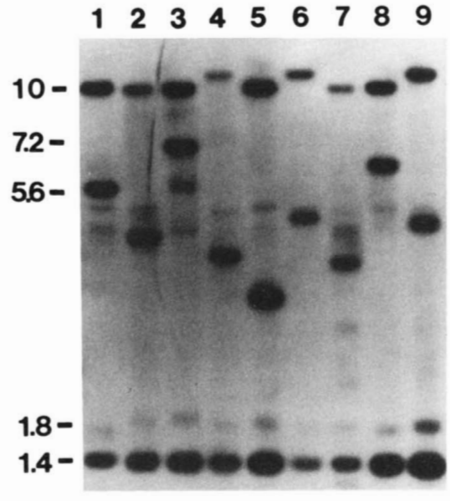

Figure 1.—Restriction fragment length polymorphisms in wild isolates of *D. discoideum* using genomic DNA cut with *Eco*RI and as probe a 340-bp fragment of pDd812 that contains a portion of the discoidin α gene. Lanes are: 1, OHIO; 2, DD61; 3, HU182; 4, HU188; 5, WS380B; 6, WS472; 7, WS576; 8, WS583; 9, WS1956. Sizes and relative positions of NC4 fragments are shown on the left.

MATERIALS AND METHODS

Strains and growth of cells: Strains were grown at 21 ± 1° either with *Klebsiella aerogenes* on SM media or with *Escherichia coli* B/r on DM media (Welker 1986; Sussman 1966; Podgorski and Deering 1980). The wild isolates were a set previously used to identify RFLPs associated with actin and tRNA genes (Welker *et al.* 1986; Dingermann *et al.* 1987). NC4 derivatives AX3K and AX3L which were known to contain RFLPs affecting the discoidin I gene family (Poole and Firtel 1984) were also used. Tester strains for the genetic crosses were HU1628 and HU1852; these are NC4 derivatives carrying genetic markers on each of the six known linkage groups (Newell 1987; Welker *et al.* 1986; Welker and Williams 1985) plus the dominant *cob-354* cobalt resistance trait.

Preparation of DNA and Southern blots: Nuclei were prepared and DNA purified using CsCl gradients as previously described (Welker *et al.* 1986; Welker, Hirth and Williams 1985), except for inclusion of a phenol-chloroform extraction prior to gradient preparation. DNA was cut with appropriate restriction enzymes, separated on 0.8% agarose gels using Tris phosphate running buffer (36 mM Tris, 30 mM NaH_2PO_4, 1 mM EDTA; pH 7.8) and blotted to nitrocellulose. Probes were nick translated using [^{32}P]dATP. The probes used were pDd812, a 340-bp *Eco*RI-*Kpn*I fragment of pDd812 that contains a portion of the α gene, or a *Bgl*II-*Eco*RI fragment of *D. discoideum* DNA from pDd17 that contains a portion of the Tdd-2 mobile genetic element (Devine, Tsang and Williams 1982; Poole and Firtel 1984). Plasmid pDd812 is a cDNA clone containing an α gene sequence; pDd17 is a genomic DNA clone containing a 7.0–7.2-kb insert carrying part of the γ gene sequence plus flanking DNA including the part of the Tdd-2 element (Devine, Tsang and Williams 1982).

Parasexual genetic techniques: For most crosses, diploids were constructed as described previously (Welker, Hirth and Williams 1985; Welker *et al.* 1986) using the dominance of *cob-354* in conjunction with a recessive conditional lethal mutation (*bsgB500*, *bsgA5* or *couA351*). For these constructions a wild isolate or other strain containing an identified RFLP was crossed to one of the tester strains, HU1628 or HU1852. In the cross of HUD239 with HUD377 the recessive conditional lethal mutations *bsgA5* and *couA351* were used. Haploid segregants were selected from the diploids constructed in the crosses using 2 μg/ml thiabendazole (Welker and Williams 1980). From these, sets of segregants with mixtures of chromosomes from the two parents were chosen on the basis of the presence or absence of the known genetic markers. Correlation of the presence of specific DNA fragments with the presence or absence of the genetic markers allows assignment of genes to linkage groups.

RESULTS

Identification of restriction fragment length polymorphisms associated with the discoidin I gene family: Polymorphisms were readily identified in the length of *Eco*RI restriction fragments carrying DNA sequences homologous to a discoidin I gene probe. Poole and Firtel (1984) identified several in wild isolates and also in laboratory derivatives of the NC4 type strain. The latter polymorphisms were attributed to insertion of copies of a repeated DNA sequence (Tdd-3) into DNA flanking the discoidin I γ gene. Analysis of a set of wild isolates confirmed that polymorphisms affecting the length of *Eco*RI fragments were common (Figure 1). Poole and Firtel (1984) identified five *Eco*RI restriction fragments in the NC4 type strain (10, 7.2, 5.6, 1.8 and 1.4 kb). Polymorphisms affecting all but the 1.4-kb fragment, which contains portions of the β and γ genes plus spacer DNA between these genes, were identified (Figure 1).

Linkage analyses of the discoidin I gene family: The discoidin I α gene was mapped using segregants

TABLE 1

Linkage analysis of the discoidin I α and γ genes using segregant haploids produced by crossing wild isolate OHIO with tester strain HU1852

Strain	Polymorphic restriction fragments (kb)	Linkage group					
		I	II	III	IV	VI	VII
HU1852	1.8, 7.2	*cycA1*	*acrA1836*	*whiB355*	*bwnA1*	*manA2*	*bsgB500*
OHIO	1.7, 5.1	*cycA*$^+$	*acrA*$^+$	*whiB*$^+$	*bwnA*$^+$	*manA*$^+$	*bsgB*$^+$
HUD179	1.7, 5.1	+	+	+	+	+	+
HUD180	1.7, 5.1	+	+	*whiB355*	+	*manA2*	+
HUD181	1.8, 7.2	+	*acrA1836*	*whiB355*	+	*manA2*	*bsgB500*
HUD182	1.8, 7.2	*cycA1*	*acrA1836*	+	+	+	+
HUD183	1.7, 5.1	+	+	+	+	+	*bsgB500*
HUD185	1.8, 7.2	*cycA1*	*acrA1836*	+	*bwnA1*	+	*bsgB500*
HUD187	1.8, 7.2	*cycA1*	*acrA1836*	*whiB355*	+	*manA2*	+
HUD188	1.8, 7.2	*cycA1*	*acrA1836*	*whiB355*	+	*manA2*	*bsgB500*
HUD189	1.7, 5.1	*cycA1*	+	*whiB355*	+	*manA2*	+
HUD191	1.7, 5.1	*cycA1*	+	+	*bwnA1*	+	*bsgB500*

Portions of the HU1852 (NC4) α and γ genes are located on *Eco*RI restriction fragments of 1.8 and 7.2 kb, respectively. The OHIO α gene is located on the 1.7-kb fragment (Figure 2). Note that segregant HUD189 has a restriction fragment of nonparental size that replaces a 5.6 kb fragment. For segregant genotypes wild isolate alleles are designated +; the full genotype for HU1852 is given elsewhere (WELKER 1986). Genetic markers used in this work for segregant characterization were: *cycA1*, cycloheximide resistance; *acrA1823*, *acrA1836* and *acrA2108*, methanol resistance; *whiB355* and *whiC351*, white spore heads; *bwnA1*, brown spore heads; *manA2*, α-mannosidase-1 deficient; *bsgA5* and *bsgB500*, unable to grow with *Bacillus subtilis* as food source; *couA351*, coumarin and temperature sensitivity.

derived from crosses of wild isolate OHIO with tester strain HU1852 (Table 1, Figure 2) and of wild isolate HU188 with tester strain HU1628 (Table 2). This gene can be mapped using polymorphisms affecting either the 1.8-kb or the 10-kb fragment. While the tester strains have patterns of restriction fragments identical to that of NC4, the wild isolate OHIO lacks the NC4 1.8-kb fragment, and isolate HU188 lacks the 10-kb fragment in the NC4 pattern. These fragments are replaced in OHIO and HU188 by fragments of different size. For example, OHIO has a 1.7-kb fragment that apparently replaces the NC4 1.8-kb fragment. With both sets of segregants the DNA markers segregated with linkage group II. In the segregants from the OHIO cross, the 1.8-kb fragment segregated with the *acrA1836* mutation from the tester strain, while the 1.7-kb fragment was present only in the *acrA*$^+$ segregants that carry the OHIO linkage group II. In the segregants from the HU188 cross, the *acrA*$^+$ segregants lacked the 10 kb fragment, while all but one of the *acrA1823* segregants had the 10-kb fragment from HU1628 (Table 2). The exceptional segregant, HUD87, may be a mitotic recombinant. Linkage group II seems prone to recombination events in crosses of wild isolates to NC4 derivatives; two other reports exist of recombinants involving linkage group II (WELKER *et al.* 1986; WILLIAMS, ROBSON and WELKER 1980).

The discoidin β and γ genes as well as the pseudogene were mapped using segregants derived from crosses of wild isolate WS380B with HU1628 (Table 3), of HU188 with HU1628 (Table 2), and of OHIO with HU1852 (Table 1). Portions of these genes are

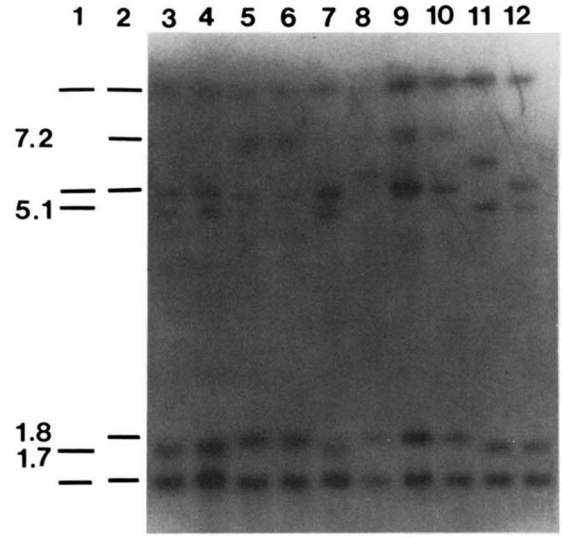

FIGURE 2.—Analysis of *Eco*RI restriction fragments in segregants of a cross of wild isolate OHIO with tester strain HU1852 using as probe plasmid pDd812 which contains a portion of the discoidin α gene (see Table 1). Lanes are: 1, OHIO pattern; 2, HU1852 pattern; 3, HUD179; 4, HUD180; 5, HUD181; 6, HUD182; 7, HUD183; 8, HUD185; 9, HUD187; 10, HUD188; 11, HUD189, 12, HUD191. The parental OHIO and HU1852 patterns and approximate sizes of the polymorphic fragments are indicated on the left.

present in NC4 on the 7.2-kb (γ), 5.6-kb (β and pseudogene), and 1.4-kb (β and γ) fragments. WS380B and HU188 did not contain 7.2- and 5.6-kb fragments, while OHIO lacked a 7.2-kb fragment but had a 5.6-kb fragment. As noted above, all three wild isolates retained a 1.4-kb fragment. These wild isolates contain fragments potentially homologous to

TABLE 2

Linkage analysis of the discoidin I gene family using segregant haploids produced by crossing wild isolate HU188 with tester strain HU1628

Strain	Polymorphic restriction fragments (kb)	Linkage group I	II	III	IV	VI	VII
HU1628	5.6, 7.2, 10	*cycA1*	*acrA1823*	*bsgA5*	*whiC351*	*manA2*	*couA351*
HU188	4, 5, 12	*cycA*$^+$	*acrA*$^+$	*bsgA*$^+$	*whiC*$^+$	*manA*$^+$	*couA*$^+$
HUD86	5.6, 7.2, 10	+	*acrA1823*	*bsgA5*	+	*manA2*	+
HUD87	4, 5, 12	+	*acrA1823*	+	+	+	+
HUD88	5.6, 7.2, 10	*cycA1*	*acrA1823*	+	+	+	+
HUD90	4, 5, 12	*cycA1*	+	+	*whiC351*	+	+
HUD91	4, 5, 12	*cycA1*	+	+	+	+	+
HUD92	4, 5, 12	+	+	+	+	+	+
HUD94	4, 5, 12	*cycA1*	+	*bsgA5*	*whiC351*	*manA2*	*couA351*
HUD95	5.6, 7.2, 10	+	*acrA1823*	*bsgA5*	*whiC351*	*manA2*	+

Portions of the HU1628 (NC4) discoidin I α, β, γ, and pseudogene are located on *Eco*RI restriction fragments of 10, 5.6, 7.2 and 5.6 kb, respectively. Note that segregant HUD87 has a linkage pattern inconsistent with the others; this segregant may be the product of a mitotic recombination event. For segregant genotypes wild isolate alleles are designated +; full genotype of HU1628 is given elsewhere (WELKER and WILLIAMS 1985; DINGERMANN *et al.* 1987; WELKER *et al.* 1986). Genetic markers are described in Table 1.

TABLE 3

Linkage analysis of the discoidin I β, γ and pseudogene using segregant haploids produced by crossing wild isolate WS380B with tester strain HU1628

Strain	Polymorphic restriction fragments (kb)	Linkage group I	II	III	IV	VI	VII
HU1628	5.6, 7.2	*cycA1*	*acrA1823*	*bsgA5*	*whiC351*	*manA2*	*couA351*
WS380B	3, 5	*cycA*$^+$	*acrA*$^+$	*bsgA*$^+$	*whiC*$^+$	*manA*$^+$	*couA*$^+$
HUD47	5.6, 7.2	*cycA1*	*acrA1823*	*bsgA5*	+	*manA2*	*couA351*
HUD48	5.6, 7.2	+	*acrA1823*	*bsgA5*	+	*manA2*	+
HUD49	5.6, 7.2	+	*acrA1823*	*bsgA5*	+	*manA2*	*couA351*
HUD53	5.6, 7.2	+	*acrA1823*	+	+	+	*couA351*
HUD54	3, 5	+	+	+	+	+	+
HUD55	3, 5	*cycA1*	+	*bsgA5*	+	*manA2*	+
HUD58	3, 5	*cycA1*	+	+	*whiC351*	+	*couA351*
HUD59	3, 5	*cycA1*	+	*bsgA5*	+	*manA2*	*couA351*
HUD60	3, 5	+	+	+	*whiC351*	+	*couA351*
HUD62	5.6, 7.2	*cycA1*	*acrA1823*	+	+	+	*couA351*

Portions of the HU1628 (NC4) discoidin I β, γ, and pseudogene are located on *Eco*RI restriction fragments of 5.6, 7.2 and 5.6 kb, respectively. In WS380B, the 5.6-kb fragment is replaced by one of 5 kb and the 7.2-kb fragment by one of 3 kb (Figure 3). For segregant genotypes wild isolate alleles are designated +. Genetic markers are described in Table 1.

the 7.2- and 5.6-kb fragments. In HU188 there are fragments of about 12, 5 and 4 kb; in OHIO there is a fragment of 5.1 kb; and in WS380B there are fragments of 5 and 3 kb. In particular, on the basis of the relative amounts of hybridization seen, the WS380B 5-kb fragment replaces the NC4 5.6-kb fragment and the 3-kb fragment replaces the 7.2-kb fragment (Figure 3). In segregants of the WS380B cross, the 7.2- and 5.6-kb fragments segregated with the *acrA1823* mutation from HU1628 while the polymorphic fragments from the wild isolate segregated with the *acrA*+ linkage group II (Figure 3, Table 3). Linkage of the 7.2-kb fragment in the segregants of the cross involving wild isolate OHIO is consistent with this result (Figure 2, Table 1), as is the data from the HU188 cross (Table 2) with HUD87 again being exceptional. Cosegregation of β, γ and the pseudogene was expected from the map of the DNA region in which these genes are located (POOLE and FIRTEL 1984).

Use of a transposon generated DNA polymorphism to study linkage of the discoidin I gene family: In strains derived from the NC4 type strain, POOLE and FIRTEL identified two DNA polymorphisms affecting *Eco*RI fragments homologous to a discoidin I gene probe (POOLE and FIRTEL 1984). POOLE and FIRTEL (1984) showed that these polymorphisms were due to integration of a mobile genetic element, Tdd-3, into a Tdd-2 element in the region near the γ gene. In the AX3K strain the NC4 7.2-kb fragment

TABLE 4

Linkage analysis of the discoidin I γ gene and Tdd-2 elements using segregant haploids produced by crossing strain AX3K with tester strain HU1852

Strain	Polymorphic restriction fragments (kb)		Linkage group					
	*Eco*RI	*Hin*dIII	I	II	III	IV	VI	VII
HU1852	7.2	15	*cycA1*	*acrA1836*	*whiB355*	*bwnA1*	*manA2*	*bsgB500*
AX3K	5.1	7.5, 16	*cycA*$^+$	*acrA*$^+$	*whiB*$^+$	*bwnA*$^+$	*manA*$^+$	*bsgB*$^+$
HUD132	5.1	16	+	+	+	*bwnA1*	+	*bsgB500*
HUD133	5.1	7.5, 16	+	+	+	+	*manA2*	*bsgB500*
HUD134	7.2	15	+	*acrA1836*	+	*bwnA1*	+	+
HUD138	5.1	7.5, 16	*cycA1*	+	*whiB355*	+	+	*bsgB500*
HUD140	7.2	15	+	*acrA1836*	*whiB355*	*bwnA1*	+	*bsgB500*
HUD143	5.1	7.5, 16	*cycA1*	+	+	+	*manA2*	+
HUD145	5.1	7.5, 16	+	+	*whiB355*	+	*manA2*	*bsgB500*
HUD148	7.2	15	+	*acrA1836*	+	*bwnA1*	*manA2*	*bsgB500*
HUD150	7.2	7.5, 15	+	*acrA1836*	*whiB355*	+	+	*bsgB500*
HUD152	5.1	7.5, 16	*cycA1*	+	+	+	+	+

A portion of the HU1852 (NC4) discoidin γ gene is located on an *Eco*RI fragment of 7.2 kb, in AX3K this fragment is replaced by one of 5.1 kb. The *Eco*RI polymorphism was detected using the pDd812 *Eco*RI-*Kpn*I 340 bp fragment; the Tdd-2 elements were mapped using *Hin*dIII polymorphisms detected with a *Bgl*II-*Eco*RI fragment from pDd17 that contains Tdd-2 sequences from the region flanking the discoidin I γ gene (Figure 5). For segregant genotypes AX3K alleles are designated +. Genetic markers are described in Table 1.

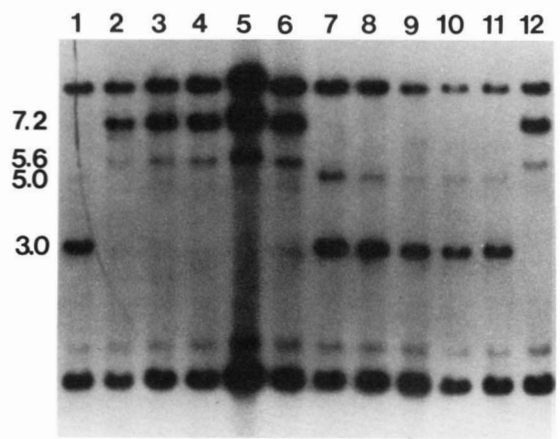

FIGURE 3.—Analysis of *Eco*RI restriction fragments in segregants of a cross of wild isolate WS380B with tester strain HU1628 using as probe a 340 bp fragment of pDd812 that contains a portion of the discoidin α gene (see Table 3). Lanes are: 1, WS380B; 2, HU1628; 3, HUD47; 4, HUD48; 5, HUD49, 6, HUD53; 7, HUD54; 8, HUD55; 9, HUD58; 10, HUD59; 11, HUD60; 12, HUD62. Approximate sizes of polymorphic fragments are indicated on the left.

is replaced by a 5.1-kb fragment. Thus the linkage of the γ gene can be confirmed using this polymorphism. Data obtained with segregants of a cross of AX3K with HU1852 was consistent with the linkage group II location; in these segregants the 5.1-kb fragment always segregated with the AX3K linkage group II and no linkage to the other groups was seen (Table 4).

Analysis of a duplication affecting the discoidin I gene family: The AX3L strain has a duplication that was known to affect at least β, γ and the pseudogene. A diagnostic 3.0-kb fragment bearing a portion of one copy of the γ gene replaces one copy of the 7.2-kb fragment and can be used to determine the linkage of the duplicated genes. In the analyses of the duplication in AX3L a complicated result was initially obtained. The stock of AX3L used appears to have been a diploid at the time of crossing to HU1628 and to HU1852. The products of both of these crosses were homozygous for at least two of the AX3L linkage groups. DUD13 (AX3L × HU1852) was homozygous for the AX3L linkage groups I and IV; DUD21 (AX3L × HU1628) was homozygous for the AX3L linkage groups III and VI. Segregants of DUD13 and DUD21 never expressed genetic markers for these linkage groups that were present in the tester strain parent. This result can be explained if the initial products of the crosses were unstable triploids that lost chromosomes by nondisjunction to form stable diploids from which the segregants were then isolated. Despite this complication, data obtained in the analysis of segregants of DUD13 and DUD21 was consistent with linkage of the duplication to linkage group II. The 3 kb fragment was always present in segregants having the AX3L *acrA*$^+$ linkage group II and no linkage of the duplication to groups I, III, IV, VI or VII was seen.

To further resolve the linkage of the duplication, an additional experiment was carried out. Haploid HUD239, which contains the AX3K linkage group II, was crossed to strain HUD377, which contains an AX3L linkage group II on which a new *acrA* mutation was selected. Segregants of the resulting diploid confirmed the linkage of the duplication to

TABLE 5

Linkage analysis of a discoidin gene duplication using segregant haploids produced by crossing strains HUD239 and HUD377

Strain	Polymorphic restriction fragments (kb)	Linkage group I	II	III	IV	VI	VII
HUD239	5.1	*cycA1*	*acrA*$^+$	*bsgA5*	*whiC*$^+$	*manA2*	*couA*$^+$
HUD377	3, 7.2	*cycA*$^+$	*acrA2108*	*bsgA*$^+$	*whiC351*	*manA*$^+$	*couA351*
HUD381	5.1	+	+	*bsgA5*	+	+	+
HUD382	5.1	+	+	*bsgA5*	*whiC351*	*manA2*	+
HUD384	3, 7.2	+	*acrA2108*	+	+	*manA2*	*couA351*
HUD386	5.1	*cycA1*	+	*bsgA5*	+	+	+
HUD387	5.1	*cycA1*	+	*bsgA5*	+	*manA2*	+
HUD391	5.1	*cycA1*	+	*bsgA5*	*whiC351*	*manA2*	+
HUD393	3, 7.2	*cycA1*	*acrA2108*	*bsgA5*	+	*manA2*	+
HUD394	3, 7.2	+	*acrA2108*	+	*whiC351*	*manA2*	+
HUD395	3, 7.2	*cycA1*	*acrA2108*	*bsgA5*	+	+	+
HUD398	5.1	*cycA1*	+	*bsgA5*	*whiC351*	+	+
HUD403	5.1	+	+	+	*whiC351*	+	*couA351*

Insertion of a copy of the Tdd-3 element into DNA flanking one of the γ genes of AX3L generated a polymorphic 3 kb *Eco*RI fragment. AX3L and its derivatives (HUD377) therefore have both a 7.2 and a 3 kb fragment. In AX3K and its derivatives (HUD239) the NC4 7.2 kb fragment is replaced by one of 5.1 kb. Thus in this cross it is possible to follow both gene copies from the duplication-bearing strain, HUD377 (Figure 4). HUD239 is a segregant of a cross of AX3K with HU1628; HUD377 is a methanol resistant mutant of HUD166, a segregant of a cross of AX3L with HU1628. Genetic markers are described in Table 1.

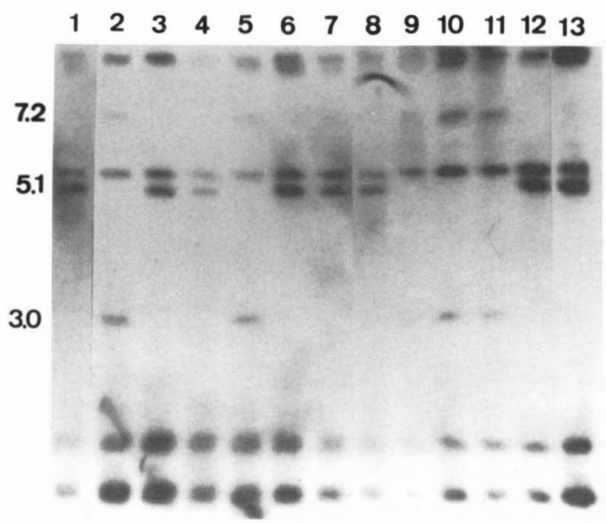

FIGURE 4.—Analysis of *Eco*RI restriction fragments in segregants of a cross of HUD239 with HUD377 using as probe pDd812, a plasmid that contains a portion of the discoidin α gene (see Table 5). Lanes are: 1, HUD239; 2, HUD377; 3, HUD381; 4, HUD382; 5, HUD384; 6, HUD386; 7, HUD387; 8, HUD391; 9, HUD393; 10, HUD394; 11, HUD395; 12, HUD398; 13, HUD403. Sizes of polymorphic fragments are indicated on the left.

linkage group II (Figure 4, Table 5). Segregants of the two parental classes, with respect to linkage group II markers, were obtained: (1) *acrA*$^+$ with the AX3K 5.1-kb fragment or (2) *acrA2108* with the AX3L 7.2- and 3.0-kb fragments.

These strains can also be used to determine whether or not the α gene is duplicated in AX3L. Fortuitously the hybridization to the 1.4-kb and 1.8-kb *Eco*RI fragments is approximately the same in the segregants containing the AX3K linkage group II. The ratio of the hybridization to the 1.8-kb (α) fragment relative to the hybridization to the 1.4-kb (β-γ) fragment ranged from 0.82 to 0.94 for DNA from HUD239 and segregants that bear the AX3K linkage group II. This corresponds to a situation where there are single copies of the α gene and the rest of the gene family. If the α gene is duplicated in AX3L, then with DNA from strains bearing the AX3L linkage group II the ratio of the hybridization to these bands should be similar to that observed with strains bearing the AX3K linkage group II. This corresponds to two copies of both the α gene and the rest of the gene family. Conversely if the α gene is not duplicated the ratio should not be similar to that observed with the strains carrying the AX3K linkage group II. This would reflect hybridization to a single 1.8-kb fragment and to two copies of the 1.4-kb fragment. The ratios of the hybridization to the 1.8-kb fragment relative to that to the 1.4-kb fragment for DNA from strains bearing the AX3L linkage group II were similar to those obtained with DNA from segregants bearing the AX3K linkage group II. The ratios ranged from 0.84 to 0.93. This indicates that the α gene is duplicated in AX3L.

Linkage analysis of Tdd-2 mobile genetic elements using DNA polymorphisms: A prediction of the insertion of the Tdd-3 element into a Tdd-2 element in the DNA flanking the discoidin gene family in AX3K is that this will generate DNA polymorphisms detectable using a probe for the Tdd-2 element. With *Hin*dIII digested genomic DNA of the segregants from the AX3K cross with HU1852, a polymorphic restriction fragment was detected that segregated as expected for a linkage group II marker.

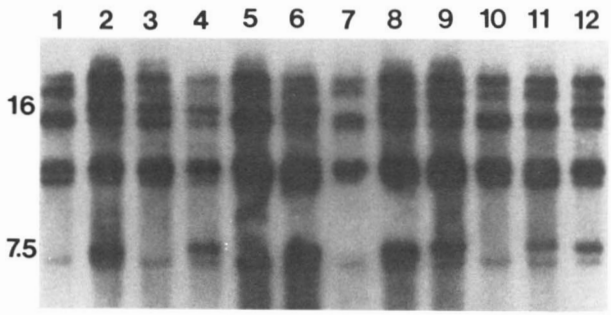

FIGURE 5.—Analysis of *Hin*dIII restriction fragments in segregants of a cross of AX3K with tester strain HU1852 using as probe an *Eco*RI-*Bgl*II fragment of pDd17 that contains a portion of the Tdd-2 sequence (see Table 4). Lanes are: 1, HU1852; 2, AX3K; 3, HUD132; 4, HUD133; 5, HUD134; 6, HUD138; 7, HUD140; 8, HUD143; 9, HUD145; 10, HUD148; 11, HUD150; 12, HUD152. Approximate sizes of polymorphic fragments are indicated on the left.

HU1852 and segregants with the HU1852 linkage group II had a doublet of about 15 kb, while segregants with the AX3K linkage group II had a pair of fragments one of about 16 kb and one of 15 kb (Figure 5, Table 4). In addition, a second polymorphic *Hin*dIII fragment of about 7.5 kb that segregates with the AX3K linkage group IV, was detected (Figure 5, Table 4). This must reflect a second independent Tdd-2 insertion or rearrangement event in AX3K.

DISCUSSION

Restriction fragment length polymorphisms allow rapid analysis of the linkage and organization of developmentally regulated gene families in *D. discoideum*. The experiments presented here establish that the entire discoidin I gene family is located on a single linkage group, linkage group II. It was known that the β, γ and pseudogene were clustered (POOLE and FIRTEL 1984) but no information on linkage to genetic markers was previously available. This work made use of RFLP analysis and parasexual crosses involving tester strains that carried genetic markers for each of the six known linkage groups. Similar analyses have been used to map actin and tRNA genes of *D. discoideum* (WELKER *et al.* 1986; DINGERMANN *et al.* 1987). DNA polymorphisms affecting restriction fragments carrying discoidin I genes were common in wild isolates, much more so than was seen in the study of the actin gene family (WELKER *et al.* 1986). This may reflect the nonessential nature of the discoidin I protein. Discoidin I is not required either for vegetative cell viability or for fruiting body formation.

Linkage of genes to their regulatory loci is potentially important for both the actual mechanism of regulation and the evolution of the regulatory process. The α gene may be clustered with the other discoidin I genes since it is duplicated along with the rest of the gene family in AX3L. Thus the gene family may be controlled by a common regulatory process, for example, one based on the presence of an enhancer or on regional chromatin changes at the time of transcription. Furthermore the gene family may be located near other loci that have been shown to influence discoidin I expression. Mutants in *disA*, a locus on group II, fail to express discoidin I, which has been taken to indicate *disA* involvement in a *trans*-acting regulatory network (ALEXANDER, CIBULSKY and CUNEO 1986). Two additional loci (*motA* and *daxA*) that affect discoidin I expression have also been mapped to linkage group II (KAYMAN, BIRCHMAN and CLARKE 1988). This linkage may be important in the coordinate maintenance through evolution of the regulatory elements and the gene family.

A duplication of the entire gene family was mapped to linkage group II. It is probable that this is a tandem duplication and that the duplicated genes lie near the normal gene family. Only one *D. discoideum* duplication other than those resulting from aneuploidy has been reported, this is a tandem duplication (WELKER, METZ and WILLIAMS 1982). One difference from the known tandem duplication is the apparent stability of the discoidin I duplication. No instability was observed with this duplication, whereas with the previously characterized tandem duplication frequent sectoring of colonies associated with loss of the duplication was observed. A second difference is the apparent absence of adverse gene dosage effects on growth with the discoidin gene duplication. These effects suggest that the discoidin gene duplication is relatively small in comparison to the previously characterized tandem duplication.

In addition to the results with the discoidin I gene family, a probe for the Tdd-2 mobile genetic element detected and allowed linkage analysis of two polymorphisms. One was linked to group II and probably is the product of a known insertion of a Tdd-3 element into a Tdd-2 element near the AX3K discoidin gene (POOLE and FIRTEL 1984). The other maps to linkage group IV and must be the result either of movement of a Tdd-2 element or of a DNA rearrangement affecting a Tdd-2 element. These results are the first mapping of putative transposable elements in *D. discoideum*. This ability to follow transposable elements will undoubtedly be employed in future mutation experiments involving transposon tagging of developmentally regulated genes, a powerful technique in other eukaryotic systems.

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