Integrated Maps of the Chromosomes in *Dictyostelium discoideum*

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ABSTRACT

Detailed maps of the six chromosomes that carry the genes of *Dictyostelium discoideum* were constructed by correlating physically mapped regions with parasexually determined linkage groups. Chromosomally assigned regions were ordered and positioned by the pattern of altered fragment sizes seen in a set of restriction enzyme mediated integration-restriction fragment length polymorphism (REMI-RFLP) strains each harboring an inserted plasmid that carries sites recognized by *Not*I, *Sst*II, *Sma*I, *Bgl*I and *Apa*I. These restriction enzymes were used to digest high molecular weight DNA prepared from more than 100 REMI-RFLP strains and the resulting fragments were separated and sized by pulsed-field gels. More than 150 gene probes were hybridized to blots of these gels and used to map the insertion sites relative to flanking restriction sites. In this way, we have been able to restriction map the 35 mb genome as well as determine the map position of more than 150 genes to with ~40 kb resolution. These maps provide a framework for subsequent refinement.

BASIC cellular and developmental processes are particularly amenable to molecular genetic analyses in Dictyostelium as a result of its small genome and unique life style (LOOMIS 1982). These characteristics are being fully exploited by recent advances in techniques for gene tagging and replacement (KUSPA and LOOMIS 1992, 1994b). Programs are underway to use saturation mutagenesis to uncover genes involved in a variety of cellular mechanisms including motility, signal transduction, tissue proportioning and morphogenesis. However, it has not been possible to construct genetic maps based on meiotic recombinational frequency because of the inefficiency of sexual reproduction (NEWELL 1978; LOOMIS 1987). Genetic mapping has been limited to the patterns of parasexual segregation of chromosomes from heterozygous diploid strains that can establish linkage groups for each chromosome but cannot determine the complete order of genes along the chromosomes (WELKER *et al.* 1986). Physical mapping with cloned portions of genes has been able to define localized regions of the genome (KUSPA *et al.* 1992). Closely linked genes can be ordered when they are colocalized within several hundred kilobases of Dictyostelium DNA cloned in yeast artificial chromosomes (YACs), and surrounding restriction sites in the genome can be recognized after digestion with rare cutting restriction enzymes and separation and sizing of the fragments by pulsed-field gel electrophoresis. Analyses of genomic fragment sizes generated by single and double restriction enzyme digestions, together with the restriction site maps of cognate large cloned regions carried in YACs, have allowed long-range maps to be constructed around various genes (KUSPA *et al.* 1992). However, this approach provides insufficient data to conclusively order genes along individual chromosomes, the smallest of which has been estimated to be 4 mb (COX *et al.* 1990). Therefore, we have generated a set of isogenic strains in which inserted plasmids provide unique sites for relational mapping (KUSPA and LOOMIS 1994a).

Transformation of *Dictyostelium discoideum* can be stimulated more than 20-fold by introducing restriction enzyme along with plasmid DNA (KUSPA and LOOMIS 1992). The restriction enzyme enters the cell during electroporation and facilitates integration of linearized foreign DNA with compatible ends into the host chromosomes. Restriction enzyme mediated integration (REMI) directs the plasmid to cognate restriction sites in the genome with little evidence of bias (KUSPA and LOOMIS 1994a,b). Because we wanted to mark as many regions as possible with an integrated plasmid, we used *Bam*HI to linearize a plasmid and carried out *Bam*HI REMI to target the several thousand *Bam*HI sites in the genome. Independent transformants (150) were isolated and used for long-range restriction fragment length polymorphism (RFLP) studies. A single copy of the plasmid carrying the selectable marker *pyr5-6* was found to be integrated in most of these strains. Because the plasmid carries an *Apa*I site in its multiple cloning region that separates the *pyr5-6* and pGEM regions, the endogenous *Apa*I fragment that now carries the plasmid is cut into two smaller fragments when digested with *Apa*I. The sizes of these fragments were determined by

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probing with sequences that flank the *Apa*I site in the integrated plasmid and thereby mapped the insertion sites relative to the flanking *Apa*I sites (KUSPA and LOOMIS 1994a). Subsequent probing with more than 100 cloned genes mapped many of the insertion sites relative to these genes and defined regions of the genome flanked by *Apa*I sites that varied from 1.5 mb to a few hundred kilobases. We have referred to this technique as REMI-RFLP analysis to distinguish it from the previous use of RFLPs, which can distinguish alleles from independent individuals but needs to be combined with other genetic data for mapping (WELKER *et al.* 1986).

To extend our regional maps in these REMI-RFLP strains to complete chromosomes, we have turned to restriction enzymes that generate larger fragments such as *Not*I, *Sst*II, and *Sma*I. The sites recognized by these enzymes contain only guanine and cytosine (pure G/C sites) and are rare in the Dictyostelium genome due to its high degree of skewing toward adenine and thymine (A/T) in noncoding regions (KIMMEL and FIRTEL 1982). Most of the fragments generated by these enzymes are outside the range where we can accurately size molecules separated by pulsed-field gel electrophoresis, but subfragments that result from insertion of a plasmid can often be accurately sized. Each insertion introduces closely spaced sites for each of these enzymes and therefore provides a unique position from which we can make several measurements to map the insertion site relative to both individual genes and flanking restriction sites. This redundancy of information overcomes problems in the preparation of very high molecular weight DNA from a large number of marked Dictyostelium strains as well as uncertainties introduced when probes recognize several different loci that contain members of small gene families.

To relate the regional maps to previously established linkage groups, we probed the REMI-RFLP set of strains with genes that had been assigned to one or another of the six linkage groups by parasexual genetics. In this way we were able to determine the linkage groups for about half of the *Apa*I fragments that had been interrupted by inserts (KUSPA and LOOMIS 1994a). We have now extended these analyses to the remaining marked *Apa*I fragments by determining the linkage group assignments on 15 more physically mapped genes using RFLP analyses of haploid segregants derived from the diploids generated in crosses between different isolates from the wild. By integrating the data on the size of individual chromosomes with the regional mapping data we have been able to connect the pieces into maps of the six chromosomes that carry the genes of Dictyostelium.

MATERIALS AND METHODS

DNA probes: The sources of the DNA probes used in this study are described in Table 1 where they are referenced. Probes were prepared as DNA fragments and labeled with [α-^{32}P] dCTP by random primed DNA synthesis (FEINBERG and VOGELSTEIN 1983).

Parasexual RFLP analysis: Procedures for defining RFLPs in independently isolated strains as well as markers defining linkage groups in *Dictyostelium* have been previously described (WELKER *et al.* 1986, 1989; WELKER 1988). Parasexual diploids were formed by fusion of a haploid tester strain (either HU1628 or HU1852) with a haploid wild isolate (either DD61, HU1852, HU188, OHIO, WS380B, WS472, WS576, WS583 or WS1956). A set of haploid segregants was obtained from each diploid population by treatment with thiabendazole and screened for the presence of genetic markers from the tester strain for each of the six known linkage groups. For each diploid, a subset of segregants with different genotypes was selected for RFLP analyses. RFLPs for each probe were identified by comparing Southern blots of restriction enzyme digested DNAs of the haploid tester strains and the wild isolates. DNAs from appropriate sets of haploid segregants were then screened to identify cosegregation of the RFLP markers with the genetic markers derived from the tester strain.

REMI-RFLP analysis: High molecular weight DNA samples prepared from the REMI-RFLP strains were digested with various restriction enzymes and separated by pulsed-field gel electrophoresis using a CHEF DRII apparatus (BioRad, Richmond, CA) and transferred to Magna NT nylon filters (MSI, Westboro, MA) as previously described (KUSPA and LOOMIS 1994a). Hybridization to ^{32}P-labeled DNA probes and size estimation of labelled fragments were also carried out as previously described (KUSPA and LOOMIS 1994a,b). Chromosomes of the yeast strain AB1380 were used as size standards. Fragment sizes recognized by specific probes are available upon request.

Nomenclature: Sites at which the plasmid DIV6 integrated in the REMI-RFLP set of strains are referred to by IS (insertion site) number. In the few cases where a single REMI-RFLP strain carries two insertions, the second insertion site was given another IS name (IS451-IS455).

RESULTS

Parasexual mapping of cloned genes: During normal asexual development of *D. discoideum*, a few of the haploid cells fuse to form stable diploids (LOOMIS 1987). When mixed aggregates are prepared from populations of cells derived from genetically dissimilar strains each carrying a selectable marker, heterozygous diploids can be selected and propagated. When such diploids are grown in the presence of microtubule destabilizing agents such as benlate or thiabendazole, they give rise to haploid progeny with random reassortments of the chromosomes. Cosegregation of markers during this parasexual cycle has been used to assign genes to individual linkage groups (LOOMIS 1987; NEWELL *et al.* 1993). Parasexual mapping can be applied to any cloned gene irrespective of whether mutant alleles generate observable phenotypes since polymorphisms in the surrounding genome can be recognized as RFLPs (WELKER *et al.* 1986). The frequency of finding RFLPs for a given gene is greatly increased when pairs of independent isolates from nature are used in parasexual crosses. Fifteen unique cloned genes have been assigned to specific linkage groups (Table 2). We have

TABLE 1

Mapped loci of *Dictyostelium discoideum*

Locus	Gene product	Chromosome	Reference
abpA	alpha actinin	*1*	NOEGEL *et al.* (1987)
abpB	p30	*3*	FECHHEIMER *et al.* (1991)
abpC	ABP120	*1*	BRINK *et al.* (1990)
abpF	actin binding protein	*6*	J. SPUDICH and K. NIEBLING, unpublished data
acaA	adenylyl cyclase-aggregation	*3*	PITT *et al.* (1992)
acgA	adenylyl cyclase-germination	*5*	PITT *et al.* (1992)
aclA	actin-like protein	*4*	P. MORINDINI and R. KAY, unpublished data
acpA	actin capping protein (cap32)	*1*	HARTMANN *et al.* (1989)
acpB	actin capping protein (cap34)	*2*	HARTMANN *et al.* (1989)
actK	actin	*5*	TITUS *et al.* (1994)
actJ	actin	*5*	TITUS *et al.* (1994)
actM	actin	*5*	TITUS *et al.* (1994)
alfA	alpha fucosidase	*3*	MULLER-TAUBENBERGER *et al.* (1989)
apeA	apurinic endoglycosidase	*2*	FREELAND *et al.* (1995)
arcA	amplified region cobalt resistant	*3*	JENSEN *et al.* (1989)
arfA	ADP ribosylation factor	*2*	C. J. WEIJER, unpublished data
arfC	ADP ribosylation factor	*3*	C. J. WEIJER, unpublished data
arfD	ADP ribosylation factor	*5*	C. J. WEIJER, unpublished data
arfG	ADP ribosylation factor	*3*	C. J. WEIJER, unpublished data
capA	cAMP binding protein	*2*	BAIN *et al.* (1991)
capB	cAMP binding protein	*2*	BAIN, GRANT and TSANG (1991)
carA	cAMP receptor 1	*2*	SAXE *et al.* (1991)
carB	cAMP receptor 2	*5*	SAXE *et al.* (1993)
carC	cAMP receptor 3	*3*	JOHNSON *et al.* (1993)
carD	cAMP receptor 4	*3*	LOUIS *et al.* (1994)
casK	casein kinase II	*5*	C. WEIJER, unpublished data
cdcB	CDC2	*2*	MICHAELIS and WEEKS (1992)
cdcC	CDC2 kinase	*4*	C. MICHAELIS, C. LUO and G. WEEKS, unpublished data
celA	cellulase (270-6)	*4*	GIORDA *et al.* (1990)
celB	cellulase (270-11)	*5*	BLUME and ENNIS (1991)
chcA	clathrin heavy chain	*2*	O'HALLORAN and ANDERSON (1992)
cigA	cAMP inducible 95 kd (BP74)	*5*	HOPKINSON *et al.* (1989)
cinA	cycloheximide induced	*5*	SINGLETON *et al.* (1988)
cmfA	conditioned media factor	*2*	JAIN *et al.* (1992)
cotA	spore coat 96	*2*	FOSNAUGH and LOOMIS (1989a)
cotB	spore coate 70	*2*	FOSNAUGH and LOOMIS (1989b)
cotC	spore coat 60	*2*	FOSNAUGH and LOOMIS (1989b)
cprA	CP1 protease	*5*	WILLIAMS *et al.* (1985)
cprB	CP2 protease	*3*	PEARS *et al.* (1985)
cprD	cysteine protease 4	*3*	G. SOUZA and H. FREEZE, unpublished
cprE	cysteine protease 5	*2*	H. FREEZE, unpublished data
crpA	CDC2 related protein	*5*	MICHAELIS and WEEKS (1993)
csaA	gp80	*5*	NOEGEL *et al.* (1986)
csbA	gp24	*2*	LOOMIS and FULLER (1990)
ctpS	CTP synthetase	*3*	A. DE LOZANNE, unpublished data
cysA	cystathionine gamma lyase	*1*	G. SHAULSKY and W. LOOMIS, unpublished data
dagA	CRAC	*4*	INSALL *et al.* (1994)
dagB	protein kinase	*4*	A. KUSPA, unpublished data
dhcA	Dynein heavy chain	*2*	KOONCE *et al.* (1992)
dhkA	histidine kinase	*6*	N. WANG and W. F. LOOMIS, unpublished data
dicA	dynein intermediate chain	*3*	R. CHISHOLM, unpublished data
DIRS	inverted repeat sequence	*2–6*	CAPPELLO *et al.* (1985)
dpyK2	tyrosine kinase	*4*	TAN and SPUDICH (1990)
dscA	discoidin	*2*	ROWEKAMP *et al.* (1980)
ecmA	ST430	*3*	MCROBBIE *et al.* (1988)
ecmB	ST310	*2*	WILLIAMS *et al.* (1987)
efaA	EF1alpha (apb50)	*1*	YANG *et al.* (1990)
erkB	extracellular response kinase 2	*4*	SEGALL *et al.* (1995)
fpaA	fucose protein	*2*	KOZAROV *et al.* (1995)
fusB	sexual fusion (gp138B)	*5*	FANG *et al.* (1993)

TABLE 1

Continued

Locus	Gene product	Chromosome	Reference
gbfA	G-box binding factor	*5*	SCHNITZLER *et al.* (1994)
gerD	germination protein (270G)	*6*	GIORDA *et al.* (1990)
gluA	beta glucosidase	*6*	BUSH *et al.* (1994)
gpaA	G-alpha 1	*4*	LILLY *et al.* (1993)
gpaB	G-alpha 2	*2*	KUMAGAI *et al.* (1989)
gpaD	G-alpha 4	*4*	HADWIGER *et al.* (1991)
gpaE	G-alpha 5	*4*	WU and DEVREOTES (1991)
gpaF	G-alpha 6	*4*	WU and DEVREOTES (1991)
gpaG	G-alpha 7	*3*	WU and DEVREOTES (1991)
gpaH	G-alpha 8	*4*	WU and DEVREOTES (1991)
gpbA	G-beta	*2*	LILLY *et al.* (1993)
guaA	GMP synthetase	*3*	CAMPAGNE *et al.* (1991)
gufB	gene unknown function (BJ22)	*3*	R. KESSIN, unpublished data
hatA	histactophilin	*1*	SCHEEL *et al.* (1989)
helA	helicase	*6*	MAHAL and NELLEN (1994)
hmgA	hmg CoA reductase A	*1*	A. DE LOZANNE, unpublished data
hmgB	hmh CoA reductase B	*2*	A. DE LOZANNE, unpublished data
hspA	heat shock 60	*5*	A. DE LOZANNE, unpublished data
hspB	heat shock 70	*1*	R. EDDY and J. CONDEELIS, unpublished data
ksnD	kinesin 4	*4*	G. MCCAFFREY and R. VALE, unpublished data
ksnH	kinesin 8	*4*	G. MCCAFFREY and R. VALE, unpublished data
lagC	signal protein	*3*	DYNES *et al.* (1994)
manA	alpha mannosidase	*6*	SCHATZLE *et al.* (1991)
mhcA	myosin heavy chain	*4*	WARRICK *et al.* (1986)
mlcE	myosin light chain	*2, 3*	CHISHOLM *et al.* (1988)
mlkA	myosin light chain kinase	*3*	RAVID and SPUDICH (1992)
mvpA	vault protein	*1*	VASU (1993)
mvpB	vault protein	*5*	VASU *et al.* (1993)
myoA	myosin IA	*3*	JUNG *et al.* (1989)
myoB	myosin IB	*5*	TITUS *et al.* (1989)
myoC	myosin IC	*2*	TITUS *et al.* (1989)
myoD	myosin ID	*2*	JUNG *et al.* (1993)
myoE	myosin IE	*5*	HAMMER (1991)
myoF	myosin IF	*5*	TITUS *et al.* (1994)
myoG	myosin IG	*2*	TITUS *et al.* (1994)
myoH	myosin IH	*5*	TITUS *et al.* (1994)
myoI	myosin II	*5*	TITUS *et al.* (1994)
myoJ	myosin IJ	*2*	TITUS *et al.* (1994)
myoK	myosin IK	*5*	TITUS *et al.* (1994)
myoL	myosin IL	*3*	TITUS *et al.* (1994)
nagA	N-acetylglucosaminidase	*4*	GRAHAM *et al.* (1988)
ndKa	nucleotide diphosphate kinase	*3*	WALLET *et al.* (1990)
ndkB	nucleotide diphosphate kinase	*2*	WALLET *et al.* (1990)
nxnA	annexin	*1*	GREENWOOD and TSANG (1991)
pegA	prestalk enriched gene (D11)	*3*	BARKLIS *et al.* (1985b)
pdhA	pyruvate dehydrogenase	*6*	A. DE LOZANNE, unpublished data
pdiA	PDE inhibitor	*3*	FRANKE *et al.* (1991)
pdsA	cAMP phosphodiesterase	*4*	PODGORSKI *et al.* (1988)
pkaC	protein kinase A catalytic	*4*	MANN *et al.* (1992)
pkaR	protein kinase A regulatory	*3*	VERON *et al.* (1988)
pkeA	protein kinase	*5*	J. DYNES, and R. FIRTEL, unpublished data
pkeB	protein kinase	*4*	J. DYNES and R. FIRTEL
pkfA	protein kinase	*5*	J. WILLIAMS, unpublished data
pkfB	protein kinase DK3	*1*	J. WILLIAMS, unpublished data
ppiA	cyclophilin	*1*	BARISIC *et al.* (1991)
pspA	prespore D19	*1*	EARLY *et al.* (1988)
pspB	prespore 14E6	*2*	POWELL-COFFMAN and FIRTEL (1994)
pspD	prespore PL3	*2*	YODER *et al.* (1994)
pspK	prepore (1F)	*5*	CORNEY *et al.* (1990)
psvA	prespore EB4	*2*	BARKLIS *et al.* (1985a)

TABLE 1

Continued

Locus	Gene product	Chromosome	Reference
ptpA	phosphotyrosine phosphatase	*2*	HOWARD *et al.* (1992)
ptpB	phosphotyrosine phosphatase	*3*	HOWARD *et al.* (1992)
ptpD	protein phosphatase (pp2A)	*5*	HARIBABU and DOTTIN (1991)
pyr5-6	UMP-synthetase	*3*	JACQUET *et al. (1988)*
rapA	rap related protein	*6*	ROBBINS *et al.* (1990)
rasB	ras homolog blue	*4*	DANIEL *et al.* (1994)
rasD	ras homolog development	*6*	REYMOND *et al.* (1984)
rasS	ras homolog S3	*4*	DANIEL *et al.* (1994)
rgcA	random genomic clone (G134)	*6*	A. KUSPA and W. F. LOOMIS, unpublished data
rpgA	ribosomal protein (V1)	*5*	SINGLETON 1989
rpgC	ribosomal protein gene (V18)	*3*	SINGLETON *et al.* (1989)
rpgE	ribosomal protein gene (p17)	*2*	SZYMKOWSKI and DEERING (1990)
sevA	severin	*5*	ANDRE *et al.* (1988)
spiA	spore coat protein	*5*	RICHARDSON *et al.* (1991)
splA	tyrosine kinase	*4*	TAN SPUDICH (1990)
tagB	serine proteae/MDR	*4*	SHAULKSY *et al.* (1995)
tfdA	transcription factor TFIID	*2*	H. ENNIS, unpublished data
tipA	aggregation gene	*3*	J. STEGE and W. LOOMIS, unpublished data
thyA	thymidine growth	*3*	DYNES and DIRTEL (1989)
topA	topoisomerase II	*3*	Y. TANAKA, unpublished data
tsuA	tsuanmi-cAMP relay	*1*	P. DEVREOTES, unpublished data
tubB	beta tubulin	*1*	TRIVINOSLAGOS *et al.* (1993)
ubqA	ubiquitin	*5*	OHMACHI *et al.* (1989)
uglA	uracil glycosylase	*3*	R. B. GUYER, T. M. FREELAND and R. A. DEERING, unpublished data
vatM	vacuolar ATPase subunit	*6*	M. CLARKE, unpublished
vseB	vegetative specific expression	*6*	SINGLETON *et al.* (1989)
wacA	water channel	*3*	G. SHAULSKY and W. LOOMIS, unpublished data

now extended these studies to map an additional 23 genes that provide anchors for assigning previously described *Apa*I fragments (KUSPA and LOOMIS 1994a) to specific linkage groups.

When a given enzyme was found to generate distinguishable bands that could be assigned to different strains, it was used to digest DNA from haploid segregants derived from the appropriate diploid strain. The NC4 derived parental strains HU1628 and HU1852 used in these crosses carry genetic markers that allow most of the linkage groups to be distinguished among the different haploid segregants (WELKER *et al.* 1986). Linkage groups III and VI can be distinguished from other linkage groups but not from each other. When the RFLPs of a gene segregate with III/VI markers, the locus is assigned to either of these groups (Table 2). All other segregation patterns allowed us to make unique assignments for the individual loci (Table 2). All five patterns of segregation were found thereby adding anchors to each chromosome.

We chose to parasexually map those genes that had been previously shown to lie within an *Apa*I fragment marked with an inserted plasmid in one or more of the REMI-RFLP set of strains (KUSPA and LOOMIS 1992). The results allowed us to assign each of the regions flanked with *Apa*I sites to specific linkage groups. Together these regions represent more than half of the Dictyostelium genome and significantly constrain the number of possible arrangements of the genes. The next step was to determine their relative order and the distances to the ends of the chromosomes.

Long-range REMI-RFLP mapping: Because the plasmid in the REMI-RFLP set of strains carries sites recognized by *Apa*I, *Not*I, *Sma*I, and *Sst*II in the multiple cloning region that separates the *pyr5-6* from the pGEM sequences, we could digest high molecular weight DNA with these enzymes and probe large fragments separated by pulsed-field electrophoresis with vector sequences to determine the distance to flanking restriction sites on either side. We have more than 100 REMI-RFLP strains in which a single copy of the plasmid was inserted randomly in the genome thereby providing a unique set of marker sites. The sequences of each of these restriction sites contain only guanine and cytosine, which are underrepresented in Dictyostelium DNA and so are rare in the genome. *Not*I recognizes an 8-base sequence that is only expected to occur once or twice in the 40 mb genome. In fact, we found that the *Not*I fragments generated from DNA of wild-type and most REMI-RFLP strains were beyond the resolution of our pulsed-field gels, being >1.6 mb. However, certain of our REMI-RFLP strains gave smaller *Not*I frag-

TABLE 2

Assignment of loci to linkage groups

Locus	Wild isolate	Enzyme/other	Linkage group	Reference
abpA	AX2	parasexual	I	WALLRAFF *et al.* (1986)
abpC	AX2	parasexual	I	BRINK *et al.* (1990)
apeA	WS380B	*Nsi*I	II	This work
arcA	WS583	*Bgl*II	III/VI	JENSEN *et al.* (1989)
carA	HU182	*Eco*RI	II	This work
cigA	DD61	*Xba*I	VII	This work
cprA	WS380B	*Hin*dIII	VII	This work
cprB	AX3	chromo blots		COX *et al.* (1990)
csaA	AX2	parasexual	VII	E. WALLRAFF, unpublished data
csbA	WS583	*Eco*RI	II	This work
dhkA	WS380B	*Xba*I	III/VI	This work
dscA	OHIO	*Eco*RI	II	WELKER (1988)
fpaA	WS380B	*Rsa*I	II	This work
gbfA	WS583	*Hin*dIII	VII	This work
gluA	AX3	parasexual	VI	LOOMIS (1980)
gpaA	OHIO	*Hin*dIII	IV	This work
gpaB	OHIO	*Hin*dIII	II	This work
gpaD	WS380B	*Hin*fI	IV	This work
gpbA	AX3K	*Eco*RI		LILLY *et al.* (1993)
lagC	WS583	*Eco*RI	III/VI	This work
manA	AX3	parasexual	VI	FREE *et al.* (1976)
mhcA	DD61	*Hin*dIII	IV	WELKER *et al.* (1989)
mlcE	OHIO	*Hin*dIII	III/VI	This work
mvpA	WS380B	*Eco*RI	I	This work
mvpB	WS472	*Taq*I	VII	This work
nagA	AX3	parasexual	IV	LOOMIS (1978)
pegA	OHIO	*Eco*RI	II	This work
pkaR	AX3	parasexual	III	ABE and YANAGISAWA (1983)
pkeA	WS380B	*Bst*BI	VII	This work
pdiA	WS472	*Hin*dIII	III/VI	This work
pdsA	AX2	parasexual	IV	BARRA *et al.* (1980)
pspA	AX3	parasexual	I	GRANT *et al.* (1985)
psvA	AX2	parasexual	II	E. WALLRAFF, unpublished
pyr5-6	WS380B	*Xba*I	III/VI	This work
rasB	WS583	*Taq*I	IV	This work
sevA	AX2	parasexual	VII	ANDRE *et al.* (1989)
tipA	WS380B	*Xba*I	III/VI	This work
thyA	WS472	*Eco*RI	III/VI	This work
tubB	WS380B	*Bcl*I	I	This work

ments and so must carry inserts near internal *Not*I sites or be positioned near the end of a chromosome. The ends of chromosomes were recognized when two or more of the rare cutting enzymes generated identical fragments to one side of an insertion site. Internal *Not*I sites were recognized when they were flanked by independent insertions and fell within fragments generated by other rare-cutting enzymes. Internal *Not*I sites were found on chromosomes *1* and *6* and provided convenient positions defining the order of genes over several megabases on these chromosomes.

Fragments generated by *Sst*II and *Sma*I could often be accurately sized after probing with various genes even when the region was not disrupted by an inserted plasmid. These results provided long-range information around each locus thereby further constraining the number of possible arrangements. However, it was the data from the insertions in the REMI-RFLP set of strains that provided the detailed information necessary to unequivocally recognize adjacent regions. Inserts that fell between different rare restriction sites affected genes to either side that would not have been seen to be linked when analyzing fragments generated by a single enzyme. In some cases, evidence for linkage was not found among the digests with *Apa*I, *Sma*I, *Sst*II, or *Not*I, and we turned to another rare-cutting restriction enzyme, *Bgl*I, which recognizes a site in the ampicilin resistance gene of the inserted plasmid. Because rare restriction sites are randomly distributed in the genome, we often found *Bgl*I would provide the necessary data when the other enzymes had failed.

Although the validity of each map depends primarily on the internal consistancy of the restriction maps defined by the gene probes and the REMI-RFLP data,

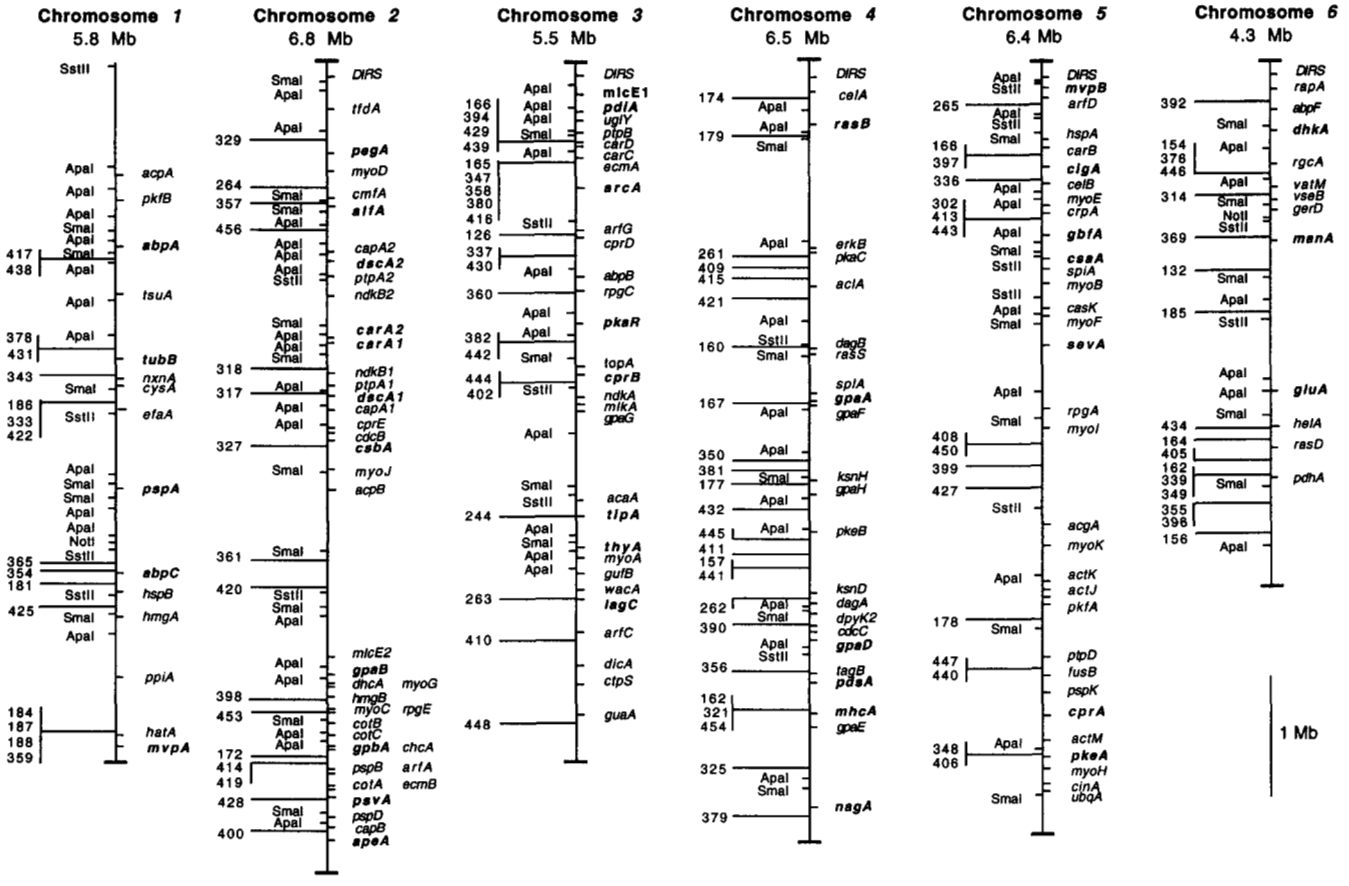

FIGURE 1.—Integrated maps of the Dictyostelium chromosomes. Loci defined in Table 1 are positioned relative to rare restriction sites and the observed ends of chromosomes, which are indicated by bars. Genes that have been parasexually mapped to a specific linkage group (Table 2) are indicated in bold. Linkage group VII is presented as chromosome *5*. Sites at which the DIV-6 plasmid integrated in individual strains of the REMI-RFLP set (*eg.*, 438) are indicated. Insertions at *Bam*HI sites separated by <100 kb are grouped. The positions of rare restriction sites are shown. Horizontal bars at the ends of chromosomes indicate that two or more rare cutting enzymes generated fragments that end at those positions.

the process of discovery of the arrangements was often complex and dependent on integrating results from diverse physical mapping techniques. *Apa*I fragments in the REMI-RFLP set of strains representing more than half of the genome have been previously described (KUSPA and LOOMIS 1994a). Moreover, we have presented detailed maps as well as contigs of large cloned regions carried in YAC vectors surrounding the dozen myosin genes (TITUS *et al.* 1994), *erkB* (SEGALL *et al.* 1995), *dagA* (INSALL *et al.* 1994), as well as various portions of each of the six chromosomes (KUSPA *et al.* 1992). This approach to whole genome physical mapping is dependent on the availability of probes for each region of about a megabase. We have used several hundred probes and mapped more than 150 genes using this approach (Table 1; Figure 1). By correlating the size of well-represented regions of these chromosomes with the estimated size of the intact chromosomes (COX *et al.* 1990), we were able to construct maps for the six chromosomes as well as position the loci to within 40 kb (Figure 1). For historical reasons, linkage group V has not been represented until recently and no cloned genes are available (LOOMIS 1987; KUSPA and LOOMIS 1992; DARCY *et al.* 1993). Because we did not find any of the cloned genes to define a linkage group other than the six established ones, we felt it best to relate chromosome *5* to linkage group VII to keep the changes to a minimum but still not to leave a numerical gap. Therefore, the genes on linkage group VII are positioned on chromosome *5* and no chromosome *7* is defined.

Confirmation: In all cases, the maps confirmed linkage of the anchor loci that were parasexually assigned to specific linkage groups. Parasexual assignment of genes to specific linkage groups reduced the number of possible arrangements of large mapped regions and provided necessary clues for solving the integrated maps. Chromosome assigned loci are found about every 1–2 mb along each of the chromosomes lending confidence to the chromosome assignments of other genes in the region. Confirmation of the maps was provided by subsequent parasexual mapping of several genes that we had mapped to specific positions on one or another of the six chromosomes. The linkage group assignments for these genes, *apeA, mlcE1, pdsA, alfA, dhkA,* and *cigA,* in every case conformed to the predictions

derived from the physical maps. We also determined the size of several restriction fragments that were predicted by the completed maps; in every case they supported the arrangement of restriction sites, loci and insertion sites. Thus, we feel that the physical maps of the Dictyostelium genome (Figure 1) provide a reliable representation of the linkage groups as well as the detailed structure of the chromosomes.

DISCUSSION

The congruence of restriction maps based on probing DNA digests from both wild-type and the REMI-RFLP set of strains with cloned genes together with the direct measurement of the distance to several different restriction sites on either side of more than 100 insertions has generated a consistent map of the six chromosomes that carry the genes of *Dictyostelium.* While the maps are linear, they are based on higher order integrations of independent data sets that are consistent with few other solutions. For instance, genes that are contiguous to insertion sites must be in the same sized *Sma*I fragment as the insertions unless a *Sma*I site intervenes. The same is true for fragment sizes generated by digestion with other restriction enzymes. Inconsistencies in some of the earlier arrangement of sites and loci indicated erroneous ordering and prompted a reassessment of the maps. The final maps are the result of several rounds of such analyses and so can be taken with a high degree of confidence.

The degree of uncertainty in map positions varies from one region to another but is never more than a few hundred kilobases. In some areas, where the density of restriction sites and insertions is high, loci can be confidently positioned within 10 kb. The average resolution is estimated to be ±40 kb or ~0.1% of the genome. The size of the individual chromosomes was determined by adding up the sizes of linked regions and so is subject to additive errors. However, the estimates can be confidently accepted with an error of a few hundred kilobases. The total size of the genome, estimated from our physical maps, is 35 mb, which agrees well with previous estimations of 34–40 mb using a variety of approaches (FIRTEL and BONNER 1972; COX *et al.* 1990).

Intact Dictyostelium chromosomes have been compared with *Schizosaccharomyces pombe* chromosomes following pulsed-field gel electrophoresis using conditions to maximize migration of very large molecules (COX *et al.* 1990). Chromosome *2* is the largest and is beyond the range where its size can be accurately measured by pulsed-field gel analyses. Our map suggests that this chromosome is 6.8 mb. Chromosomes *4* and *5* appear to be >6 mb while the other chromosomes are smaller in agreement with our maps. The additive size of our maps indicate a consistent over-estimation in the pulse-field gel electrophoretic studies of whole chromosomes. Because size measurements are more accurate for fragments of <1 mb, we feel that our chromosome size estimates are likely to be more accurate than sizes obtained from pulsed-field gels of intact chromosomes.

While we have mapped only a few percent of the Dictyostelium genes, we can already see some patterns in the maps that shed light on the genetic history of this organism. A megabase region near the center of chromosome *2* underwent an inverted duplication at about the time that the progenitor of our mapping strain was isolated from the wild-strain NC-4 (KUSPA *et al.* 1992). We have mapped five genes to this region, which is likely to include at least a hundred more genes. However, there does not seem to be any serious consequences to the duplication because strains carrying the duplication such as our mapping strain, AX4, grow and develop in a manner indistinguishable from that of a related strain, AX2, which did not suffer this duplication. Further down on chromosome *2* there is a cluster of related genes that encode the proteins found in the spore coats and surrounding matrix of the sorus. The primary sequences of the predicted products of these genes, *cotA, cotB, cotC, pspB,* and *pspD,* are all related and show evidence for duplication and divergence from a common ancestoral gene (FOSNAUGH and LOOMIS 1989a,b; POWELL-COFFMAN and FIRTEL 1994; YODER *et al.* 1994). The fact that they are clustered within a megabase indicates that the duplications occurred in the local chromosomal vicinity. On chromosome *3* we find two genes encoding surface cAMP receptors, *carC* and *carD,* tightly linked within 40 kb. Although these genes are expressed at different stages during development of Dictyostelium, they are likely to have arisen from a duplication earlier in evolution. Chromosome *4* carries five of the eight genes encoding small GTP binding proteins; *gpaA* and *gpaF* are within 40 kb of each other, while *gpaD, gpaE,* and *gpaH* are spread over the bottom half of chromosome *4,* indicating that the initial divergence was followed by subsequence rounds of duplication and divergence. This chromosome also carries two ras-related genes, *rasB* and *rasS,* and two kinesin related genes, *ksnD* and *ksnH.* The most striking clustering is found among the family of myosin I genes; six of the 12 myosin I genes are found on chromosome *5.* All but *myoE* are linked to pairs of actin genes (TITUS *et al.* 1994), suggesting that the original duplicated unit included a myosin I gene and a pair of actin genes.

Another multigene family, DIRS, has an interesting arrangement on the chromosomes. There are ~40 copies of this element in the genome (CAPPELLO *et al.* 1985). By analyzing YAC clones that carry DIRS elements, we have been able to show that they fall into seven clusters with five to seven members each (A. KUSPA and W. F. LOOMIS, unpublished data). Six of the clusters carry an intact DIRS element that can be recognized as a 4.2-kb fragment in *Eco*RI digests. The intact element encodes a product related to reverse

transcriptase indicating the possible retroviral origin of these elements (CAPPELLO *et al.* 1985). The seventh cluster does not contain an intact element and is smaller than the others. It has been mapped between *rasB* and *erkB* on chromosome *4*. The other DIRS clusters that we have mapped all lie at the extremities of chromosomes *3, 4, 5,* and *6* (Figure 1). Because centromeres are often associated with repeated elements and the Dictyostelium chromosomes all appear to be telocentric, it is possible that DIRS is centromere associated. We would then expect chromosomes *1* and *2* to carry DIRS clusters as well. Although we do not have REMI-RFLP or YAC contig evidence directly indicating the presence of DIRS clusters near the ends of chromosomes *1* or *2*, the long-range restriction maps that we have been able to generate from the YAC contigs carrying DIRS suggest that each of these chromosomes carries a DIRS cluster at the upper end of the maps shown in Figure 1 (unpublished data). Moreover, *Apa*I digests of total genomic DNA show six distinct fragments recognized by DIRS. Probing a blot of intact chromosomes separated by pulsed-field electrophoresis with DIRS showed that each of the bands including the largest, chromosome *2*, carries multiple DIRS elements. However, direct genetic determination of DIRS-dependent mitotic stability will be required before we can confidently assign function to these regions.

Maps of the six chromosomes that carry the genes of Dictyostelium provide a convenient way to identify genes that have already been cloned as well as newly isolated genes. They are also useful for recognizing new members of multigene families. When interacting genes are found to map to different chromosomes, parasexual reassortment of chromosomes can be used to generate double mutants. The maps provide a framework for positioning contigs built up from YAC clones such that gaps as well as juxtaposed contigs that do not happen to have an overlapping probe can be recognized. At present our YAC contigs cover ~85% of the genome and increase the accuracy of positioning loci by a factor of two (A. KUSPA and W. F. LOOMIS, unpublished data). Maps based solely on YAC contigs confirm many regions of the genome. Ultimate refinement of the maps will come when the nucleotide sequence of the complete genome is determined.

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