THE USE OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AND DNA DUPLICATIONS TO STUDY THE ORGANIZATION OF THE ACTIN MULTIGENE FAMILY IN *DICTYOSTELIUM DISCOIDEUM*

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ABSTRACT

The techniques of restriction fragment length polymorphism analysis and examination of gene copy number in duplication-bearing *Dictyostelium discoideum* strains have been used to map four actin genes of the wild-type strain NC4 to specific linkage groups. In part, this was accomplished by identification of restriction fragments corresponding to particular cloned actin genes using gene-specific probes from unique sequence 5′ and 3′ untranslated regions. Cloned gene Actin 8 (designation *act-8*) maps to linkage group I; Actins 12 (*act-12*) and M6 (*actM6*) to linkage group II. An uncloned gene (*act-100*) also maps to linkage group II in the same region as *actM6*, as defined by a chromosomal duplication. From analysis of other wild isolates of *D. discoideum*, it was determined that in these isolates at least two actin genes map to linkage group I and at least four map to linkage group II. These results demonstrate the utility of molecular techniques in genetic analysis of Dictyostelium, particularly for developmentally regulated genes that have been cloned but that have no identified mutant phenotypes.

ACTIN and the actin gene family have received considerable attention by researchers working with *Dictyostelium discoideum*. This protein is abundant (SPUDICH and SPUDICH 1982), and the individual genes are differentially expressed during vegetative growth and the *D. discoideum* developmental cycle (MCKEOWN and FIRTEL 1981a, 1982; MCKEOWN *et al.* 1982; ROMANS, FIRTEL and SAXE 1985). Many of the approximately 20 genes of the actin multigene family from the NC4 isolate have been cloned and characterized at the molecular level (KINDLE and FIRTEL 1978; FIRTEL *et al.* 1979; MCKEOWN and FIRTEL

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1981a,b, 1982; ROMANS and FIRTEL 1985a; ROMANS, FIRTEL and SAXE 1985). Actin genes are dispersed in the genome, as evidenced by the lack of conserved restriction sites in the DNA surrounding the protein coding regions. However, at least three pairs of actin genes are closely linked; in two cases, DNA clones containing the paired actin genes have been isolated (MCKEOWN *et al.* 1978; ROMANS and FIRTEL 1985a,b). Since much information was already available for this gene family, we chose it to investigate the usefulness of DNA polymorphisms and duplications for genetic analysis of the *D. discoideum* genome. The results so obtained thereby providing additional information on the organization of this gene family. In particular, we correlated the cloned actin genes with specific restriction fragments of genomic DNA. Then, we analyzed the genomic organization of several genes using restriction fragment length polymorphisms (RFLPs) exhibited by different wild isolates of *D. discoideum* and/or by comparison of DNA amounts in different actin-gene-containing restriction fragments in genetic duplication *vs.* nonduplication-bearing strains. Our results establish that, as is the case in other organisms, notably humans (BOTSTEIN *et al.* 1980; GUSELLA *et al.* 1983; WHITE *et al.* 1985), analysis of RFLPs in Dictyostelium is a powerful technique for the study of gene location and organization.

MATERIALS AND METHODS

Strains and growth of amoebae: Twelve wild isolates of *D. discoideum* were used. NC4, V12, WS10 (HU182), WS51, WS380B, WS472, WS576, WS583, WS585 (HU188) and WS1956 were obtained from K. B. RAPER, University of Wisconsin, Madison. The origins of these strains are presented elsewhere (ERDOS, RAPER and VOGEN 1973; METZ *et al.* 1983; ROBSON and WILLIAMS 1979, 1980). Isolate DD61 was from D. WADDELL, Gesamthochschule Wuppertal, Wuppertal, Federal Republic of Germany, and the OHIO isolate was from D. FRANCIS, University of Delaware, Newark. The duplication-bearing strains HU329 and HU1257 and their haploid sectors—HU440 and HU1310, respectively—have been described previously (WELKER, METZ and WILLIAMS 1982; WILLIAMS, ROBSON and WELKER 1980). AX3, a derivative of NC4 which is capable of axenic growth, has been used in some studies. The isolates of this strain maintained in the KESSIN laboratory (AX3K) and LOOMIS laboratory (AX3L) exhibit considerable differences (POOLE and FIRTEL 1984). The multiply marked strain HU1628 was described previously (WELKER and WILLIAMS 1985).

Nomenclature: Actin loci have been designated *act.* In the case of loci identified in strain NC4, the allele numbers assigned are the same as the number identifying the cloned gene if the cloned gene has been correlated with a particular genomic *Hin*dIII fragment. For example, the designation for the cloned gene Actin 8 is *act-8*. For NC4 loci not identified with a cloned gene and for actin genes from the other wild isolates, allele numbers were assigned according to standard *D. discoideum* genetic nomenclature.

Parasexual genetic techniques: Diploids were constructed between the NC4-derived tester strain HU1628 (WELKER and WILLIAMS 1985) and the haploid wild isolates. Diploids were distinguished from the mixed population of parental haploids as they express the dominant cobalt resistance mutation *cob-354* and fail to express the recessive *Bacillus subtilis* sensitivity mutation *bsgA5* (WELKER and WILLIAMS 1985). In addition to these two "selector" mutations, strain HU1628 also carries easily scorable markers on each of the six established linkage groups. Because such selectors are not available in them, direct crosses between pairs of wild isolates were not possible. Haploid segregants bearing different combinations of chromosomes from the parental strains were selected from the diploids, using the antimicrotubule agent thiabendazole (2 μg/ml in SM agar;

WELKER and WILLIAMS 1980). Genetic markers were scored using standard methods (NEWELL 1982). Construction of segregants with mixtures of chromosomes from the V12 and NC4 wild isolates has been described previously (ROBSON and WILLIAMS 1979; WILLIAMS, ROBSON and WELKER 1980).

DNA preparation and analysis of Southern blots: Amoebae were harvested from approximately 20 SM agar plates, lysed for the preparation of nuclei, and nuclear DNA prepared as described previously (FIRTEL *et al.* 1976; FIRTEL, KINDLE and HUXLEY 1976; WELKER, HIRTH and WILLIAMS 1985). Genomic DNA was digested with restriction enzymes, electrophoresed through 0.8% w/v agarose gels using a Tris-borate buffer, transferred to nitrocellulose (Schleicher and Schuell BA85, SOUTHERN 1975) and hybridized with DNA probes labeled with [α^{32}P]dATP by nick translation (RIGBY *et al.* 1977). For mapping studies, the entire *D. discoideum* Actin 8 plasmid (MCKEOWN and FIRTEL 1981a) was used as the probe. For the identification of particular actin genes with specific restriction fragments of genomic DNA, restriction fragments containing unique sequence at the 5′ and 3′ ends of cloned actin genes were isolated. The fragments were purified from agarose gels by electrophoresis onto DE81 paper, followed by elution with 1M LiCl and ethanol precipitation. The fragments were labeled by nick translation and were hybridized to a set of blots of AX3K DNA separately digested with *Eco*RI, *Hin*dIII and *Sau*96I. To facilitate gene identification the same set of blots was subsequently hybridized to an actin coding sequence probe from the cDNA clone Actin B1 (MCKEOWN *et al.* 1978) after being washed several times in boiling 5 mM EDTA (pH 7.2), 0.5% SDS to release the previous probe. By comparing the hybridization pattern, we identified which actin gene band corresponded to a specific cloned gene. Isolation and restriction maps of the Dictyostelium actin genes are described elsewhere (ROMANS and FIRTEL 1985a,b).

RESULTS

Identification of cloned actin genes with genomic restriction fragments: We have correlated particular cloned actin genes with genomic DNA fragments produced by separate digestion of AX3K DNA with *Eco*RI, *Hin*dIII and *Sau*96I. Gene-specific probes were prepared by isolation of restriction fragments containing unique DNA sequence from the 5′ and 3′ ends of the protein coding regions (see MATERIALS AND METHODS). The results of these hybridizations and extensive prior restriction mapping allowed us to make the identifications summarized in Table 1 and Figure 1.

Mapping actin genes using DNA polymorphisms: Polymorphisms in the DNA sequences surrounding *D. discoideum* actin genes were easily identified. Digestion of nuclear DNA from 12 wild isolates including NC4 with either *Hin*dIII or *Eco*RI, followed by probing DNA blots with an actin-coding sequence probe, showed significant differences in the pattern of hybridized bands (Figure 2). The NC4 pattern was not identical to that of any of the other 11 isolates. However, particular bands from the NC4 pattern were common to all or were shared with some wild isolates. In order to map actin genes using restriction fragment length polymorphisms, diploids were constructed by parasexual genetic crosses between a haploid tester strain (HU1628) derived from NC4 and the wild isolate haploids OHIO, DD61 and WS380B. Haploid segregants were obtained from these diploids and DNA prepared from segregants that contained different combinations of the NC4 and wild isolate-derived chromosomes, as identified by genetic markers from HU1628.

Assignment of *act-8* to linkage group I: Segregants with mixtures of chro-

TABLE 1

Correlation of specific cloned actin genes with genomic actin-containing restriction fragments

Cloned gene	Restriction fragment (size in kb)		
	*Eco*RI	*Hin*dIII	*Sau*96I
Actin M6	4.25[a]	3.2[a]	1.45[a]
Actin 2-sub 1	9.4–9.7[a]	1.45–1.5[a]	2.0[a]
2-sub 2		ND	1.45[a]
Actin 3-sub 1	9.7–10.0[a]	ND	3.25[a]
3-sub 2			2.0[a]
Actin 4	7.1[a]	ND	4.62–4.65[a]
Actin 5	12.5[b]	7.3–7.4	5.05
Actin 6	5.6–5.7[a]	5.4–5.5	ND
Actin 7	5.05–5.1[a]	ND	4.4–4.5
Actin 8	4.6[a]	1.35–1.4[a]	ND
Actin 9	7.05–7.1[a]	7.4	3.4–3.5
Actin 10	2.65[a]	10	ND
Actin 11	5.15[a]	1.95	≥15
Actin 12	5.6–5.7[a]	2.0–2.1	1.95–2.0[a]
Actin 13	8.4–8.5[a]	11–12	8.6–8.8

[a] Fragment sizes were determined directly from the restriction maps of the cloned genes and, in most cases, confirmed using 5′ or 3′ unique sequence fragments. These were purified, nick-translated and used to probe genomic Southern blots of *D. discoideum* strain AX3K DNA cut with *Eco*RI, *Hin*dIII and *Sau*96I to determine the size of the genomic fragment in each digest corresponding to a specific cloned actin gene (Figure 1). Details of the cloning of actin genes and restriction maps are presented elsewhere (ROMANS and FIRTEL 1985b). Blots were also hybridized with a probe derived from the coding region of the cDNA clone Actin B1 to aid in correlation of bands (Figure 1). Sequencing data suggest that B1 may be derived from *Actin 13* or the very closely related Actin 9 (ROMANS and FIRTEL 1985b).

[b] The *Eco*RI fragment size derived from the map of Actin 5 is 5.5 kb, rather than 12.5 kb indicated with a gene-specific probe. Analysis indicates that there is a deletion of DNA 5′ to the coding region of the Actin 5 (10a1) clone.

ND—Size not determined.

mosomes derived from the WS380B wild isolate and HU1628 allowed *act-8* (the cloned gene Actin 8) to be assigned to linkage group I. All segregants (HU2302, HU2303 and HU2321) carrying the HU1628-derived linkage group I marker, *cycA1*, also carried *act-8*, whereas segregants carrying the WS380B-derived linkage group I did not carry *act-8* (Table 2, Figure 3). The presence of two bands derived from WS380B, *act-356* and *act-357*, was correlated with the presence of the WS380B linkage group I. The absence of a band in the position of *act-8* and the presence of two new bands in *Hin*dIII digests were also observed in the wild isolates WS51, WS472, WS576, WS585 (HU188) and WS1956 (Figure 2A). Likewise, a 4.6-kb *Eco*RI restriction fragment that in NC4 carries the Actin 8 gene (Table 1) is absent from these wild isolates (Figure 2B).

Assignment of *act-12* and other genes to linkage group II: The locus defined by the Actin 12 gene could be mapped because at least four of the wild isolates (OHIO, DD61, V12 and WS583) lacked the 2-kb *Hin*dIII restriction fragment identified in NC4 (Table 1, Figure 2A). Diploid DU2933 was constructed by crossing HU1628 with the OHIO wild isolate. Analysis of se-

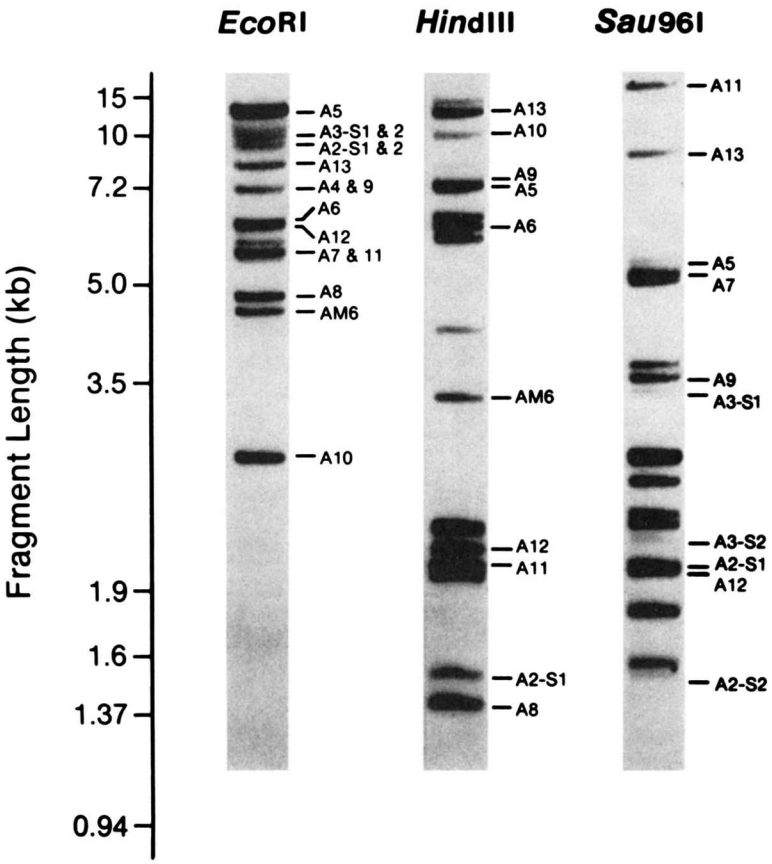

FIGURE 1.—Correlation of specific cloned actin genes with actin-gene-containing restriction fragments. DNA was isolated from strain AX3K, digested with *Eco*RI, *Hin*dIII or *Sau*96I, electrophoresed, blotted and probed with the gene-containing fragment of the Actin B1 DNA clone. Fragments corresponding to specific genes are designated to the right of each lane using abbreviations, *e.g.*, A8 is Actin 8. This figure summarizes several experiments in which unique sequence DNA fragments 5′ or 3′ to the protein coding regions of individual cloned actin genes were isolated, nick-translated and hybridized with the indicated genomic DNAs, as described in MATERIALS AND METHODS [see ROMANS and FIRTEL (1985b) for additional information on the hybridization conditions]. To confirm the identity of each gene-specific fragment relative to the entire pattern of actin gene-containing restriction fragments, the blots were probed with the Actin B1 fragment.

gregants from this diploid indicates that *act-12* is on linkage group II (Table 3, Figure 4). Segregants HUD14, HUD17 and HUD32 carry both *act-12* and the *acrA1823* marker for linkage group II derived from HU1628. The other segregants from this diploid do not carry either *act-12* or the *acrA1823* marker. The mapping of *act-12* to linkage group II was confirmed using haploids with mixtures of chromosomes from the NC4 and V12 wild isolates (data not shown). Data from diploid DU2885 (HU1628 × DD61) are consistent with the assignment of *act-12* to linkage group II, with the exception of a single segregant (Table 4, Figure 5). This segregant, HU2236, is probably a haploid

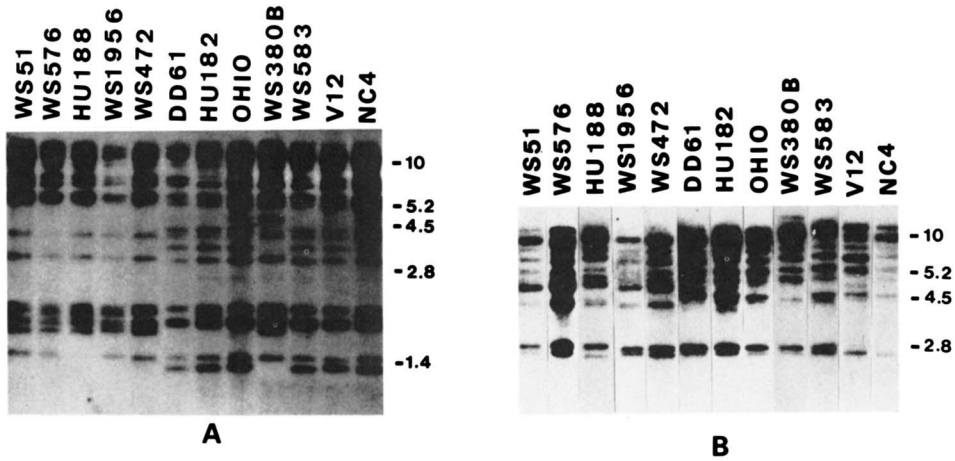

FIGURE 2.—*Hin*dIII (A) and *Eco*RI (B) restriction fragments homologous to the Actin 8 probe from the nuclear DNA of 12 different wild isolates. Restriction fragment length polymorphisms are clearly present in both sets of digests. Approximate sizes in kb are given on the right. Note that strain HU188 does have a 1.45- to 1.5-kb *Hin*dIII fragment, which was visible on longer exposure of this blot. The NC4 *Hin*dIII pattern (A) includes an atypical faint band of approximately 3.5 kb that is probably due to hybridization to a restriction fragment with low homology to the Actin 8 probe DNA. However, a similarly sized band was not observed in Southern blots of NC4 derivatives. This may reflect a change that occurred in laboratory culture; such changes are known at other *D. discoideum* loci (POOLE and FIRTEL 1984).

derived from a diploid mitotic recombinant involving linkage group II. Such unselected haploid mitotic recombinants have been reported previously (WELKER and WILLIAMS 1982, 1983; WILLIAMS, ROBSON and WELKER 1980).

Several *Hin*dIII fragments that are polymorphic in the OHIO or DD61 isolates relative to NC4 also map to linkage group II. The *act-351* and *act-352* genes are present only in segregants carrying linkage group II from the OHIO strain and were absent from those carrying the HU1628 *acrA1823* marker (Table 3, Figure 4). Restriction fragments similar in size to those of *act-351* and *act-352* were seen in the blots of *Hin*dIII cut DD61, HU182, WS583 and V12 DNA (Figure 2A). In the segregants of DU2885 these fragments (*act-354* and *act-355*) also mapped to linkage group II, again with the exception of segregant HU2236 (Table 4, Figure 5). The OHIO isolate carries two additional actin genes mapping to linkage group II, *act-350* and *act-353* (Figure 4).

The presence of *act-358* from WS380B was correlated with the presence of the WS380B linkage group II. Segregants that carried the HU1628 linkage group II (HU2280, HU2305, HU2306) did not carry *act-358*; instead, two faint bands were present in these segregants (Table 2, Figure 3). These bands were about the same size as that of the *act-358* band and may be due to hybridization to restriction fragments from HU1628 that have lower homology to the Actin 8 probe DNA. A similar sized band(s) is seen in DNA of NC4 and its derivatives on long exposure of the DNA blots or after lower stringency hybridization (P. ROMANS and R. A. FIRTEL, unpublished data). We have not yet established whether *act-358* corresponds to one of these bands from NC4.

TABLE 2

Genotypes of HU1628, WS380B and haploid segregants of diploid DU2889, which was constructed from the haploid tester strain HU1628 and the wild isolate WS380B

Strain	Linkage group					
	I	II	III	IV	VI	VII
HU1628	*cycA1*	*acrA1823* *axeA1* *axeC1* *oaaA1*	*bsgA5*	*whiC351*	*manA2*	*couA351* *frtB353*
WS380B	+	+	+	+	+	+
HU2274	+	+	+	+	+	+
HU2275	+	+	+	*whiC351*	+	+
HU2280	+	*acrA1823*	*bsgA5*	*whiC351*	*manA2*	+
HU2281	+	+	*bsgA5*	+	*manA2*	*couA351*
HU2302	*cycA1*	+	*bsgA5*	+	*manA2*	*couA351*
HU2303	*cycA1*	+	*bsgA5*	*whiC351*	*manA2*	*couA351*
HU2305	+	*acrA1823*	*bsgA5*	+	*manA2*	*couA351*
HU2306	+	*acrA1823*	+	*whiC351*	+	+
HU2321	*cycA1*	+	+	*whiC351*	+	+

The haploid segregants were derived from the diploid, following thiabendazole-induced nondisjunction and chromosome loss. In this work segregants of diploids are denoted HU or HUD, followed by a number. They contain mixtures of the six established linkage groups (chromosomes) from HU1628 and the wild isolate; for historical reasons, the unmarked linkage group is group V. Complete genotypes are given for HU1628 and the wild isolate; in the segregants, the *axeA1*, *axeC1*, *oaaA1* and *frtB353* markers were not scored. Phenotypes of mutations at these loci are: *acrA*, resistance to acriflavin (100 μg/ml), methanol (2–3%) and benzimidazole carbamate derivatives; *axeA*, *axeC* ability to grow in axenic medium (requires an additional mutation at the *axeB* locus); *bsgA*, inability to use *B. subtilis* as a food source; *couA*, sensitivity to 1.3 mM coumarin with pleiotropic temperature sensitivity (*tsgK21*); *cycA*, resistance to cycloheximide (500 μg/ml); *frtB*, distribution of fruiting bodies in concentric rings; *manA*, α-mannosidase-1 deficient; *oaaA*, ability to develop in the presence of ω-aminocarboxylic acids; *whiC*, absence of the normal yellow spore pigment. Additional information on the phenotypes of mutations at these loci is reviewed elsewhere (NEWELL 1982). Alleles from the wild isolate are denoted +.

Mapping actin genes within linkage group II using a DNA duplication: Since several actin genes mapped to linkage group II, a strain that carries a duplication for part of linkage group II (WILLIAMS, ROBSON and WELKER 1980) was used to examine more precisely the location of actin genes on this chromosome. We used densitometer tracings of the relative hybridization of excess ^{32}P-labeled Actin 8 probe DNA to compare blots of DNA from the duplication-bearing strain HU329 [a strain derived from a diploid of NC4 and V12 (WILLIAMS, ROBSON and WELKER 1980)] and its haploid sector HU440. Two nonpolymorphic actin genes, *actM6* and *act-100*, are present in the duplication (Figure 6). This is consistent with the finding that the cloned Actin M6 gene (*actM6*) is tightly linked to another actin gene (MCKEOWN and FIRTEL 1981b; MCKEOWN *et al.* 1978), which we presume to be *act-100*. Hybridization to actin genes on restriction fragments of similar size provides the necessary internal standards for this comparison. Against these standards the approximately two-fold difference in hybridization to the *actM6* and *act-100* bands is striking. In

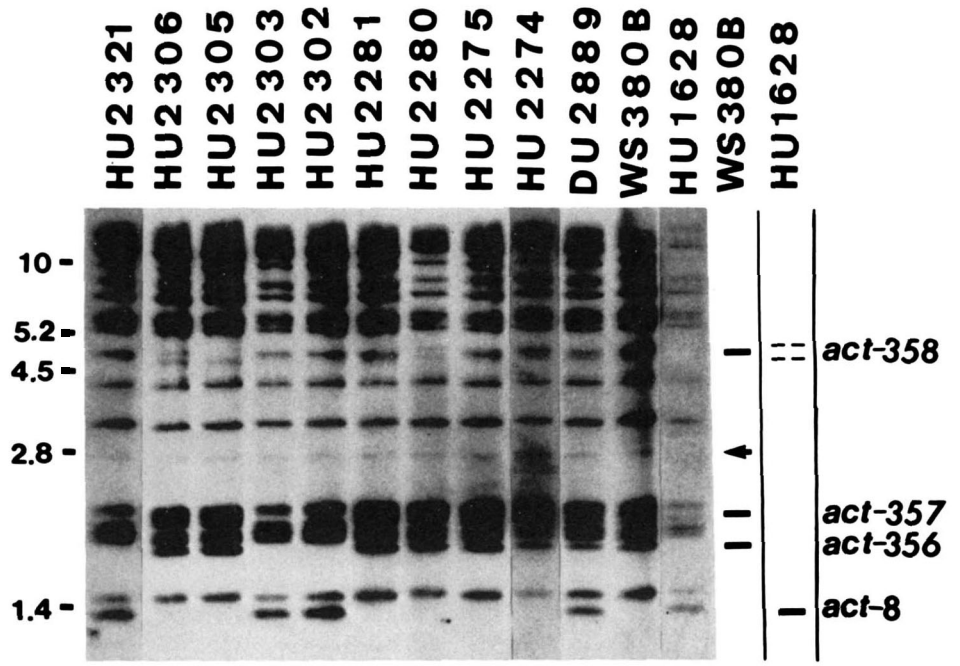

FIGURE 3.—Actin-containing *Hin*dIII restriction fragments in nuclear DNA of HU1628, WS380B, diploid DU2889 (HU1628 × WS380B) and segregant haploids (HU2274-HU2321) derived from DU2889. Polymorphic restriction fragments from WS380B and HU1628 are indicated on the right; for genotypes of segregants, see Table 2. *Act-8* maps to linkage group I, because segregants (HU2302, HU2303 and HU2321) either carry both *act-8* and the genetic marker for linkage group I, *cycA1*, or carry neither *act-8* nor *cycA1* (all other segregants shown). The arrow on the right indicates a faint band common to WS380B, DU2889 and the segregants of DU2889; a band of similar size is also seen in DNA prepared from NC4 derivatives after low stringency hybridization (25% formamide). The presence of faint bands in HU2280, HU2305 and HU2306, indicated by the broken lines on the right, are also probably due to hybridization of the probe DNA to restriction fragments with low homology to the probe DNA. These appear to map to the HU1628 linkage group II. Approximate sizes in kb are given on the left.

combination with previous data (WILLIAMS, ROBSON and WELKER 1980), these results place *actM6* and *act-100* between the centromere and the *acrA* locus on linkage group II of NC4 and of V12.

A second feature of this duplication permitted determination of the positions of *act-12* and of the V12 genes *act-360* and *act-361* (which correspond to *act-351* and *act-352* of OHIO respectively) within linkage group II. This linkage group is recombinant in HU329; the centromere and adjacent sequences from NC4 are coupled to V12-derived material extending from a point distal to *whiA* but proximal to *acrA* through to the telomere. HU329 also carries a V12-derived centromeric fragment of linkage group II that accounts for the duplication (WILLIAMS, ROBSON and WELKER 1980). The nonduplication-bearing haploid HU440 has lost the V12-derived centromeric fragment. It retains the recombinant linkage group II chromosome. Since HU329 and HU440 lack

TABLE 3

Genotypes of HU1628, OHIO and haploid segregants of diploid DU2933, which was constructed from the haploid tester strain HU1628 and the wild isolate OHIO

Strain	Linkage group					
	I	II	III	IV	VI	VII
HU1628	*cycA1*	*acrA1823* *axeA1* *axeC1* *oaaA1*	*bsgA5*	*whiC351*	*manA2*	*couA351* *frtB353*
OHIO	+	+	+	+	+	+
HUD14	*cycA1*	*acrA1823*	*bsgA5*	*whiC351*	*manA2*	*couA351*
HUD16	*cycA1*	+	+	+	+	*couA351*
HUD17	*cycA1*	*acrA1823*	+	*whiC351*	+	*couA351*
HUD28	*cycA1*	+	*bsgA5*	+	*manA2*	+
HUD31	+	+	+	+	+	+
HUD32	+	*acrA1823*	+	*whiC351*	+	*couA351*
HUD33	*cycA1*	+	+	*whiC351*	+	+
HUD34	+	+	+	*whiC351*	+	+

For further explanation of the genotypes, see Table 2.

act-12 from NC4 and retain the V12-derived *act-360* and *act-361*, these three genes reside in the distal portion of the linkage group.

Part of linkage group III contains no actin genes: A duplication that covered part of linkage group III between the *whiB* and *radB* loci was also tested. Comparison of the relative hybridization of ^{32}P-labeled Actin 8 probe to Southern blots of DNA obtained from the duplication-bearing strain HU1257 and its sector HU1310 did not reveal any striking differences (data not shown). Therefore, it appears that no actin genes are present within this region of linkage group III.

DISCUSSION

Four of the actin genes of the NC4 wild-type strain were mapped to particular linkage groups; *act-8* (cloned gene Actin 8) to linkage group I; *act-12* (Actin 12), *actM6* (Actin M6) and *act-100* (cloned gene not yet determined) to linkage group II. The mapping of *act-8* and *act-12* made use of *Hin*dIII restriction fragment length polymorphisms affecting DNA sequences near these genes. Such polymorphic differences are readily detectable in the DNA of different wild isolates. The overall *Hin*dIII and *Eco*RI restriction patterns of NC4 genomic DNA are not identical to those of 11 other wild isolates, although many individual restriction fragments appear to be shared among various isolates. Two common DNA polymorphisms involving *act-8* and *act-12* were identified. Of 12 isolates examined, one (NC4) had both a 1.4-kb *Hin*dIII fragment (*act-8*) and a 2.0-kb *Hin*dIII fragment (*act-12*), five (OHIO, DD61, V12, WS583 and probably HU182) had a 1.4-kb fragment but lacked a 2.0-

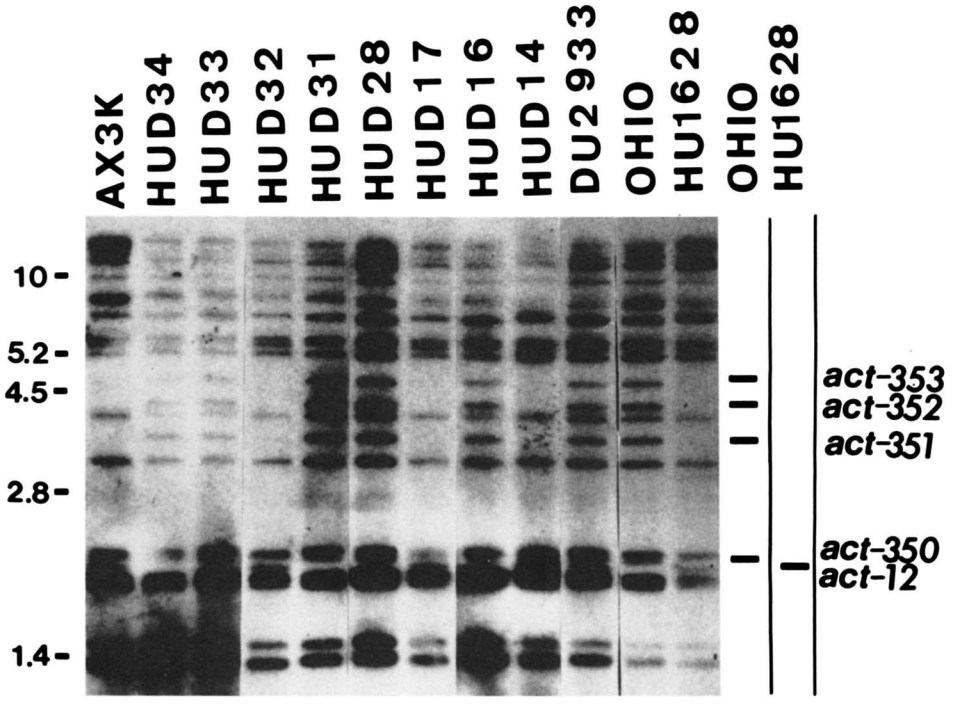

FIGURE 4.—Actin-containing *Hin*dIII restriction fragments in nuclear DNA of HU1628, OHIO, diploid DU2933 (HU1628 × OHIO), segregant haploids derived from DU2933 (HUD14-HUD34), and the NC4-derived strain AX3K. Polymorphic restriction fragments from OHIO and HU1628 are indicated on the right; for genotypes of the segregants, see Table 3. *Act-12* maps to linkage group II, because segregants (HUD14, HUD17 and HUD32) either carry both *act-12* and the genetic marker for linkage group II, *acrA1823*, or carry neither *act-12* nor *acrA1823* (all other segregants shown). Approximate sizes in kb are given on the left.

kb fragment, and six (WS380B, WS51, WS472, WS576, WS1956, and HU188) lacked a 1.4-kb fragment but apparently retained a 2-kb fragment. Less common polymorphisms were also identified; for example, in strains OHIO and WS380B the presence of a 4.6-kb *Hin*dIII fragment and in strain HU182 that of a 2.1-kb *Hin*dIII fragment. These shared and unshared restriction fragments undoubtedly reflect divergence from an ancestral form.

Restriction fragment length polymorphisms also permitted several actin genes in other wild isolates to be assigned to linkage groups. Two genes from strain WS380B were assigned to linkage group I; four genes from strain OHIO and one gene from WS380B mapped to linkage group II. It has not been determined which, if any, of these correspond to *act-8* and *act-12*, nor has it been determined whether the actin genes of WS380B assigned to linkage group I and those of OHIO assigned to linkage group II represent duplication of the NC4 *act-8* and *act-12* genes, respectively. DNA from strains WS380B and OHIO digested with *Hin*dIII and hybridized with Actin 8 and Actin 12 specific probes, respectively, should resolve these issues. The finding of such duplications in the different wild isolates is not unprecedented in *D. discoideum*.

TABLE 4

Genotypes of HU1628, DD61 and haploid segregants of diploid DU2885, which was constructed from the haploid tester strain HU1628 and the wild isolate DD61

Strain	Linkage group					
	I	II	III	IV	VI	VII
HU1628	*cycA1*	*acrA1823* *axeA1* *axeC1* *oaaA1*	*bsgA5*	*whiC351*	*manA2*	*couA351* *frtB353*
DD61	+	+	+	+	+	+
HU2235	*cycA1*	+	*bsgA5*	*whiC351*	*manA2*	*couA351*
HU2236	*cycA1*	+	+	*whiC351*	+	+
HU2245	+	*acrA1823*	*bsgA5*	*whiC351*	*manA2*	*couA351*
HU2246	*cycA1*	+	+	+	+	+
HU2248	+	*acrA1823*	+	*whiC351*	+	+
HU2259	+	+	*bsgA5*	+	*manA2*	*couA351*
HU2260	+	+	+	+	+	+

For further explanation of the genotypes, see Table 2. Segregant HU2236 is probably a mitotic recombinant of linkage group II (see text).

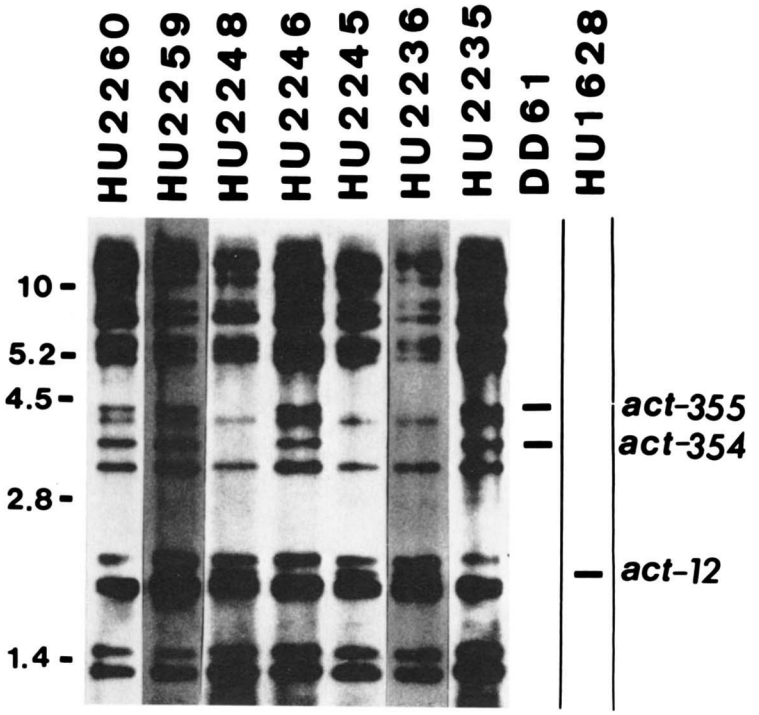

FIGURE 5.—Actin-containing *Hin*dIII restriction fragments in nuclear DNA of segregants of diploid DU2885 (HU1628 × DD61). Polymorphic restriction fragments from DD61 and HU1628 are indicated on the right; for genotypes of the segregants, see Table 4. This data supports the mapping of *act-12* to linkage group II, except for that obtained from segregant HU2236. This segregant is probably an unselected mitotic recombinant. Approximate sizes in kb are given on the left.

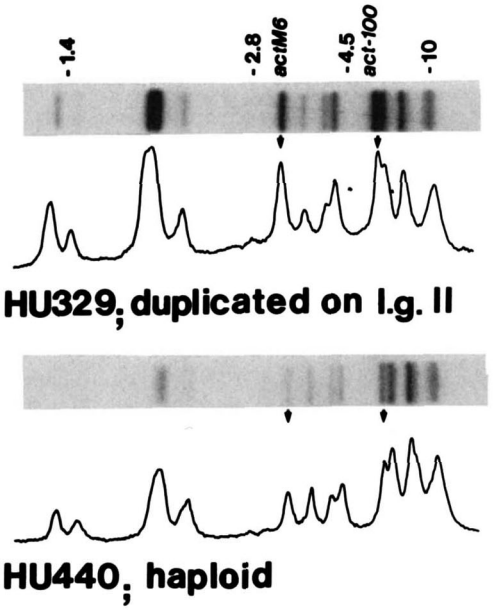

FIGURE 6.—Densitometer tracings of autoradiograms of actin-containing *Hin*dIII restriction fragments of nuclear DNA from the duplication-bearing strain HU329 and its nonduplication-bearing haploid sector HU440. These strains carry a linkage group II formed by recombination in the *whiA* to *acrA* interval. The centromere and proximal sequences are derived from NC4, the distal sequences from V12. HU329 also carries a fragment chromosome corresponding to the proximal part of the V12 linkage group II. Arrows indicate the *actM6* and *act-100* genes present in the duplication. From this data, we conclude *actM6* and *act-100* map on linkage group II, proximal to the *acrA* locus. Approximate sizes in kb are given at the top.

It has been shown that some strains derived from NC4 contain different numbers of discoidin I genes (POOLE and FIRTEL 1984).

The *actM6* and *act-100* genes were mapped by comparison of the relative DNA amounts in the duplication-bearing strain HU329 and its nonduplication-bearing haploid sector HU440. Since it is known that this duplication affects the region of linkage group II between the centromere and the *acrA* locus (WILLIAMS, ROBSON and WELKER 1980), *actM6* and *act-100* must map within this region. In addition, since *act-12* maps to linkage group II but is not included in the duplication, it must lie distal to the duplicated region. This technique has great potential since it does not require diploid formation and laborious segregation analyses, nor does it require screening of multiple strains to identify polymorphisms. Additional duplication-bearing strains maintained by complementation of recessive lethal mutations are now being characterized (D. L. WELKER and K. L. WILLIAMS, unpublished results).

The experiments described have permitted the mapping of several actin genes using RFLPs detectable following digestion of genomic DNA with *Hin*dIII. However, not all of the actin genes lie within the available duplications or on polymorphic *Hin*dIII restriction fragments. Therefore, in order to map the other actin loci, it will be necessary to use RFLPs identified using

other restriction enzymes. Clearly, such polymorphisms exist (Figure 2B). This type of analysis in *D. discoideum* may be somewhat limited by the exceedingly AT-rich nature of its DNA, 78% overall and 85% A + T in extragenic regions (FIRTEL and BONNER 1972; KIMMEL and FIRTEL 1982, 1983). As a consequence, many restriction enzymes that recognize six base sequences cut *D. discoideum* DNA very infrequently, often within genes, and for actin yield a genomic pattern of fragments that consists of a few bands in the 30- to 60-kb range (P. ROMANS and R. A. FIRTEL, unpublished results), which cannot be resolved adequately even on 0.4% agarose gels. However, from these digests a particular cloned actin gene can often be identified as an individual band. For example, Actin 7 is contained within a 1.8-kb *Kpn*I fragment in AX3K DNA (data not shown). Using such data together with RFLPs and duplications probed with unique sequence DNA from the immediate 5′ and 3′ regions of specific actin genes, as was done to characterize AX3K DNA in the present work, it will be possible to assign most, if not all, of the actin genes in NC4 to a linkage group or to a portion of one.

Our findings are consistent with the interpretation that the actin gene family of *D. discoideum* is moderately dispersed (KIMMEL and FIRTEL 1982; KINDLE and FIRTEL 1978; MCKEOWN and FIRTEL 1982; MCKEOWN *et al.* 1978). We have established that actin loci map to at least two of the seven linkage groups. Additional linkage groups are undoubtedly involved; preliminary evidence with *Eco*RI digested DNA of segregants of DU2889 (WS380B × HU1628) indicates that at least one actin locus may map to linkage group VII (data not shown). Within mitotic linkage groups the actin loci do not map to a single site. The three known actin loci on the NC4 linkage group II map to at least two regions of this chromosome. One of these regions is proximal to the *acrA* locus and probably includes the actin *actM6* and the *act-100* genes, while the third gene, *act-12*, maps distally. The finding of multiple sites for actin loci on linkage group II is not unexpected. This linkage group carries the largest number of mapped genes (NEWELL 1982); thus, it is one of the larger chromosomes, and it is not unreasonable that it should carry multiple sites at which actin loci map. This organization is consistent with that seen in other species where members of actin multigene families are distributed on several chromosomes (CLEVELAND *et al.* 1980; FYRBERG *et al.* 1980; LEE *et al.* 1984; SCHEDL and DOVE 1982).

This work establishes the usefulness of both restriction fragment length polymorphisms and duplication-bearing strains in genetic analysis of *D. discoideum*. Similar experiments have already proven useful in genetic characterization of other species, particularly man (BOTSTEIN *et al.* 1980; GUSELLA *et al.* 1983; WHITE *et al.* 1985). With these techniques it is now possible to expand the *D. discoideum* genetic map to include the numerous loci for which cloned DNA probes are already available but for which no phenotypes or mutations are known. These include not only loci required for growth such as tRNA genes (T. DINGERMANN, personal communication) but also developmentally regulated genes (MEDHY, RATNER and FIRTEL 1983; ROWEKAMP and FIRTEL 1980; WILLIAMS and LLOYD 1979) and gene families such as discoidin I (DEVINE, TSANG

and WILLIAMS 1982; POOLE and FIRTEL 1984; POOLE *et al.* 1981; ROWEKAMP, POOLE and FIRTEL 1980).

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