Mapping Flagellar Genes in Chlamydomonas Using Restriction Fragment Length Polymorphisms

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ABSTRACT

To correlate cloned nuclear DNA sequences with previously characterized mutations in Chlamydomonas and, to gain insight into the organization of its nuclear genome, we have begun to map molecular markers using restriction fragment length polymorphisms (RFLPs). A *Chlamydomonas reinhardtii* strain (CC-29) containing phenotypic markers on nine of the 19 linkage groups was crossed to the interfertile species *Chlamydomonas smithii*. DNA from each member of 22 randomly selected tetrads was analyzed for the segregation of RFLPs associated with cloned genes detected by hybridization with radioactive DNA probes. The current set of markers allows the detection of linkage to new molecular markers over approximately 54% of the existing genetic map. This study focused on mapping cloned flagellar genes and genes whose transcripts accumulate after deflagellation. Twelve different molecular clones have been assigned to seven linkage groups. The α-1 tubulin gene maps to linkage group *III* and is linked to the genomic sequence homologous to pcf6-100, a cDNA clone whose corresponding transcript accumulates after deflagellation. The α-2 tubulin gene maps to linkage group *IV*. The two β-tubulin genes are linked, with the β-1 gene being approximately 12 cM more distal from the centromere than the β-2 gene. A clone corresponding to a 73-kD dynein protein maps to the opposite arm of the same linkage group. The gene corresponding to the cDNA clone pcf6-187, whose mRNA accumulates after deflagellation, maps very close to the tightly linked *pf-26* and *pf-1* mutations on linkage group *V*.

GENETIC mapping has recently become much more powerful by the use of restriction fragment length polymorphisms (RFLPs) to follow the segregation of particular DNA fragments in the genome (Botstein *et al.* 1980). Segregation of many RFLPs can be followed in a single cross, overcoming the practical limitation of traditional genetic approaches in which only a small number of mutant phenotypes may be scored. Extensive genetic maps have been constructed using RFLPs in a number of organisms including tomato (BERNATZKY and TANKSLEY 1986), maize (HELENTJARIS *et al.* 1986; BURR *et al.* 1988), lettuce (LANDRY *et al.* 1987), and human (DONIS-KELLER *et al.* 1987). In Chlamydomonas, RFLP analysis has been used to study the inheritance of DNA from the chloroplast and mitochondrial genomes (METS 1980; LEMIEUX, TURMEL and LEE 1980; BOYNTON *et al.* 1987) and to demonstrate linkage of a cloned gene to the nuclear mating type locus (FERRIS and GOODENOUGH 1987). We are using RFLP analysis to map cloned nuclear sequences throughout the *Chlamydomonas reinhardtii* genome, beginning with cloned flagellar genes and genes whose mRNAs accumulate after deflagellation (SILFLOW and ROSENBAUM 1981; SCHLOSS, SILFLOW and ROSENBAUM 1984; YOUNGBLOM, SCHLOSS and SILFLOW 1984; SILFLOW *et al.* 1985; WILLIAMS, MITCHELL and ROSENBAUM 1986).

The ability to recover all of the products of a meiosis and to follow the segregation of markers in complete tetrads is a major advantage of Chlamydomonas. Tetrad analysis enables the determination of the frequency of recombination, and thus the map distance, between a marker and its centromere. Centromeres of the 19 different linkage groups add an additional 19 markers to the 13 phenotypic and molecular markers used in this study. When linkage between a known marker and a new molecular marker is detected, the new marker can often be mapped to the right or left arm and distal or proximal the centromere relative to the established marker. In cases where linkage between a known marker and an unknown molecular marker is not detectable, an estimate of the centromere distance of the unknown marker can still be determined. Tetrad analysis also allows a direct examination of the distribution of crossovers between the four chromatids, enabling estimates of both chromatid interference and gene conversion. The data are interlocking and thus increase the confidence with which judgments can be made about the segregation of markers.

Chlamydomonas is an ideal organism for the combined biochemical and genetic analysis of eukaryotic flagella (LUCK 1984; HUANG 1986; LEFEBVRE and ROSENBAUM 1986). More than 50 independent mutant loci affecting flagellar assembly and function have been described in this organism, including many mutations which produce paralyzed flagella or abnormal swimming patterns. *C. reinhardtii* has also been used as a model system for studying the expression of genes coding for flagellar proteins. When the two flagella are removed, they regenerate synchronously, reaching full length within 1–2 hr (ROSENBAUM, MOULDER and RINGO 1969). Of the estimated 200 polypeptides in the flagellum (LUCK 1984), the synthesis of at least 30–50 proteins, including the tubulins, the dyneins and the radial spoke proteins, has been shown to increase after deflagellation (WEEKS and COLLIS 1976; LEFEBVRE *et al.* 1978; REMILLARD and WITMAN 1982). Post-deflagellation increases in the levels of flagellar protein mRNAs have facilitated the isolation of cDNA clones corresponding to α- and β-tubulin and other flagellar proteins (SILFLOW and ROSENBAUM 1981; MINAMI *et al.* 1981; SCHLOSS, SILFLOW and ROSENBAUM 1984).

While Chlamydomonas is an excellent organism in which to study both the regulation of gene expression and flagella assembly and structure, there has been little information relating the molecular studies to the genetic analysis of flagellar mutants. One solution, illustrated by the work of WILLIAMS, MITCHELL and ROSENBAUM (1986), is to prepare antibodies to flagellar proteins and to isolate the corresponding genes from expression libraries. Another approach is to correlate the map positions of cloned flagellar genes with known flagellar mutations on the genetic map. Our results show that RFLP mapping in Chlamydomonas can greatly facilitate such correlations.

To date, we have assigned twelve molecular clones to seven different linkage groups. The α-1 tubulin gene maps to linkage group *III* and the α-2 tubulin gene to linkage group *IV*. We have also shown that the two β-tubulin genes and a gene encoding the 73kd dynein protein found in the outer dynein arm are linked. Although these genes did not map to any of the linkage groups which were marked in our study, we place them all on linkage group *XII* because β-tubulin mutants have been mapped recently to the right arm of linkage group *XII* (BOLDUC, LEE and HUANG, 1988). Some of the clones we have mapped may correspond to, or at least map very close to, previously characterized flagellar mutations.

MATERIALS AND METHODS

Strains: The *C. reinhardtii* strains (CC-29, CC-410 and CC-1418) as well as *C. smithii* (CC-1373) were obtained from ELIZABETH HARRIS at the Chlamydomonas Genetics Center, Department of Botany, Duke University, Durham, North Carolina 27706 (genetics center numbers are indicated in parentheses). The *C. reinhardtii* strain (CC-29) containing multiple genetic markers (*msr-1, ac-17, nit-2, pyr-1, act-2, mt⁻, can-1, sr-1, nit-1, nic-13, pf-2* and *y-1*) was constructed by SMYTH, MARTINEK and EBERSOLD (1975) from mutants which were isolated from the laboratory strain 137c. *C. reinhardtii* strains CC-410 and CC-1418 were collected from the Caroline Islands and Florida respectively. The *C. reinhardtii* strains containing *nit-1* (305^- and $305d^+$) and *nit-2* (203^- and $203d^+$) markers were obtained from EMILIO FERNÁNDEZ (FERNÁNDEZ and MATAGNE 1984). *C. smithii* was isolated by GILBERT M. SMITH from a sample collected near South Deerfield, Massachusetts (HOSHAW and ETTL 1966).

Media: Vegetative cells were grown under continuous light, approximately 4000 lux, in either liquid TAP medium (GORMAN and LEVINE 1965) containing 2 μg/ml nicotinamide, or on solid TAP + nicotinamide medium containing appropriate amounts of GIBCO agar. Progeny of the *C. reinhardtii* × *C. smithii* cross were stored in stabs containing TAP medium, 2 μg/ml nicotinamide, 1% GIBCO agar, and 4 g/liter Difco yeast extract. The *C. reinhardtii* × *C. smithii* crosses were plated and germinated on minimal medium I (SAGER and GRANICK 1953), supplemented with 22 mM sodium acetate and 2 μg/ml nicotinamide.

Genetic analysis: Crosses, maturation of zygotes, and tetrad analysis were carried out as described by LEVINE and EBERSOLD (1960) except that the cells were allowed to mate for 16 h due to the inefficiency of the mating reaction between *C. reinhardtii* and *C. smithii*. If linkage between two markers was detected by high ratios of parental ditype (PD) to nonparental ditype (NPD) tetrads according to PERKINS (1953), the map distance between those markers was estimated using the formula 1/2 T + 3NPD/(PD + NPD + T) where PD, NPD and T equal the number of parental ditype, nonparental ditype and tetratype (T) tetrads, respectively. Centromere distances of phenotypic and molecular markers were approximated by comparing their segregation with the segregation of the centromere-linked marker *y-1* using the equation 1/2 T/(PD + NPD + T) (GOWANS 1965).

Scoring progeny for genetic markers: Drug-resistance markers were scored on solid medium containing drug concentrations lethal to both *C. smithii* and wild-type *C. reinhardtii*: *msr-1* was resistant to 500 μg/ml methionine sulfoximine, *pyr-1* and *act-2* grew on 10 μg/ml pyrithiamine and cycloheximide, respectively, and *sr-1* on 20 μg/ml streptomycin. All drugs were purchased from Sigma Chemical Company (St. Louis, Missouri).

The *ac-17* (acetate requiring) and the *nic-13* (nicotinamide requiring) mutants were scored by their inability to grow in the absence of acetate and nicotinamide respectively. The *pf-2* (paralyzed flagella) marker was most easily scored by inoculating cells from solid medium into sterile water, exposing them to light for two hrs, and then examining the cells for motility using a phase contrast microscope. The *y-1* mutant was scored by its yellow color after 5–7 days of growth on solid medium (TAP + nicotinamide) in the dark. Mating type (*mt*) was scored by test crosses with mt^+ and mt^- wild-type strains; the presence or absence of zygotes was monitored to determine which mating reactions had been successful.

Two unlinked, recessive nitrate reductase mutations (*nit-1* and *nit-2*) in CC-29 (derived from the *C. reinhardtii* 137c wild-type strain) alone or together prevent growth on medium containing nitrate as the sole nitrogen source. *C. smithii* is $nit\text{-}1^+$ and $nit\text{-}2^+$. Each of the tetrad progeny unable to grow on nitrate as the sole nitrogen source was assayed for one or both of the *nit* mutations by separate test crosses.

DNA Isolation: Cells of individual tetrad products were

grown in 1600 ml of liquid TAP medium, harvested by centrifugation at late log phase, washed once in 1× SSC (0.15 M NaCl, 0.015 M Na-citrate, pH 7.0), and frozen as pellets in liquid nitrogen. DNA was isolated using a modification of the method of CHIANG and SUEOKA (1967). The volume of packed frozen cells was brought to 5 ml with 1× SSC, 3 ml of a solution containing 1× SSC and 50% sucrose added, and the pellet thawed on ice. Cells were lysed for 2 min on ice by the addition of 2 ml of 25% Sarkosyl and gentle mixing. Samples were diluted by adding 9 ml of 1× SSC, and digested for 30 min at 37° with 0.12 mg/ml α-amylase as well as 0.2 mg/ml RNase A and 20 units/ml T1 RNase (RNase stock solution was boiled 10 min before addition), 0.05 mg/ml of proteinase K was then added and the samples incubated for an additional 30 min at 37°. Samples were occasionally mixed during both incubations. The samples were then extracted with an equal volume of phenol (saturated with 1× SSC) for 30 min at 25°. After centrifugation of samples to separate the phases, the aqueous phase was removed, extracted with an equal volume of chloroform (saturated with 1× SSC), and DNA precipitated by adding 1/10 volume of 3 M Na-acetate (pH 6.5) and 2/3 volume of 25° isopropanol. DNA was recovered by spooling onto a glass rod and was then resuspended in 10 ml TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The samples were gently shaken overnight to resuspend the DNA, and then redigested with α-amylase and proteinase K and reextracted as described above. After precipitating and spooling the DNA a second time, the samples were resuspended in a small volume (0.5–2.0 ml) of TE. DNA which could not be spooled was recovered by centrifugation, washed with 70% ethanol, and resuspended as described above.

Restriction enzyme digestion and gel electrophoresis: Restriction enzymes were obtained from IBI or Promega Biotec and used according to supplier recommendations. DNA was digested with 4–5 units of enzyme per μg for approximately 16 hr at 37°, loaded onto 1% agarose gels (12.7 × 19.0 × 0.6 cm), 5 μg/lane and subjected to electrophoresis at 30 V for *ca.* 16 hr in TBE buffer (0.45 M Tris, 0.44 M boric acid, and 0.01 M EDTA, pH 8.0). Lambda phage DNA digested with *Ava*I or *Hin*dIII was used as a molecular weight standard.

DNA blotting and hybridization: Following electrophoresis, the DNA fragments were blotted onto nylon membranes (Zeta Probe; Bio-Rad Laboratories; Richmond, California) according to the procedure of REED and MANN (1985). The DNA on the filters was hybridized to labeled probes in a solution containing: 100 μg/ml calf thymus DNA, 5× Denhardt solution (1× Denhardt solution is 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone), 6× SSC, 1% sodium dodecyl sulfate (SDS), and 20 mM sodium phosphate (pH 6.5) at 64° for 16 to 20 hr. The filters were washed first in 2× SSC, 1% SDS at 25° for 20 min, then in three changes of 0.2× SSC, 0.2% SDS at 60° for a total of 90 min. Radioactive probes were stripped from the filters by the method of REED and MANN (1985). Filters were reused as many as ten times. Two sets of blots, containing the 88 DNA samples digested with *Eco*RI/*Xho*I or *Sal*I were used to map the molecular markers. The enzyme used to detect an RFLP for each probe is listed in Table 4.

Hybridization probes: A number of cDNA clones (pcf6-2, pcf6-8, pcf6-100, pcf6-135, pcf6-175 and pcf6-187) corresponding to transcripts whose levels increase after deflagellation were used as hybridization probes (SCHLOSS, SILFLOW and ROSENBAUM 1984). A "constitutive" cDNA clone, pcf8-13, corresponding to a transcript whose abundance is unchanged by deflagellation, was isolated in the same study. The α-tubulin probes (pcf10-2 and pcf4-2) and β-tubulin probes (pcf8-13 and pcf9-12) are full length cDNA clones representing the two α-tubulin and two β-tubulin genes in the *C. reinhardtii* genome (YOUNGBLOM, SCHLOSS and SILFLOW 1984; SILFLOW *et al.* 1985). A radial spoke clone (Rsa-1), a clone (Db-1) thought to correspond to the β-heavy chain dynein protein, and a clone (Da-1) corresponding to the 73-kD outer arm dynein were selected from a genomic λgt11 expression library by WILLIAMS, MITCHELL and ROSENBAUM (1986). Another genomic clone corresponding to the mutant gene in radial spoke mutant *pf-14* was provided by B. WILLIAMS and J. ROSENBAUM (personal communication). The radial spoke and dynein clones were subcloned into the *Eco*RI site of the SP65 plasmid by B. WILLIAMS. The gs-1 probe (of unknown identity) is a genomic clone isolated from an EMBL4 genomic library (J. LARKIN and J. YOUNGBLOM, unpublished data). The ef-12e and ef-3a probes were isolated by E. FERNÁNDEZ (unpublished data) from the genomic λgt11 expression library prepared by WILLIAMS, MITCHELL and ROSENBAUM 1986). An unidentified probe j-134 was isolated by J. SPANIER and J. JARVIK (unpublished data) from the genomic λgt11 expression library prepared by WILLIAMS, MITCHELL and ROSENBAUM (1986). The above clones were nick translated according to SILFLOW and ROSENBAUM (1981) or labeled by the random-primer technique outlined by FEINBERG and VOGELSTEIN (1983). For the *pf-14*, Da-1, and Rsal clones, repetitive sequences in the cloned DNA prevented the detection of RFLP segregation. Small fragments from each of the original clones were used to provide a unique sequence probe for RFLP analysis. The plasmid containing the *pf-14* clone was digested with *Sst*I, fragments were separated by gel electrophoresis, and a 700-bp fragment was eluted from the gel and nick translated. The plasmid containing the Rsa-1 DNA was digested with *Pst*I and a 700-bp fragment was subcloned into the pUC119 vector for nick translation. The plasmid containing the Da-1 clone was digested with *Eco*RI and *Xba*I and a 1.2 Kb fragment was subcloned into pUC119 and labeled using the random-primer method.

Scoring of molecular markers: Technical problems occasionally made it difficult to score all of the molecular markers for each of the tetrad progeny analyzed in this study (see Table 1). Barring rare events such as gene conversion, one can predict from the segregation of a marker in three- or sometimes two-tetrad progeny what the segregation of that marker will be for the rest of the tetrad progeny. Because in tetrads the scorable phenotypic and molecular markers segregate 2:2, we extrapolated the genotype for some members of tetrads when they could be predicted from the genotypes of the other members. The numbers of tetrads in which genotypes were extrapolated for a given marker comparison are listed in parentheses in Tables 3 and 4.

To ensure the detection of linkage between markers, to minimize errors, and to show that unlinked markers do not show aberrant linkage among the tetrad progeny, a method was developed to carry out pairwise comparisons of the segregation of all markers using a micro computer. Lotus 1-2-3, version 2.0 (Lotus Development Corporation, Cambridge, Massachusetts) was used with two sets of spreadsheets designed by Dr. David J. Mangen (Mangen Research Associates, Maple Grove, MN) to distinguish between PD, NPD, and T tetrad classes. Copies of the spreadsheets as well as details regarding their use are available upon request.

RESULTS

Strains: A number of different strains obtained from the Chlamydomonas Genetics Center were sur-

TABLE 1

Marker segregation data

Marker	Tedrad[a]																																											
	1a (2125)	1b (2126)	1c (2127)	1d (2128)	2a (2129)	2b (2130)	2c (2131)	2d (2132)	3a (2133)	3b (2134)	3c (2135)	3d (2136)	4a (2137)	4b (2138)	4c (2139)	4d (2140)	5a (2141)	5b (2142)	5c (2143)	5d (2144)	6a (2145)	6b (2146)	6c (2147)	6d (2148)	7a (2149)	7b (2150)	7c (2151)	7d (2152)	8a (2153)	8b (2154)	8c (2155)	8d (2156)	9a (2157)	9b (2158)	9c (2159)	9d (2160)	10a (2161)	10b (2162)	10c (2163)	10d (2164)	11a (2165)	11b (2166)	11c (2167)	11d (2168)	12a (lost)	12b (2169)	12c (2170)	12d (2171)
msr-1	1[b]	2[b]	1	2	1	2	2	1	2	2	1	1	2	1	1	2	1	2	2	1	1	1	2	2	2	2	1	1	2	1	1	2	1	2	2	1	2	2	1	1	1	2	1	2	1	2	2	1
nit-2	2	2	1	1	2	2	1	1	1	2	2	1	2	1	2	1	2	2	1	1	1	2	1	2	1	1	2	2	2	1	1	2	0	0	0	0	1	1	2	2	1	2	2	1	0	1	2	2
nit-1	2	2	1	1	1	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	1	1	1	2	2	1	2	1	2	0	0	0	0	1	2	1	2	2	1	2	1	0	2	1	2
act-2	2	2	1	1	2	2	1	1	2	1	1	2	1	2	2	1	1	1	2	2	1	2	2	1	2	1	1	2	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	1	1	2	2
sr-1	2	2	1	1	1	0[c]	0	2	1	2	2	1	2	1	2	1	2	2	1	1	1	1	2	2	1	2	1	2	1	2	2	1	2	1	1	2	2	2	1	1	2	1	2	1	1	2	2	1
nic-13	1	1	2	2	2	1	2	1	2	1	1	2	2	1	2	1	1	2	1	2	1	2	1	2	2	1	2	1	1	2	1	2	2	1	1	2	2	1	2	1	2	1	2	1	2	1	2	1
ac-17	2	2	1	1	2	2	1	1	1	2	2	1	2	1	2	1	2	2	1	1	1	2	1	2	1	2	1	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	1	1	2
y-1	2	2	1	1	1	2	1	2	2	1	1	2	2	1	2	1	1	1	2	2	1	2	1	2	2	1	2	1	2	1	2	1	2	1	1	2	2	1	2	1	2	1	2	1	1	2	2	1
pf-2	2	2	1	1	1	2	1	2	2	1	1	2	1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	1	2	2	1	2	1	2	1	1	2	1	2	1	2	2	1
mt	2	1	1	2	2	1	2	1	2	2	1	1	1	2	1	2	2	1	2	1	1	2	1	2	1	2	1	2	2	1	1	2	2	2	0	1	2	0	1	2	1	0	0	1	2	1	2	1
pyr-1	2	2	1	1	2	1	2	1	2	1	1	2	2	1	2	1	1	1	2	2	1	2	1	2	1	2	1	2	2	1	2	1	2	1	1	2	2	1	2	1	1	2	2	1	2	1	1	2
α-1	2	2	1	1	2	2	1	1	1	2	2	1	2	1	2	1	2	2	1	1	1	2	1	2	2	1	2	1	1	2	1	2	1	2	2	1	1	1	2	2	2	1	2	1	1	1	2	2
α-2	2	2	1	1	2	1	2	1	2	1	1	2	2	1	2	1	1	1	2	2	1	2	1	2	2	1	1	2	2	1	2	1	2	1	2	1	2	1	2	1	1	1	2	2	2	1	1	2
β-1	1	1	2	2	2	1	2	1	1	2	1	2	2	1	2	1	2	2	1	1	1	2	1	2	1	2	1	2	2	2	1	1	1	2	1	2	1	2	0	0	1	2	1	2	2	1	2	1
β-2	1	1	2	2	2	1	2	1	2	1	1	2	2	1	2	1	2	2	1	1	1	2	1	2	1	2	1	2	1	2	1	2	1	2	2	1	1	2	1	2	1	2	2	1	2	1	1	2
Db-1	2	1	2	1	2	1	1	2	1	2	2	1	2	1	1	2	2	2	1	1	1	1	2	2	1	1	2	2	1	2	1	2	2	2	1	1	2	2	1	1	1	2	2	1	1	2	2	1
pcf6-8	2	2	1	1	1	0	1	2	2	1	1	2	2	1	2	1	1	1	2	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	2	1	0	0	0	0	1	0	1	2	2	1	1	2
gs-1	2	2	1	1	2	1	2	1	2	1	1	2	2	1	2	1	1	1	2	2	1	0	1	2	1	2	1	2	2	1	2	1	2	1	1	2	2	1	2	1	1	0	1	2	2	1	1	0
pf-14	2	2	1	1	0	2	1	1	2	1	1	2	1	2	2	1	1	1	2	2	1	0	2	1	2	1	1	2	2	1	2	1	2	1	1	2	0	1	2	1	2	1	1	2	2	1	2	2
Rsa-1	1	1	2	2	0	1	2	1	2	1	1	2	1	1	2	2	2	2	1	1	1	2	1	2	1	1	2	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	2	1	2	1	1	0
pcf6-100	2	2	1	1	2	2	1	1	1	2	2	1	2	1	2	1	2	2	1	1	1	2	1	2	2	1	2	1	2	1	1	2	1	2	2	1	1	1	2	2	2	1	2	1	1	1	2	2
ef-3a	2	2	1	1	2	1	2	1	2	1	1	2	1	2	1	2	1	1	2	2	1	0	1	2	2	1	2	1	1	2	1	2	1	2	2	1	0	0	1	0	1	2	1	2	1	2	2	1
pcf6-175	1	1	2	2	2	1	2	1	1	2	2	1	2	1	2	1	1	1	2	0	1	0	1	2	1	2	1	2	2	1	2	1	1	2	2	1	0	2	1	0	2	0	2	1	1	2	2	1
pcf6-2	1	2	1	2	1	2	1	2	2	1	1	2	1	2	1	2	2	1	2	1	2	1	2	1	1	2	2	1	0	1	2	1	2	1	2	1	1	1	0	2	1	2	1	2	1	2	2	1
ef-12e	2	2	1	1	0	2	0	1	1	2	2	1	2	1	2	1	2	2	1	1	1	0	1	2	1	1	2	2	2	1	1	2	1	2	1	2	0	0	2	0	1	2	2	1	1	1	2	2
pcf6-135	1	1	2	2	2	1	2	1	1	2	2	1	2	1	2	1	1	1	2	2	1	2	1	2	1	2	1	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1	2	1	1	2	2	1
pcf-187	1	1	2	2	2	1	2	1	2	1	1	2	1	1	2	2	2	2	1	1	1	2	1	2	1	1	2	2	1	2	2	1	1	2	2	1	2	1	2	1	1	0	1	0	2	1	1	2
pcf8-13	2	2	1	1	0	1	0	1	2	2	1	1	1	2	1	2	1	1	2	2	1	0	1	2	1	2	1	2	2	1	2	1	2	1	1	2	2	1	2	0	2	1	2	1	2	1	2	2
j-134	2	1	1	2	2	2	0	1	1	1	2	2	2	1	1	2	0	0	1	0	2	1	1	2	2	1	1	2	2	1	0	1	2	2	1	1	2	1	0	1	2	0	1	0	1	1	2	0
da1	1	2	2	1	0	0	0	2	1	2	1	2	2	1	1	2	1	2	2	1	1	0	2	2	1	2	1	2	1	2	1	2	2	1	1	2	0	0	1	0	2	0	1	1	1	2	0	1

TABLE 1—Continued

Tedrad[a]

Marker	13a (2172)	13b (2173)	13c (2174)	13d (2175)	14a (2176)	14c (2178)	14b (2177)	14d (2179)	15a (2180)	15b (2181)	15c (2182)	15d (2183)	16a (2184)	16b (2185)	16c (2186)	16d (2187)	17a (2188)	17b (2189)	17c (2190)	17d (2191)	18a (2192)	18b (2193)	18c (2194)	18d (2195)
msr-1	1	2	2	1	1	2	1	2	1	1	2	2	2	1	2	1	2	1	2	1	1	2	1	2
nit-2	2	1	2	1	1	2	0	2	1	2	2	1	2	1	2	0	2	1	2	1	1	2	2	1
nit-1	2	2	1	1	2	1	0	1	1	2	2	1	1	1	2	0	2	1	2	1	1	1	2	2
act-2	1	2	1	2	1	2	1	2	1	1	2	2	2	1	1	2	1	2	1	2	2	1	1	2
sr-1	2	1	2	1	2	1	1	2	2	2	1	1	1	1	2	2	2	1	2	1	1	2	2	1
nic-13	2	1	2	1	1	0	0	0	1	2	2	1	1	2	1	2	1	2	1	2	1	2	2	1
ac-17	2	1	2	1	1	2	1	2	1	2	2	1	2	2	1	1	2	1	2	1	1	2	2	1
y-1	2	1	2	1	2	1	2	1	2	1	1	2	1	1	2	2	1	2	1	2	1	2	2	1
pf-2	2	1	1	2	2	2	1	1	1	2	2	1	2	2	1	1	2	0	2	1	2	1	1	2
mt	2	1	1	2	2	1	1	2	1	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1
pyr-1	2	1	2	1	1	2	1	2	2	1	1	2	1	1	2	2	2	1	2	1	1	2	2	1
α-1	2	1	2	1	1	2	1	2	1	2	2	1	2	2	1	1	2	1	2	1	1	2	2	1
α-2	2	1	2	1	1	2	1	2	2	1	1	2	1	1	2	2	2	1	2	1	1	2	2	1
β-1	2	1	2	0	1	2	2	1	2	1	1	2	2	1	1	2	2	1	2	1	2	1	1	2
β-2	2	1	2	0	1	2	1	2	2	1	1	2	2	2	1	1	2	1	2	1	2	1	1	2
Db-1	2	1	2	1	2	1	1	2	2	2	1	1	2	1	2	1	2	1	1	2	1	2	2	1
pcf6-8	1	0	0	0	1	1	2	2	2	1	1	2	1	1	2	2	0	0	0	0	2	1	2	1
gs-1	2	1	2	1	1	2	1	2	2	1	1	2	1	1	2	2	0	1	2	1	1	0	0	1
pf-14	1	2	1	2	1	2	1	2	2	1	1	2	2	1	1	2	1	2	1	2	2	1	1	2
Rsa-1	2	1	2	1	1	2	1	2	2	1	2	1	1	1	2	2	2	2	1	1	2	1	2	1
pcf6-100	2	1	2	0	1	2	1	2	1	2	2	1	2	2	1	1	2	1	2	1	1	2	2	1
ef-3a	1	0	0	0	0	0	0	0	2	1	1	2	1	1	2	2	2	1	2	1	2	1	1	2
pcf6-175	1	0	0	2	2	1	2	1	1	2	2	1	2	1	2	1	1	0	1	0	1	0	0	1
pcf6-2	2	1	1	2	2	2	1	1	1	2	2	1	2	1	2	1	2	1	2	1	2	0	0	1
ef-12e	2	1	2	1	1	2	1	2	1	2	2	1	2	1	2	1	2	1	2	1	1	2	2	1
pcf6-135	1	2	1	2	2	1	2	1	1	2	2	1	1	1	2	2	1	2	1	2	0	0	0	1
pcf6-187	2	1	2	0	1	2	1	2	2	1	2	1	1	1	2	2	2	2	1	1	0	0	0	0
pcf8-13	2	0	0	2	2	1	1	2	2	1	1	2	2	2	1	1	1	1	2	2	2	2	1	1
j-134	2	2	1	0	2	0	1	1	1	2	1	2	2	1	2	1	0	0	0	0	0	0	0	0
dal	2	0	0	1	1	2	1	2	2	1	1	2	2	2	1	1	1	0	2	0	1	0	0	0

Marker	19a (2196)	19b (2197)	19c (2198)	19d (2199)	20a (2200)	20b (2201)	20c (2202)	20d (2203)	21a (2204)	21b (2205)	21c (2206)	21d (2207)	22a (2208)	22b (2209)	22c (2210)	22d (2211)	23a (2212)	23b (2213)	23c (2214)	23d (2215)	24a (2216)	24b (2217)	24c (2218)	24d (2219)
msr-1	1	1	2	2	1	2	2	1	2	1	1	2	2	2	1	1	1	1	2	2	2	2	1	1
nit-2	1	1	2	2	1	1	2	2	2	1	1	2	2	1	1	2	1	1	2	2	2	2	1	1
nit-1	2	2	1	1	2	1	1	2	2	1	2	1	1	2	1	2	2	2	1	1	2	1	2	1
act-2	2	1	2	1	2	1	2	1	1	1	2	2	1	2	2	1	2	1	2	1	2	1	2	1
sr-1	2	2	1	1	2	2	1	1	1	1	2	2	2	1	1	2	1	2	2	1	2	2	1	1
nic-13	1	1	2	2	1	1	2	2	1	2	1	2	1	2	2	1	1	2	2	1	1	1	2	2
ac-17	1	1	2	2	1	1	2	2	1	2	1	2	2	1	1	2	1	2	2	1	2	2	1	1
y-1	2	2	1	1	1	1	2	2	2	1	2	1	1	2	2	1	2	1	1	2	2	2	1	1
pf-2	1	2	2	1	2	2	1	1	2	1	2	1	1	2	2	1	2	1	1	2	1	2	2	1
mt	2	2	1	1	1	1	2	2	1	2	1	2	1	2	1	2	1	2	2	1	0	2	1	1
pyr-1	1	1	2	2	2	2	1	1	2	1	2	1	1	2	2	1	2	1	2	1	2	1	1	2
α-1	1	1	2	2	1	1	2	2	2	1	1	2	2	1	1	2	0	1	2	2	2	2	1	0
α-2	1	1	2	2	2	2	1	1	2	1	2	1	1	2	2	1	0	2	2	1	1	1	2	0
β-1	2	1	1	2	1	1	2	2	2	1	2	1	2	1	2	1	0	2	2	1	1	1	2	0
β-2	2	1	1	2	1	1	2	2	2	1	2	1	1	2	2	1	0	2	2	1	1	1	2	0
Db-1	2	1	1	2	2	2	1	1	1	2	2	1	2	1	1	0	0	2	2	1	2	2	1	0
pcf6-8	2	1	1	2	2	2	1	1	0	1	2	1	2	1	1	2	0	1	1	2	0	0	0	0
gs-1	1	1	2	2	2	2	1	1	0	1	2	1	1	2	2	1	0	1	0	1	0	1	1	0
pf-14	2	1	2	1	2	1	2	1	1	1	2	2	1	2	2	1	0	0	2	1	2	1	0	0
Rsa-1	1	1	2	2	1	1	2	2	2	2	1	1	1	2	2	1	0	2	2	1	0	1	2	0
pcf6-100	1	1	2	2	1	1	2	2	0	1	1	2	2	1	1	2	0	0	2	2	2	2	1	0
ef-3a	2	2	1	1	1	1	2	0	1	2	1	2	1	2	2	1	0	1	1	2	2	2	1	0
pcf6-175	1	1	2	2	0	0	0	0	2	1	2	1	1	2	0	0	0	0	0	0	0	0	0	0
pcf6-2	1	2	2	1	0	0	0	0	0	1	2	1	1	2	2	1	0	0	0	0	0	0	0	0
ef-12e	1	1	2	2	1	0	2	2	0	0	1	2	2	1	1	2	0	0	2	2	2	2	1	0
pcf6-135	1	1	2	2	1	1	2	2	2	1	2	1	1	2	2	1	0	2	2	1	0	0	0	0
pcf6-187	1	1	2	2	1	1	2	2	0	2	0	1	1	2	2	1	0	2	2	1	1	2	1	0
pcf8-13	2	2	1	0	1	0	2	2	0	2	1	2	0	2	2	1	0	2	2	1	2	2	1	0
j-134	2	2	1	1	1	2	2	1	0	0	0	0	2	1	0	0	0	1	1	2	0	0	2	0
dal	2	2	1	1	2	1	2	1	0	0	1	2	1	2	2	1	0	0	0	0	1	1	0	0

[a] Chlamydomonas Genetic Center numbers are indicated in parentheses.
[b] "1" and "2" indicate the *C. reinhardtii* and *C. smithii* segregation of various markers, respectively.
[c] "0" indicates that no data were obtained for the segregation of that marker.

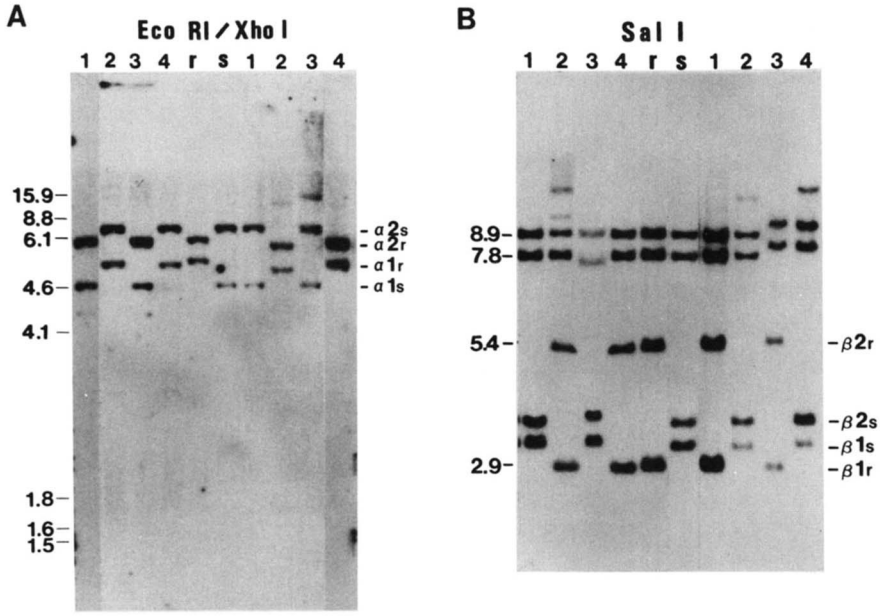

FIGURE 1.—Southern blots of genomic DNA isolated from tetrad progeny. DNA was isolated from *C. reinhardtii* (lane R) and *C. smithii* (lane S), and from the tetrad progeny of crosses between the two strains (lanes numbered 1–4). A, The DNA was digested with *Eco*RI/*Xho*I, gel fractionated, blotted, and probed with the α-tubulin cDNA clones pcf10-2 (α-1) and 4-2 (α-2) (SILFLOW et al. 1985). Migration of DNA fragments containing the α-1 and α-2 genes in each strain are indicated at the right of the panel. The tetrad on the left is a nonparental ditype; on the right is a parental ditype. B, The DNA was digested with *Sal*I, blotted as above, and probed with β-tubulin cDNA clones pcf9-12 (β-1) and pcf8-31 (β-2) (YOUNGBLOM, SCHLOSS and SILFLOW 1984). The R and S lanes each contain two smaller tubulin fragments representing the two genes, as indicated on the right of the panel. The two tetrads shown are parental ditypes.

veyed for RFLPs. Genomic DNA was isolated from four different strains of *C. reinhardtii* derived from three independent isolates. These strains included two common laboratory strains NO^+ (CC-620), and 21GR (CC-1690), strain CC-410 from the Caroline Islands, and strain CC-1418 from Florida. In addition, we also examined the DNA from *C. smithii* (CC-1373), a related species of Chlamydomonas which is interfertile with *C. reinhardtii*. We isolated and digested DNA from the strains described above with *Sst*I, *Xho*I, *Hin*dIII, and *Sma*I. Southern blot analysis using α- and β-tubulin sequences as probes revealed RFLPs between the *C. reinhardtii* and *C. smithii* isolates but not among the different *C. reinhardtii* strains (data not shown). RFLPs are common between *C. reinhardtii* and *C. smithii*; out of 33 different cloned probes examined, we identified RFLPs for 26 of them using only three different restriction enzymes or double enzyme combinations. Because of the high frequency of RFLPs between *C. reinhardtii* and *C. smithii*, tetrad progeny generated from a cross of these two species were used in the RFLP mapping project. An example of the segregation of the α- and β-tubulin genes in representative tetrads is shown in Figure 1.

Markers: The 12 phenotypic markers, on 9 of the 19 linkage groups in the *C. reinhardtii* strain (CC-29), were examined in *C. smithii*, which displayed contrasting phenotypes for all of the markers except canavanine resistance; this marker (*can-1*) was not used. The Rsa-1 clone was used as a marker for linkage group *V* (WILLIAMS, MITCHELL and ROSENBAUM 1986). Rsa-1 corresponds to the gene for radial spokehead protein 6; this gene contains the *pf-26* mutation whose map location was previously determined by genetic analysis (HUANG *et al.* 1981). As a marker for linkage group *VI*, we used a cloned genomic fragment known to correspond to the radial spoke gene containing the *pf-14* mutation (B. WILLIAMS and J. ROSENBAUM, personal communication) which was previously characterized by LUCK *et al.* (1977). The segregation data for all of the markers are listed in Table 1. Tetrads numbered 1 to 22 were used to determine the linkage relationships and map positions summarized in Tables 2, 3 and 4. Data from tetrads 23 and 24 are incomplete but are included in Table 1 as an aid in the discussion of the map position of the α-2 tubulin gene.

Linkage detection limits: The feasibility of the mapping project depended on obtaining reliable data from a manageable number of tetrads (20–25 tetrads equaling 80–100 DNA samples). To estimate the distance over which linkage would be detectable with this number of tetrads, genetic linkage data compiled by E. HARRIS (in press) was used to predict the PD:NPD ratios expected from analyzing 22 tetrads. For example, the previously compiled PD:NPD:T ratio for crosses involving the *ac-17* and *y-8* markers on linkage group *III* was 29:0:40. If only 22 tetrads had been analyzed and the ratio remained constant, the numbers would be approximately 9:0:13 and the PD:NPD ratio of 9:0 would predict linkage at the 99% confidence level (PERKINS 1953). In two cases, using the data compiled by E. HARRIS, we predicted linkage would be detectable with 95% confidence up to 38 map units from *pf-14* (linkage group *VI*) and *nic-13* (linkage group *X*). In all cases examined, when two markers were within 30 map units of one another, linkage was detectable. Therefore, 30 cM was chosen as a conservative estimate of the distance from a given marker which would allow us to detect linkage at the 95% confidence level (broken lines in Figure 2). Using both the phenotypic markers and the molecular markers mapped in this study, approximately 54% of the known linkage map is covered.

TABLE 2

Independent assortment of unlinked markers

	ac-17	*pyr-1*	*act-2*	*mt(−)*	*sr-1*	*nic-13*	*pf-2*	*y-1*	(*pf-26*) *Rsa-1*
msr-1	4:2:16	4:2:16	2:3:17	3:2:14	4:5:12	1:4:16	3:1:17	1:5:16	4:2:14
ac-17		10:11:1	5:7:10	4:3:12	9:6:6	12:5:4	6:10:5	9:12:1	9:5:6
pyr-1			7:4:11	3:5:11	9:6:6	10:8:3	8:9:4	14:8:0	9:5:6
act-2				1:0:18	3:7:11	5:4:12	6:1:14	8:3:11	4:6:10
mt(−)					3:5:10	5:4:9	4:5:9	3:5:11	2:3:12
sr-1						5:7:8	3:8:9	9:6:6	3:8:9
nic-13							5:11:4	12:6:3	9:2:8
pf-2								9:8:4	5:5:9
y-1									7:7:6

The PD:NPD:T ratios between markers known to map to different chromosomes are shown above. All of these markers were scored in the progeny of the cross between the multiply marked *C. reinhardtii* strain (CC-29) and *C. smithii*.

TABLE 3

Linkage data

	PD:NPD:T	Partial tetrads[a]	Linkage confidence[b]	Marker distance (map units)
Linkage Group III:				
ac-17/α-1 tubulin	19:0:3	(0)	>99%	7
ac-17/pcf6-100	18:0:4	(2)	>99%	9
ac-17/ef-12e	13:0:6	(2)	>99%	16
ac-17/*nit-2*	14:0:7	(3)	>99%	17
α-1 tubulin/pcf6-100	21:0:1	(2)	>99%	2
α-1 tubulin/ef-12e	14:0:5	(2)	>99%	13
α-1 tubulin/*nit-2*	17:0:4	(3)	>99%	10
pcf6-100/ef-12e	15:0:4	(2)	>99%	11
pcf6-100/*nit-2*	18:0:3	(5)	>99%	7
ef-12e/*nit-2*	20:0:0	(7)	>99%	≤2
Linkage Group IV:				
pyr-1/α-2 tubulin	19:0:3	(0)	>99%	7
pyr-1/gs-1	22:0:0	(6)	>99%	≤2
α-2 tubulin/gs-1	19:0:3	(6)	>99%	7
Linkage Group V:				
Rsa-1/pcf6-187	20:0:0	(4)	>99%	≤2
Linkage Group VI:				
pcf8-13/*act-2*	6:0:16	(9)	97.5%	36
pcf8-13/pf-14	8:0:14	(9)	>99%	32
pcf8-13/*mt−*	13:0:9	(10)	>99%	20
act-2/pf-14	20:0:2	(3)	>99%	5
act-2/*mt−*	1:0:21	(3)		
pf-14/*mt−*	0:0:22	(5)		
Linkage Group IX:				
Db-1/*sr-1*	11:0:11	(2)	>99%	25
Linkage Group XI:				
pcf6-2/*pf-2*	15:0:6	(5)	>99%	14
Linkage Group XII:[c]				
β-1 tubulin/β-2 tubulin	16:0:5	(1)	>99%	12
β-1 tubulin/Da-1	3:0:14	(4)		41
β-2 tubulin/Da-1	6:0:11	(4)	97.5%	32
Unknown Linkage Group A:				
pcf6-135/pcf6-175	16:0:1	(4)	>99%	3

[a] The number of tetrads for which tetrad type data were extrapolated based on segregation data for two or three members of the tetrad.

[b] According to PERKINS (1953).

[c] Identified by published map position of β-tubulin mutants (BOLDUC, LEE and HUANG 1988).

Unlinked markers in CC-29 segregated independently in crosses to *C. smithii* as shown by the segregation ratios of the unlinked phenotypic markers and the molecular marker, Rsa-1 (Table 2). No aberrant linkage was detected in our sample, indicating that the chromosomes marked in this study behave normally; that is, the unlinked markers segregated independently of one another.

Tubulins and linked genes: Three molecular markers map to linkage group *III* (Table 3 and Figure 2): α-1 tubulin, cDNA clone pcf6-100 (selected as a transcript which accumulates after deflagellation), and ef-12e (a clone of unknown function). The ef-12e sequence is located within approximately 2 map units of *nit-2*. Use of *y-1* as a centromere marker throughout the study enabled us to treat the progeny as ordered tetrads and thus to distinguish between first and second division segregations. Analysis of recombinant tetrads demonstrates that the α-1 tubulin gene and the gene represented by the pcf6-100 cDNA clone are also located on the same side of the centromere as *nit-2*, based on the following reasoning. If the α-1 and pcf6-100 genes are located on the same side of the centromere as *nit-2*, eight single crossovers had to occur between *nit-2* and the centromere to explain the segregation of the markers in the 22 tetrads. If α-1 and pcf6-100 are on the opposite side of the centromere from *nit-2*, however, four single crossovers and four double crossovers would have had to occur between the markers. The second possibility is much less likely. Crossovers between *ac-17* and α-1, between α-1 and pcf6-100, between pcf6-100 and *nit-2* and between pcf6-100 and ef-12e allowed us to order the genes from the centromere as follows: *ac-17*—α-1 tubulin—pcf6-100—*nit-2* or ef-12e. The α-1 tubulin gene and the gene corresponding to the pcf6-100 cDNA clone map approximately 9 and 11 map units from the centromere, respectively (Table 4). Both the α-1 tubulin gene and the putative flagellar gene pcf6-100, map to the same region of linkage group *III* as

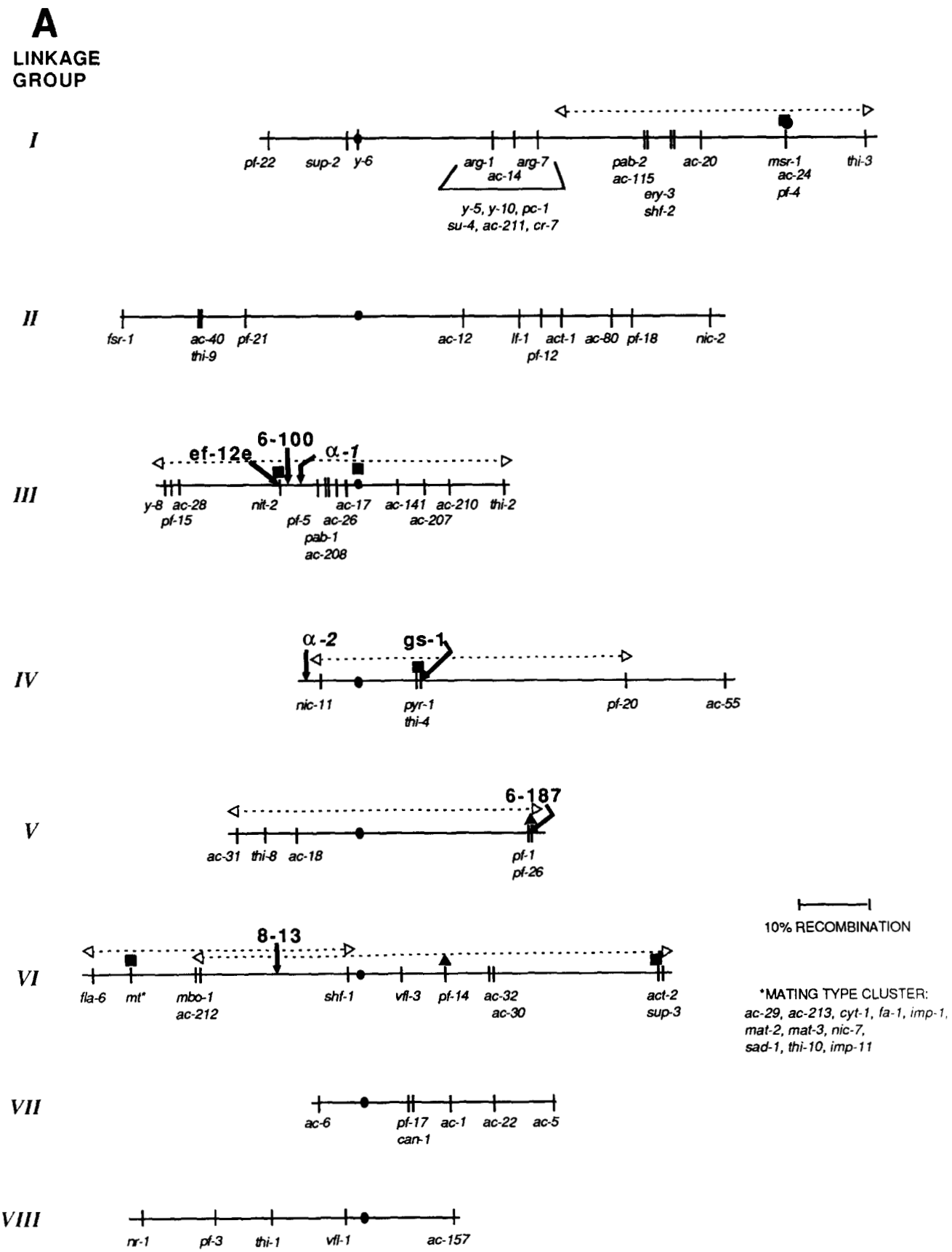

FIGURE 2.—Chlamydomonas genetic map adapted from Harris, Boynton and GILLHAM (1987). (1) Solid squares indicate the genetic markers used in the multiply marked *C. reinhardtii* strain (CC-29); (2) solid triangles indicate the two molecular markers known to correspond to the *pf-26* and *pf-14* mutations that were used to detect linkage on linkage groups *V* and *VI*; (3) solid circles mark each centromere; (4) the molecular markers mapped in this study are shown above their respective linkage groups with their approximate locations indicated with arrows; and (5) linkage detection limits from the markers used (at the 95% confidence level) are indicated with dotted arrows.

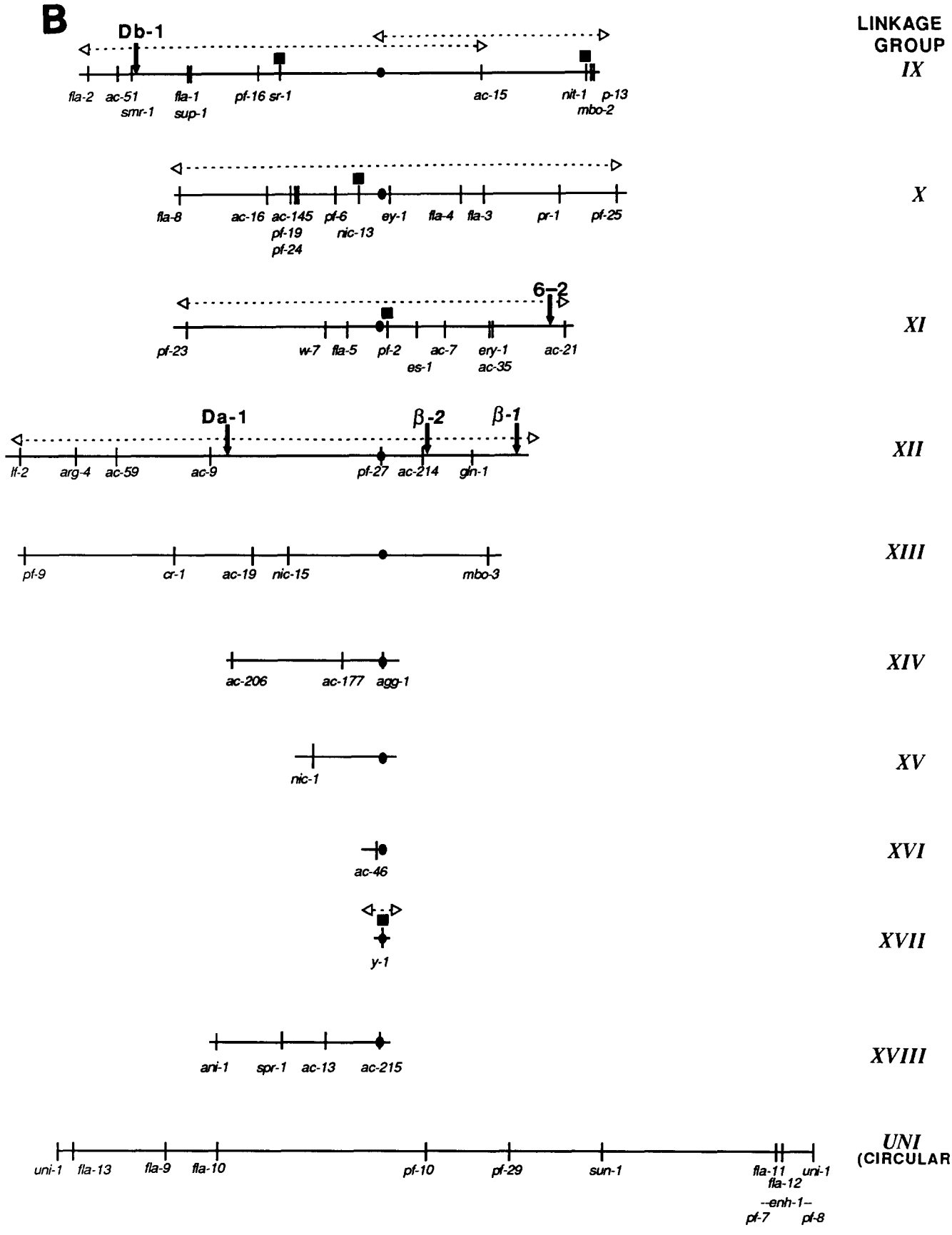
B
LINKAGE GROUP
IX
Db-1
fla-2 ac-51 smr-1 fla-1 sup-1 pf-16 sr-1 ac-15 nit-1 p-13 mbo-2
X
fla-8 ac-16 ac-145 pf-19 pf-24 pf-6 nic-13 ey-1 fla-4 fla-3 pr-1 pf-25
XI
6-2
pf-23 w-7 fla-5 pf-2 es-1 ac-7 ery-1 ac-35 ac-21
XII
Da-1 β-2 β-1
lf-2 arg-4 ac-59 ac-9 pf-27 ac-214 gln-1
XIII
pf-9 cr-1 ac-19 nic-15 mbo-3
XIV
ac-206 ac-177 agg-1
XV
nic-1
XVI
ac-46
XVII
y-1
XVIII
ani-1 spr-1 ac-13 ac-215
UNI (CIRCULAR)
uni-1 fla-13 fla-9 fla-10 pf-10 pf-29 sun-1 fla-11 fla-12 uni-1 --enh-1-- pf-7 pf-8

FIGURE 2

TABLE 4

Centromere distances

Marker	Restriction enzyme	Linkage Group	Arm	*Y-1*/Marker PD:NPD:T	Partial tetrads[a]	Centromere distance (cM)
Tubulins:						
α-1	*Eco*RI/*Xho*I	*III*	Left	8:10:4	(0)	9
α-2	*Eco*RI/*Xho*I	*IV*	Left	13:6:3	(0)	7
β-1	*Sal*I	*XII*	Right	6:7:8	(1)	19
β-2	*Sal*I	*XII*	Right	8:11:3	(1)	7
Radial Spoke:						
Rsa-1	*Eco*RI/*Xho*I	*V*	Right	7:9:6	(2)	14
pf-14	*Sal*I	*VI*	Right	10:3:9	(3)	20
Dyneins:						
Da-1	*Sal*I	*XII*	Left	5:4:7	(4)	22
Db-1	*Eco*RI/*Xho*I	*IX*	Left	3:5:14	(1)	32
Regulated Clones:						
pcf6-2	*Eco*RI/*Xho*I	*XI*	Right	6:5:10	(4)	24
pcf6-8	*Sal*I	Unknown	Unknown	8:7:3	(3)	8
pcf6-100	*Sal*I	*III*	Left	8:9:5	(2)	11
pcf6-135	*Eco*RI/*Xho*I	Unknown[b]	Unknown	12:9:0	(0)	≤2
pcf6-175	*Sal*I	Unknown[b]	Unknown	10:7:1	(6)	3
pcf6-187	*Sal*I	*V*	Right	7:9:5	(3)	12
Other Clones:						
pcf8-13	*Sal*I	*VI*	Left	11:6:5	(9)	11
ef-3a	*Sal*I	Unknown	Unknown	9:10:0	(2)	≤3
ef-12e	*Sal*I	*III*	Left	6:7:7	(3)	18
j-134	*Eco*RI/*Xho*I	Unknown	Unknown	3:0:14	(8)	41
gs-1	*Eco*RI/*Xho*I	*IV*	Right	14:8:0	(6)	≤2

[a] The number of tetrads for which tetrad type data were extrapolated based on segregation data for two or three members of the tetrad.
[b] pcf6-135 and pcf6-175 are linked to one another but are unlinked to any of the other markers.

does the previously mapped paralyzed-flagella mutant *pf-5* (HUANG *et al.* 1981), raising the possibility that the gene containing the *pf-5* mutation corresponds to the α-1 tubulin gene or to the pcf6-100 gene.

The α-2 tubulin gene, as well as a genomic sequence represented by the gs-1 clone, are linked to the *pyr-1* marker on linkage group *IV* (Table 3, Figure 2). The α-2 tubulin gene maps approximately 7 cM from *pyr-1* and from its centromere (Table 4). In the *C. reinhardtii* × *C. smithii* cross, *pyr-1* maps within 2 cM of the centromere instead of the 10 cM on the *C. reinhardtii* genetic map (Figure 2). Some evidence indicates that the α-2 tubulin gene is on the opposite side of the centromere from the *pyr-1* and gs-1 markers. Data from two partial tetrads (23 and 24, Table 1), show recombination between the *pyr-1* and gs-1 markers and their centromere. If the α-2 tubulin gene is located on the opposite side of the centromere from these markers, two single crossovers explain the segregation of all three markers in these tetrads. If, however, the α-2 tubulin gene is located on the same side of the centromere as *pyr-1* and gs-1, then two double crossovers would be required to explain the results. The genomic sequence homologous to the gs-1 clone maps within 2 cM on either side of the *pyr-1* marker (Table 3, Figure 2). We have not observed recombination between these two markers among the tetrads analyzed to date.

The two β-tubulin genes found in Chlamydomonas are linked to one another as indicated by a PD:NPD:T ratio of 16:0:5 (Table 3). The β-2 gene maps approximately 7 cM from the centromere, while the β-1 gene is distal to the β-2 gene and about 19 cM from the centromere (Tables 3 and 4). The gene for a 73-kD dynein polypeptide represented by the Da-1 clone also shows linkage to the β-2 tubulin gene. Crossover data indicate that the sequence homologous to the Da-1 clone is on the opposite side of the centromere from the two β-tubulin genes, and approximately 22 cM from the centromere. Although we did not detect linkage of these genes with any markers in the CC-29 strain, we have placed them all on linkage group *XII* (Figure 2) because β-tubulin mutations have recently been mapped to the right arm of linkage group *XII* (BOLDUC, LEE and HUANG 1988).

Other flagellar genes: Radial spokehead protein 6 is encoded by a gene identified by the *pf-26* mutation (LUCK *et al.* 1977). The wild-type allele of the *pf-26* gene was cloned by WILLIAMS, MITCHELL and ROSENBAUM (1986), and the clone, Rsa-1, was used in this study as a marker for linkage group *V*. The pcf6-187 cDNA clone, which represents a transcript whose levels increase after deflagellation (SCHLOSS, SILFLOW and ROSENBAUM 1984) is closely linked to the Rsa-1 marker (Table 3, Figure 2). Among 20 tetrads examined, no recombinants occurred between the pcf6-

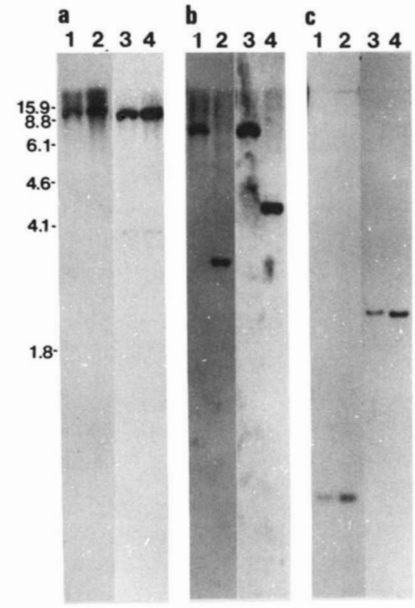

FIGURE 3.—Southern blot of genomic DNA probed with Rsa-1 and pcf6-187. DNA was isolated from *C. smithii* (lanes marked with 1 and 3) and *C. reinhardtii* (lanes marked with 2 and 4), digested with *Bam*HI (panel a), *Eco*RI/*Xho*I (panel b), or *Pst*I (panel c), gel fractionated, transferred to a nylon membrane which was probed successively with the Rsa-1 (lanes marked with 1 and 2) and pcf6-187 (lanes marked with 3 and 4) clones. Between hybridizations the radioactive probes were stripped from the filters and the filters were exposed to X-ray film to ensure that the probe had been successfully removed.

187 and Rsa-1 sequences, placing the two genes within approximately 2 cM of one another. Southern blots of *C. reinhardtii* and *C. smithii* DNA probed sequentially with the pcf6-187 and Rsa-1 clones refine this distance further, suggesting that these clones are located no more than 8 kb apart (Figure 3). Both clones hybridize to a 12 kb *Bam*HI fragment (Figure 3, panel a), a 9-kb *Sma*I fragment, and a 20-kb *Bgl*II fragment (data not shown), all of which are present in both *C. reinhardtii* and *C. smithii* DNA. In DNA digested with *Eco*RI/*Xho*I, the two clones hybridize to the same 8-kb fragment in *C. smithii*. In *C. reinhardtii*, however, the Rsa-1 and pcf6-187 clones hybridize to 3.5-kb and 4.5-kb fragments respectively (Figure 3, panel b), suggesting that the *C. reinhardtii* DNA homologous to the 8-kb *C. smithii* fragment carries an additional *Xho*I or *Eco*RI site in the region between the two molecular markers. The fragments to which the Rsa-1 and pcf6-187 clones hybridize are not the same size in *C. reinhardtii* or *C. smithii* when DNA is digested with *Pst*I (Figure 3, panel c), *Hin*dIII, and *Sal*I (data not shown), indicating that these three enzyme sites lie between the two markers in both *C. reinhardtii* and *C. smithii* DNA.

A clone (Db-1) thought to represent a gene encoding one of the high molecular weight dynein polypeptides was mapped in this study. The *sup-pf-1* mutation, which was previously mapped to linkage group *IX*, was isolated as a suppressor mutation which restores flagellar activity to paralyzed radial spoke or central pair mutants. Correlated with *sup-pf-1* is an alteration in the electrophoretic mobility of a β-heavy chain dynein protein found in the outer dynein arms of Chlamydomonas flagella (HUANG, RAMANIS and LUCK 1982; BROKAW, LUCK and HUANG 1982). The Db-1 clone isolated by WILLIAMS, MITCHELL and ROSENBAUM (1986), is thought to represent the *sup-pf-1* gene, although the large size of the corresponding protein product prevented a conclusive demonstration of its identity by hybridization selection and in vitro translation. Our data indicate that the genomic sequence corresponding to Db-1 maps to the left arm of linkage group *IX*, distal to the streptomycin resistance marker *sr-1*, and approximately 35 cM from its centromere (Tables 3 and 4, Figure 2). This map location is the average of the map position of Db-1 relative to its centromere and to *sr-1*. Although our data place the Db-1 clone 8 map units distal to the site of the *sup-pf-1* mutation mapped by HUANG, RAMANIS and LUCK (1982), these data are indirect as *sup-pf-1* was not used as a marker in this cross. The difference in map position between Db-1 and *sup-pf-1* can be accounted for by mapping error (see discussion below). Our mapping data support the conclusion that the Db-1 clone corresponds to the wild-type allele of the *sup-pf-1* locus and thus to the β-heavy chain dynein protein found in the outer dynein arms.

The genomic sequence corresponding to the cDNA clone pcf6-2, whose mRNA accumulates sevenfold after deflagellation (SCHLOSS, SILFLOW and ROSENBAUM 1984), maps to linkage group *XI* (Table 3, Figure 2). Its position is estimated at 24 cM from the centromere (Table 4). We place this marker on the right arm of linkage group *XI* as a result of analyzing recombinant tetrads. Ten single crossovers account for the segregation of the *pf-2* and pcf6-2 markers when placed on the same side of the centromere. If these markers are on opposite sides of the centromere, however, six single and four double crossovers must be invoked to explain their segregation. Sequences corresponding to the pcf6-135 and pcf6-175 clones (SCHLOSS, SILFLOW and ROSENBAUM 1984) both of whose corresponding transcripts accumulate after deflagellation, are linked to each other at greater than the 99% confidence level (Table 3). These sequences, however, are unlinked to any of the other phenotypic or molecular markers used in our study. No crossovers were observed between the sequence corresponding to the pcf6-135 clone and its centromere, placing it within 2 cM of the centromere (Table 4). One crossover between the gene represented by the pcf6-175 clone and its centromere places it approximately 3 cM from the centromere. An additional clone (pcf6-8) whose transcripts accumulate after deflagellation

(SCHLOSS, SILFLOW and ROSENBAUM 1984) does not show linkage to any of the other markers (phenotypic or molecular) used in this study. Its approximate centromere distance is listed in Table 4.

Other molecular markers: The markers *act-2* and mating type (*mt*), as well as a molecular clone corresponding to the radial spoke mutant *pf-14*, were used to mark linkage group *VI*. A sequence homologous to a constitutive clone, pcf8-13 (SCHLOSS, SILFLOW and ROSENBAUM 1984), also maps to this linkage group (Tables 3 and 4, Figure 2). Map distances calculated from the recombination frequency between the pcf8-13 sequence and *act-2*, *pf-14*, and *mt* are consistent with its placement on the left arm of linkage group *VI* approximately 20 map units from the mating type locus (Table 3) and 11 map units from its centromere (Table 4). When the crossovers for each tetrad were analyzed to account for the segregation of all four of the markers, the possible double crossovers were minimized when the pcf8-13 marker was placed on the left arm. Two additional unidentified molecular markers (ef-3a and j-134) do not show linkage to one another or to any of the other markers (phenotypic or molecular) used in this study. Their approximate centromere distances are listed in Table 4.

Centromere markers *ac-17* and *y-1*: The *ac-17* and *y-1* mutations are often used as centromere markers in Chlamydomonas. Our data indicate that *ac-17* is located on the *nit-2* side of the centromere of linkage group *III*. When the *ac-17* data were compared with data of another unlinked centromere marker, *y-1* (linkage group *XVII*) also present in (CC-29), one tetratype tetrad was seen (tetrad 2, Table 1), indicating that a crossover had occurred between either *ac-17* or *y-1* and their respective centromeres. For this same tetrad, tetratype segregation was observed between the *ac-17* and *pf-2* (linkage group *XI*) while parental ditype segregation was observed between *y-1* and *pf-2*, a marker approximately 2 cM from its centromere in *C. reinhardtii*. Tetratype segregation was also observed in this tetrad when *ac-17* was compared with *pyr-1* (linkage group IV) and *nic-13* (linkage group X) which are located within 10 map units of their respective centromeres. Parental-ditype or nonparental-ditype segregation was observed when *y-1* was compared with *pyr-1* and *nic-13*. The simplest explanation for these results postulates a single crossover between *ac-17* and its centromere rather than single crossovers between all four of the other markers listed above and their respective centromeres. Corroborating this conclusion are the centromere distances of *ac-17*, *y-1*, and *pf-2* calculated from the tetratype frequencies obtained when following three markers on different chromosomes according to WHITEHOUSE (1950). Centromere distances calculated using the WHITEHOUSE method are 10, 3 and 0 cM for *pf-2*, *ac-17* and *y-1*, respectively. Because recombination between the molecular markers on linkage group *III* and *ac-17* did not occur in this particular tetrad, it is likely that *ac-17* is located on the *nit-2* side of the centromere along with the α-1 tubulin, pcf6-100, and ef-12e sequences.

Accuracy of map position: To test the accuracy of linkage data generated from 22 tetrads, and to examine the possibility that the genetic map of *C. smithii* may be different from that of *C. reinhardtii*, we calculated the centromere distances of the markers used in our study and compared them with the map positions which were assigned as a result of previous *C. reinhardtii* × *C. reinhardtii* crosses (HARRIS 1988) (Figure 2). The *msr-1* marker was excluded because its map position is too far from its centromere for this analysis to be meaningful. Of the nine markers analyzed, three of them (*mt*, *sr-1* and *ac-17*) mapped to within 3 map units of their location on the *C. reinhardtii* map. In five cases (*pyr-1*, *pf-26*, *pf-14*, *nic-13* and *pf-2*) the markers mapped within 4–10 cM of their *C. reinhardtii* map positions. In contrast, our data place the *act-2* marker 25 map units from its centromere on linkage group VI, or 21 map units closer to its centromere than the position indicated by previous *C. reinhardtii* × *C. reinhardtii* crosses. The centromere distance obtained in this study is also consistent with the linkage distance between *act-2* and *pf-14* (20:0:2 or 5 map units) calculated here. In eight of the nine examples we tested, data generated from the analysis of the 22 *C. reinhardtii* × *C. smithii* tetrads placed markers within ten map units of their *C. reinhardtii* map positions. As the order of additional molecular markers is determined on a linkage group, the localization of specific markers will become more accurate.

DISCUSSION

Mapping the growing number of cloned nuclear sequences from Chlamydomonas will increase the utility of this important experimental system by bridging the gap between genetic and molecular studies. In addition, mapping studies generate fundamental information about the organization of the genome. Once the DNA from the progeny of the *C. reinhardtii* × *C. smithii* cross is isolated, digested, and blotted onto nylon membranes it is possible to use the same set of filters to map a number of different sequences by repeatedly removing old probes and rehybridizing with different clones. The library of filters and DNA samples can be used to map genes for which clones become available in the future. The markers originally used in this study allowed us to detect linkage over 47% of the existing genetic map. Additional molecular markers placed on the map have increased the percentage of the map covered to 54%. We are currently analyzing DNA from progeny of another mul-

tiply marked *C. reinhardtii* strain crossed to *C. smithii* which will make linkage detectable over 78% of the known genetic map. As additional molecular markers are added to the map this percentage will continue to increase and the mapping of newly cloned sequences will become more accurate. The progeny of the *C. reinhardtii* (CC-29) × *C. smithii* cross described above have been sent to the Chlamydomonas Genetics Center for distribution to anyone wishing to use them for mapping purposes.

The use of RFLPs to map genes in Chlamydomonas will greatly facilitate correlations between the increasing number of molecular clones available for Chlamydomonas and the large number of phenotypic mutations which have been reported. For example, information regarding the map positions of all of the tubulin genes has proven extremely useful for the characterization of putative tubulin mutants resistant to antimicrotubule drugs (JAMES *et al.* 1988). In another example, the α-1 tubulin gene and the gene represented by the pcf6-100 cDNA clone map near the *pf-5* mutation on linkage group *III* which is missing multiple flagellar proteins including two radial spoke proteins, an inner dynein arm protein and an unidentified protein (HUANG *et al.* 1981). Thus the *pf-5* phenotype may be caused by a mutation in either the α-1 tubulin gene or the gene represented by the pcf6-100 clone. Further experiments to order the genetic and molecular markers in this region will test this hypothesis.

Previous genetic studies have shown that the *pf-26* mutation maps very close to the *pf-1* locus. In 990 zygote clones or dissected tetrads no recombination between these two loci has been observed (HUANG *et al.* 1981) placing these two mutations within 0.05 cM of each other. The *pf-26* mutation has been shown to alter the primary structure of radial spokehead protein 6 (HUANG *et al.* 1981). Revertant analysis of the *pf-1* mutation (LUCK *et al.*, 1977) provides strong evidence that radial spokehead protein 4 is the mutant gene product of *pf-1*. Our data suggest that the gene represented by the pcf6-187 cDNA clone is within 8 kb of the Rsa-1 sequence (*pf-26*) (WILLIAMS, MITCHELL and ROSENBAUM 1986). Preliminary northern blot data demonstrate that the Rsa-1 and pcf6-187 clones hybridize to different sized transcripts suggesting that they represent different genes (data not shown). The size of the pcf6-187 transcript (1910 nucleotides), as estimated by SCHLOSS, SILFLOW and ROSENBAUM (1984) is consistent with its ability to encode radial spoke protein 4, estimated at 76 kD (PIPERNO *et al.* 1981). The pcf6-187 clone may correspond to the wild-type allele of *pf-1* or to another unidentified flagellar gene which is tightly linked to the genes encoding radial spokehead proteins 4 and 6.

With the possible exception of one region of linkage group *VI*, the genomes of *C. reinhardtii* and *C. smithii*, as sampled in this study, appear to have very similar overall genomic organization. The fact that the two species are interfertile argues against extensive chromosome rearrangements. In addition, our results suggest that most of the RFLPs seen between *C. reinhardtii* and *C. smithii* are due to single base-pair changes, and not to insertions or deletions. Additional evidence indicating that these genomes are similar comes from hybridization experiments using gene specific probes for sequences 5′ and 3′ to the tubulin genes. The hybridization signals we observe are comparable for both *C. reinhardtii* and *C. smithii* DNA (data not shown), indicating that there is homology not only within highly conserved coding regions of the genes, but also within non-coding regions whose sequences are presumably not subject to high selection pressure.

As the number of mapped genomic sequences increases, it will become more feasible to use chromosome walking techniques to clone other genes and specialized regions of interest. For example, genomic sequences corresponding to four different clones (pcf6-135, pcf6-175, ef-3a and gs-1) map within approximately 3 cM of three different centromeres (Table 3). The map positions of the pcf6-135 and pcf6-175 sequences are of particular interest because both of these sequences map near the same centromere. If further experiments indicate that these sequences map to opposite sides of their centromere, then that centromere could be cloned by chromosome walking from the pcf6-135 to the pcf6-175 sequence.

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