Molecular Studies of Linkage Group XIX of *Chlamydomonas reinhardtii*: Evidence against a Basal Body Location

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Abstract. Linkage group XIX (also known as the UNI linkage group) in the green alga, *Chlamydomonas reinhardtii*, exhibits a number of unusual properties that have lead to the suggestion that it represents a basal body-associated chromosome. To begin a molecular analysis of this linkage group, we have identified DNA sequences from it and used them to determine the copy number of linkage group XIX within the cell. We find that linkage group XIX is present in the same copy number per cell as nuclear linkage groups in both haploid and diploid strains. We also find that the copy number of linkage group XIX is unchanged in mutants lacking basal bodies. We conclude that there is no convincing evidence that linkage group XIX localizes to the basal bodies of *Chlamydomonas reinhardtii* cells.

CENTRIOLES are cylindrical organelles composed of triplet arrays of microtubules. They are found at the mitotic poles of dividing cells or adjacent to the nucleus in interphase cells. They can also be found at the proximal ends of flagella as basal bodies, where they are required for flagellar assembly. Apart from this, both their function and their mode of replication are unknown and the subject of some controversy (for reviews see Fulton, 1971; Vorobjev and Nadezhdina, 1987).

The premise that basal bodies and centrioles are at least in part genetically autonomous organelles has been advanced almost since their discovery. The belief is based on two aspects of their behavior through the cell cycle. First, centrioles and basal bodies usually exist in pairs, and new centrioles appear to form adjacent to preexisting centrioles. Second, centrioles appear to duplicate just before nuclear division. These two properties suggested that centrioles control their own duplication, and therefore, could be partially genetically autonomous as well.

Conflicting views hold that there is no compelling evidence to regard centrioles as genetically independent organelles. Pickett-Heaps (1971) argued that their observed distribution simply reflects an efficient mechanism whereby the cell can partition centrioles at mitosis. In this model no genetic autonomy is necessary to explain centriolar replication. Consistent with this interpretation are the observations of de novo centriolar formation in many organisms. *Naegleria*, for example, lack centrioles during the amoeboid phase of their life cycle, but generate them during the transition to the flagellated phase (Fulton and Dingle, 1971). Similarly, no centrioles are detectable in early mouse embryos until the blastocyst stage (Calarco-Gillam et al., 1983). These examples suggest that the genetic information necessary for centriole formation is extrinsic to the assembled organelle.

The possibility that the centrioles, like mitochondria and chloroplasts, contain a distinct genetic entity has spurred numerous attempts to identify nucleic acids in centrioles and basal bodies. These studies have been at best inconclusive (reviewed by Fulton, 1971). Several workers report finding DNA or RNA, but such results have been difficult to evaluate because of problems with the purity of the preparations, the specificity of the signal observed, the sensitivity of the methods used, and the reproducibility of the studies (Hartman et al., 1974; Heidemann et al., 1975; for additional references see Fulton, 1971).

Recently, interest in the question of centriolar autonomy has risen anew, resulting from investigations of the single-celled, bi-flagellated green alga, *Chlamydomonas reinhardtii*. Its basal bodies have been well described (Ringo, 1967; Johnson and Porter, 1968; Cavalier-Smith, 1974; Coss, 1974; Treimer and Brown, 1974; Gould, 1975). Many mutations have been identified that affect flagellar assembly and function, and several of these appear to act at the level of the basal body (Huang et al., 1982; Dutcher, 1986). One of these, the *bald2* mutation, results in the lack of any detectable basal bodies in >98% of mutant cells examined (Goodenough and St. Clair, 1975). Genetic analyses have led to the discovery of a linkage group, linkage group XIX or the UNI linkage group, with three unusual properties (Ramanis and Luck, 1986; Dutcher, 1986). First, genetic analysis of this linkage group generates a meiotically circular map. Second, nearly all loci mapping to this linkage group affect microtubule-based processes. Finally, recombination between loci on this linkage group, but not between loci on other linkage groups, has a temperature-sensitive period early in zygotic maturation. An attractive hypothesis is that this linkage group is localized within the basal body.

Testing this hypothesis has required isolating DNA sequences from the chromosome corresponding to this linkage group. Hall et al. (1989) have reported that DNA sequences

from linkage group XIX identify a 6–9-megabase linear chromosome that localizes to each of the two basal bodies, but not to the nucleus, of *Chlamydomonas reinhardtii* cells. We have independently begun a molecular analysis of linkage group XIX and have found that linkage group XIX is present in the same copy number as nuclear linkage groups. We argue that this contradicts the assignment of a basal body location for this linkage group.

Materials and Methods

Chlamydomonas Strains, Growth Conditions, and Genetics

Chlamydomonas reinhardtii strains used included CC-125 (Harris, 1989), CC-1952 (Gross et al., 1988), and strains carrying the following mutations and associated phenotypes. The *pf10* mutation causes cells to pellet in low light (Randall and Starling, 1971; Dutcher et al., 1988). *fla10* mutants are flagellated and motile at 21°C but aflagellated after 24 h at 32°C (Huang et al., 1977; Adams et al., 1982). Mutations at the *apm1* locus confer resistance to a variety of microtubule inhibitors (James et al., 1988). The *apm1* allele used in this study, *apm1-111*, was isolated in a *Chlamydomonas reinhardtii* strain CC-125 background (Lux and Dutcher, 1991), and was scored by testing for growth on 15 μM oryzalin. The *bald2* strain used in this study was an aflagellate segregant from a cross between the original *bald2* isolate (Goodenough and St. Clair, 1975) and CC-125. This cross was performed using the method of Pasquale and Goodenough (1987). Diploid strains were provided by F. Lux and had been constructed using complementing arginine markers by the method of Ebersold (1967). Diploid strain A was constructed from *arg2 fla10-1 act2 mtP* (plus A) and *arg7 fla10-1 mtM* (minus A) parents. Diploid strain B was constructed from *arg7 fla10-1 mtP* (plus B) and *arg2 mtM* (minus B) parents.

Some *Chlamydomonas reinhardtii* strains were stored frozen at −70°C after growth to high density in liquid media, concentration to one-fifth to one-tenth their original volume, and dimethyl sulfoxide to 7% (vol/vol) added. Details of this procedure will be published elsewhere. *Chlamydomonas reinhardtii* cells were grown in medium I as described by Sager and Granick (1953) with the modification of Dutcher et al. (1988). Genetic techniques were carried out as described by Levine and Eversold (1960). Genetic linkage was determined using the method of Perkins (1952).

DNA Manipulations

Isolation of Chlamydomonas reinhardtii DNA. *Chlamydomonas reinhardtii* DNA was isolated using modifications of a procedure provided by Dr. Curtis Wilkerson (Rockefeller University, New York). Cells were resuspended in ddH_2O and mixed with 4 vol of a solution containing 5% SDS, 10 mM Tris-Cl pH 7.6, 20 mM EDTA, and 1 mg/ml pronase E (Sigma Chemical Co., St. Louis, MO). After overnight incubation at 50°C, one-fifth volume 5 M ammonium acetate was added, the lysate was extracted with an equal volume of phenol/chloroform (50:50), and the aqueous phase was mixed with an equal volume of isopropanol. The precipitate was resuspended in a solution containing 10 mM Tris-Cl pH 7.6, 10 mM EDTA, and 5 μg/ml RNase A and digested for a minimum of 1 h at 37°C. The DNA was then reextracted with phenol/chloroform (50:50), precipitated with isopropanol, and resuspended in 10 mM Tris-Cl pH 8.0, 1 mM EDTA.

Hybridization Conditions. Genomic DNA was digested with the restriction enzymes indicated in the text and then electrophoresed through 0.8% agarose gels prepared in Tris-phosphate-EDTA buffer (Maniatis et al., 1982). The DNA was then transferred to Zetaprobe (Bio-Rad Laboratories, Richmond, CA) membranes in 10× SSC (1× SSC: 0.15 M NaCl, 0.15 M NaCitrate) following denaturation in 0.5 M NaOH–1.5 M NaCl. Filters were prehybridized in 2× SSPE (1× SSPE: 0.18M NaCl, 10 mM sodium phosphate pH 7.6, 1 mM EDTA) 0.2% SDS, 0.5 mg/ml heparin (Singh and Jones, 1984), and 0.3 mg/ml denatured salmon sperm DNA. Hybridizations were carried out with oligo-nucleotide-labeled probes (Feinberg and Vogelstein, 1984) for at least 16 h in 2× SSPE, 0.2% SDS, 0.5 mg/ml heparin and 5–10% dextran sulfate. After hybridization filters were washed once for 5 min in 2× SSC, 0.1% SDS, and then for 90 min in 0.2× SSC, 0.1% SDS at 65°C with three wash changes. Filters were exposed to Kodak XRP x ray film.

Isolation of a DNA Hybridization Probe from the Gulliver H Element. A DNA probe for the Gulliver H element was isolated from the plasmid pGullH, derived from the phage λTcL26 (obtained from Patrick Ferris, Washington University, St. Louis, MO). This phage contains the left end of the Gulliver element copy H and ~4.5 kb of flanking genomic sequences. A 2.5-kb fragment defined by an internal EcoRI site 700 bp from the terminus of the element and a HindIII site in the flanking genomic DNA was isolated and ligated to EcoRI-HindIII-digested pGEM7Z (Promega Biotec, Madison, WI). The resulting plasmid was named pGullH. A probe specific for Gulliver sequences was isolated by digesting pGullH with EcoRI and BglI, which cuts internally in the element 40 bp from the terminus, and isolating the relevant 600-bp fragment.

AMBIS Quantitations

Filters were scanned for 6–16 h on an AMBIS Radioanalytic Imaging System (AMBIS Systems, San Diego, CA). Individual lanes were then traced, and peaks corresponding to each band identified and quantified. The base of each peak was omitted using the multilevel background procedure recommended by the manufacturer.

Results

Identification of DNA Sequences from Linkage Group XIX

We identified DNA sequences that map to linkage group XIX by comparing restriction fragment length polymorphism patterns among three types of *Chlamydomonas reinhardtii* strains: CC-125, CC-1952, and the 18000 series of congenic strains. The first two strains are highly polymorphic in nucleotide sequence (Gross et al., 1988). The 18000 series carry small regions of CC-125 DNA in genetic backgrounds that are otherwise composed mainly of CC-1952 DNA; we refer to these as congenic strains (Ruvkun et al., 1989). The congenic strains were constructed by crossing CC-125 strains carrying mutations in two linkage group XIX genes to CC-1952 (Fig. 1). Progeny that retained the linkage group XIX markers were identified and crossed again to CC-1952. In addition to the regions on linkage group XIX, two other regions of the CC-125 strain were unavoidably retained in the congenic strains. First, the CC-1952 strain carries the mating-type minus allele. Because mating requires opposite mating-types, the mating-type plus allele from the CC-125 parent was selected in each generation. Second, the CC-1952 isolate contains a recessive meiotic mutation that we have designated *ger2*. Diploid zygotes homozygous for this mutation fail to germinate. Therefore, germinating zygotes must carry the CC-125 *GER2* allele as well as the CC-125 mating-type plus allele. These CC-125 genes and surrounding DNA will be present in one-half of the resulting progeny after each cross.

After four rounds of crosses to the CC-1952 parent, approximately 15/16 of the nonselected genome in the congenic strain was, on average, derived from the CC-1952 parent. The 1/16 of the nonselected genome remaining from the CC-125 parent was predicted to be present at random locations, which should differ in independently derived congenic strains. CC-125 DNA sequences should be present near CC-125 markers retained in the construction of the congenic strains. Therefore, a DNA sequence identifying a CC-125 RFLP in several independent congenic strains is likely to be linked to a selected marker. Establishing congenic strains enables one to identify quickly RFLPS linked to loci of interest, especially when independent congenic strains are generated. For example, the chance of seeing a CC-125

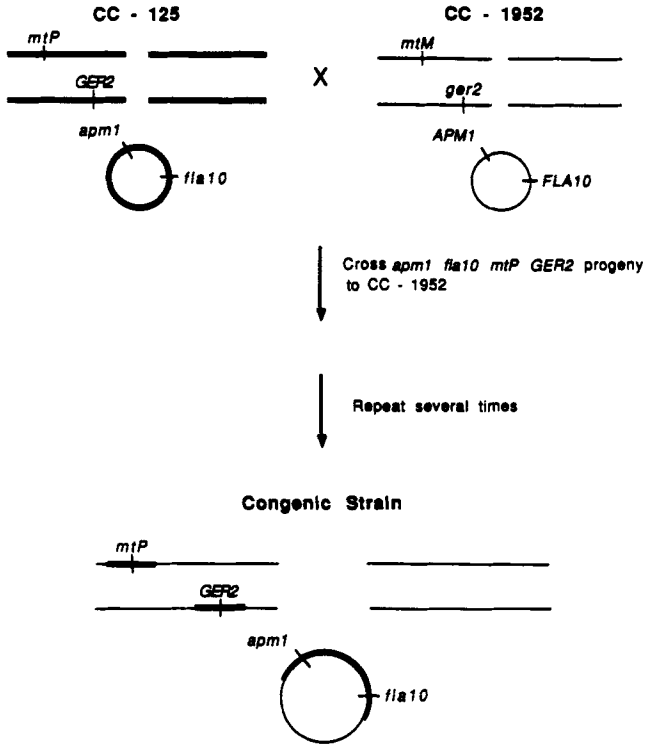

Figure 1. Construction of congenic lines. Congenic strains were constructed following the schematic procedure outlined above. Thick and thin lines represent linkage groups from the CC-125 and CC-1952 parents, respectively. In this example, congenic strains were constructed with the *apm1* and *fla10* mutant alleles. Repeated crosses of spores with the mating-type plus, *GER2*, and *CC-125* linkage group XIX mutant alleles to CC-1952 led to the replacement of CC-125 DNA sequences with CC-1952 sequences in regions not closely linked to the selected markers. In this map the region of the map between the *apm1* and *fla10* loci is shown as being derived from the CC-125 parent. For this region to contain CC-1952 DNA, a double exchange would have had to occur between the selected CC-125 loci. This is unlikely, because the *apm1* and *fla10* loci are sufficiently close to each other that double exchange events are unlikely to occur (Dutcher, 1986; James et al., 1988). Congenic strains were also established by repeatedly selecting for either the *apm1* and *pf10* mutant alleles, or the *fla10* and *pf10* mutant alleles.

RFLP pattern from a region unlinked to the selected markers in three independently derived fourth generation congenic strains is $((1/2)^4)^3$, or 1 in 4,096.

We examined the congenic strains for copies of two previously described *Chlamydomonas reinhardtii* transposons, TOC1 (Day et al., 1988) and Gulliver (Ferris, 1989). Both elements are present in multiple copies in CC-125. In CC-1952, TOC1 is present in low copy number and Gulliver is absent. Only one congenic strain out of four independent strains examined contained a *TOC1* element from the CC-125 parent (data not shown). We did, however, find several copies of the Gulliver sequence in fourth and fifth generation congenic strains. A representative hybridization pattern of Gulliver probes to DNA isolated from the CC-125 strain and congenic strains is shown in Fig. 2. The congenic strains shown are 18200, which carries the *apm1* and *pf10* mutations; 18303, a fourth generation congenic strain which carries *apm1* and *fla10* mutations; and 18475, a fourth generation strain, which carries the *pf10* and *fla10* mutations. Gulliver

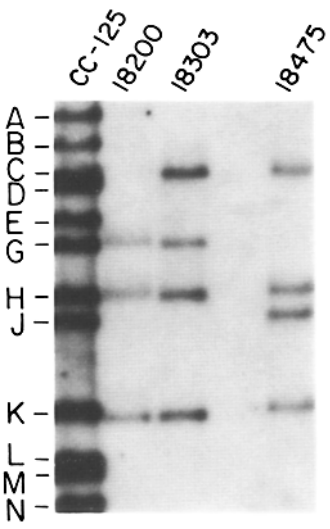

Figure 2. Hybridization pattern of Gulliver elements in congenic lines. DNA was isolated from the CC-125 parent and three congenic strains, and digested with HindIII. After electrophoresis through a 0.8% agarose gel, the DNA was transferred to a nylon membrane and hybridized to a 700-bp EcoRI-BglI fragment from the Gulliver element H. The resulting autoradiogram is shown. Bands G through N are labeled as described by Ferris (1989), while bands of higher molecular weight than this may correspond to different bands.

elements C and K were found in 3 and 5 of 13 independent congenic strains examined, respectively. The Gulliver element G was found in three congenic strains carrying the *apm1* and *pf10* mutations and in two strains carrying the *apm1* and *fla10* mutations. The five strains had been separated from each other for a total of 19 generations; therefore, the chance of element G residing in a nonselected CC-125 region of the congenic strains is $(1/2)^{19}$. Similarly, Gulliver element H was found in two strains carrying the *fla10* and *apm1* mutations, and in eight strains carrying the *pf10* and *fla10* mutations. The chance of obtaining this pattern in these 10 strains for an unlinked CC-125 sequence is $(1/2)^{31}$. Thus, copies of Gulliver elements C, G, H, and K are linked to loci on linkage group XIX or to another selected CC-125 region in the congenic strains.

To determine which selected regions from CC-125 contain the Gulliver elements, the Gulliver elements were scored as genetic markers in crosses between CC-125 strains carrying mutations from linkage group XIX and the CC-1952 strain. The resulting tetrad analysis is shown in Table 1. We found that elements C and K are unlinked to linkage group XIX markers. They are linked to the *GER2* locus. In 20 out of 22 random spores the Gulliver element K cosegregated with the *GER2* allele (data not shown). The remaining two elements, G and H, map to linkage group XIX (Table I). Gulliver ele-

Table I. Tetrad Mapping of DNA Sequences to Linkage Group XIX

	apm1	*fla10*	*pf10*	Gulliver G	Gulliver H
Gulliver G	11:0:3	3:0:20	0:0:14	–	5:0:20
Gulliver H	2:0:14	21:0:15	18:0:8	–	–

Linkage of Gulliver element G, and Gulliver element H to linkage group XIX loci *apm1*, *pf10*, and *fla10* was determined by assessing the segregation of these loci in crosses of CC-125 strains carrying two linkage group XIX mutations to CC-1952 strains. CC-125 parents in the crosses carried either the *apm1* and *fla10* mutations, the *fla10* and *pf10* mutations, or the *pf10* and *apm1* mutations. The number of parental ditype (PD), nonparental ditype (NPD), and tetratype (T) tetrads is shown (PD:NPD:T). Gulliver elements C and K were found to be unlinked to linkage group XIX markers (element C to *apm1*: 0:3:7, and element K to *apm1*: 0:3:6). By $\chi 2$ tests, Gulliver element G and Gulliver element H show linkage to at least one other locus on linkage group XIX ($P = 0.01$).

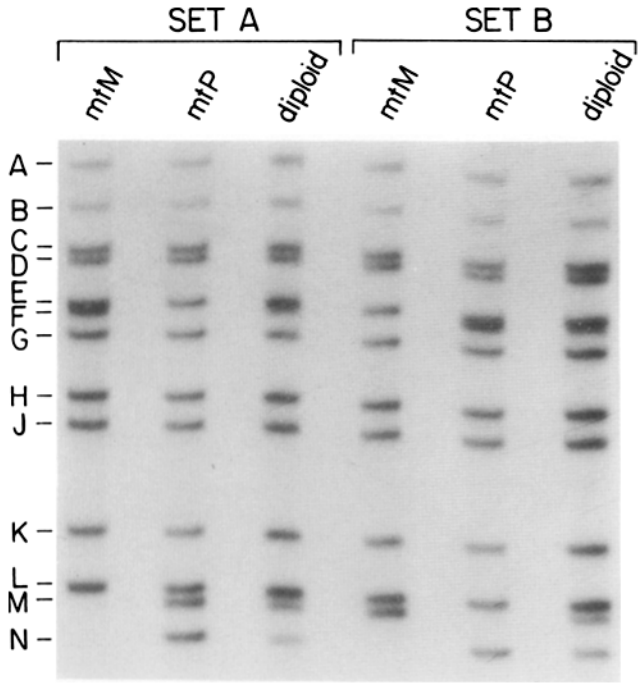

Figure 3. Hybridization pattern of Gulliver elements in haploid and diploid strains. DNA was isolated from two diploid strains, diploid A and diploid B, and from the haploid strains used to construct them (plus A and minus A, and plus B and minus B, respectively). The Gulliver hybridization pattern was then determined as described in the legend to Fig. 2. The resulting autoradiogram is shown. The minus A parent lacks Gulliver elements M and N, while the plus A parent lacks Gulliver element F. The minus B parent lacks elements N and F, and the plus B parent lacks element M. Gulliver elements G and H map to linkage group XIX.

ment G maps 11 centiMorgans from the *apm1* locus, and Gulliver element H maps 15 centiMorgans from the *pf10* locus.

Linkage Group XIX Is Present in the Same Copy Number as Nuclear Linkage Groups

Hall et al. (1989) have proposed that linkage group XIX is located within the basal bodies themselves and acts as a basal body-associated genome. A simple test for establishing whether linkage group XIX represents a basal body chromosome is to determine its copy number in the cell relative to nuclear linkage groups. If each basal body contains a copy of linkage group XIX, then haploid cells will contain at least two copies of DNA from this linkage group. Alternatively, if linkage group XIX DNA resides in the nucleus, then its copy number should be the same as other nuclear linkage groups in both haploid and diploid strains. To distinguish between these hypotheses requires an assay that is sensitive enough to distinguish a twofold difference in copy number between DNA sequences on different chromosomes.

The presence of Gulliver elements on linkage group XIX, as well as on other linkage groups in strain CC-125 (Ferris, 1989), makes this analysis straightforward. By comparing the relative hybridization intensity of Gulliver elements on linkage group XIX to the hybridization intensity of Gulliver elements that reside on other linkage groups, we could determine directly the copy number of linkage group XIX. In this way, the relative amounts of DNA sequences from different linkage groups were compared within the same DNA sample using the same probe. The choice of the appropriate probe in this experiment is crucial because it is possible to underestimate the copy number of linkage group XIX if Gulliver elements from linkage group XIX do not hybridize to the probe sequence as well as Gulliver elements that reside on other linkage groups. To avoid this problem, we used a probe derived from the Gulliver H element, which maps to linkage group XIX. The use of a probe from this element ensured that hybridization to at least one of the elements on linkage group XIX was not underestimated if sequences in the probe have diverged from other Gulliver elements.

We first established that a twofold difference in hybridization intensity between Gulliver elements could be detected. To determine whether our conditions were sufficiently sensitive, we compared the signal intensities of Gulliver elements

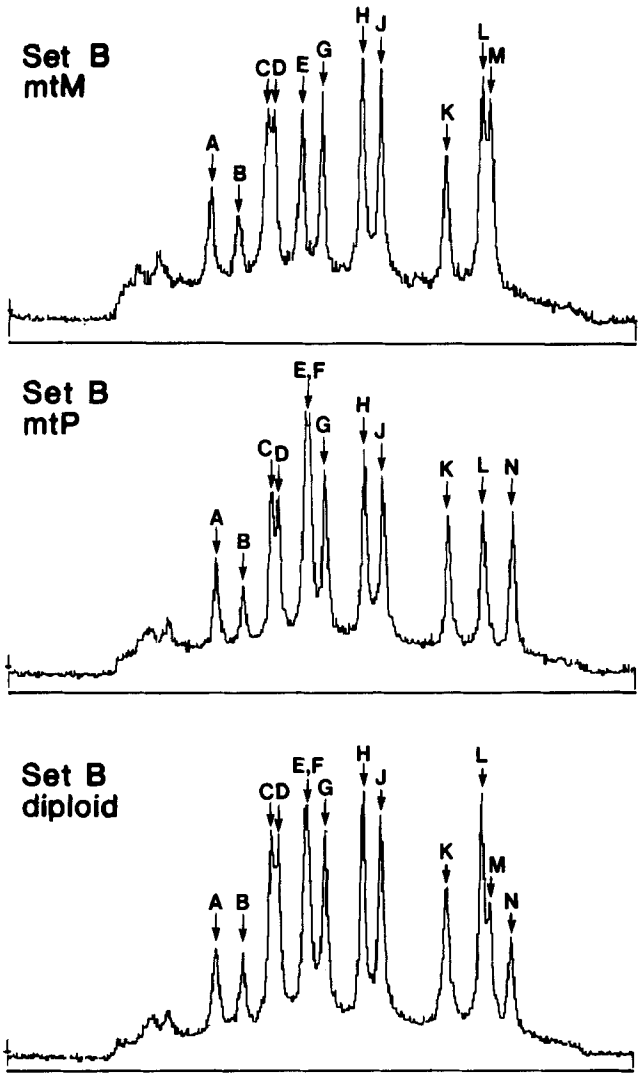

Figure 4. AMBIS scanning profiles of Gulliver hybridization patterns in haploid and diploid strains. To determine the relative hybridization intensity of each Gulliver band, the filter that generated the autoradiogram in Fig. 3 was scanned with an AMBIS Radioanalytic Imaging System. Representative scans of the filter containing DNA from the diploid B strain and its two haploid parents are shown. The arrows indicate the peaks that correspond to each Gulliver band.

Table II. Relative Intensities of Representative Gulliver Elements

Elements compared	Set A			Set B		
	Minus A	Plus A	Diploid A	Minus B	Plus B	Diploid B
G/E	ND	0.86 ± 0.1	ND	0.98 ± 0.2	ND	ND
H/J	0.86 ± 0.1	1.05 ± 0.2	1.08 ± 0.1	1.10 ± 0.2	1.06 ± 0.2	1.06 ± 0.03
M/L	ND	0.92 ± 0.3	0.40 ± 0.1	0.88 ± 0.3	ND	0.46 ± 0.1
N/L	ND	0.80 ± 0.2	0.23 ± 0.1	ND	0.93 ± 0.2	0.43 ± 0.1

The number of counts corresponding to the indicated Gulliver elements was determined using an AMBIS Radioanalytic Imaging System, and the relative signal of different elements is expressed as a ratio. The ratio represents the mean from four trials. The error represents one standard deviation from the mean. The ratio of Gulliver element G to element E was only determined in strains lacking element F, which comigrates with element E. The ratio of Gulliver element M to L and of Gulliver element N to L could not be determined in the haploid strains lacking M or N.

M or N to Gulliver element L in two different diploid strains and in the haploid strains used for their construction. In these strains, elements M and N were present in only one of the haploid parents, while element L was present in all haploid parents. Thus, the ratio of M to L, and of N to L, in a diploid strain should be one-half that observed in a haploid strain. The relatively lower hybridization intensity of Gulliver element M and N in diploid strains is evident from the autoradiogram (Fig. 3).

To confirm independently that a twofold difference in hybridization intensity could be detected, the experiment shown in Fig. 3 was repeated three additional times. For each trial, the filter was scanned with an AMBIS Radioanalytic Imaging System and the signal corresponding to each Gulliver element quantified. A profile of the gel in Fig. 3 that contains the diploid B strain and its two parents is shown in Fig. 4. The areas of the peaks corresponding to elements M or N, compared to the peak for element L, are markedly lower in the diploid strain compared to the same peaks in haploid parents. The twofold drop in hybridization intensity is further illustrated in Table II. For the diploid A strain, the ratio of Gulliver element M signal to that of element L was 0.40 ± 0.1, while the M to L ratio in the mating-type plus A parent was 0.92 ± 0.3. A similar twofold drop was observed in the M to L ratio in the diploid B strain relative to the haploid mating-type minus B parent. The signal ratio corresponding to element N relative to that of element L also decreased by one-half in both diploid strains relative to their mating-type plus parents. We concluded that our conditions were sensitive enough to detect a twofold difference in relative hybridization intensity.

The relative copy number of linkage group XIX was then determined by comparing the hybridization of Gulliver elements from linkage group XIX to Gulliver elements from other linkage groups that exhibited similar gel mobilities. We compared the relative signal intensity of element H, which maps to linkage group XIX, to element J, which maps to linkage group VI (Ferris, 1989). Similarly, we compared the relative hybridization intensity of element G to element E in the mating-type plus A parent and in the mating-type minus B parent. These strains lack element F, which comigrates with element E (Fig. 3). The hybridization signal observed to elements G and H was no stronger than that observed to Gulliver elements E and J, respectively (Fig. 3; Table II). For element G, the hybridization relative to element E was 0.86 ± 0.1 in the mating-type plus A strain and 0.98 ± 0.2 for the mating-type minus B strain. Gulliver elements H and J show nearly identical hybridization efficiencies in all strains examined, including both haploid and diploid strains (Table II). We concluded that linkage group XIX is present in the same copy number per cell as other linkage groups in both haploid and diploid cells.

We also examined the relative copy number of Gulliver elements in strains with a mutation that affects basal body number. One such mutation is *bald2*, in which over 98% of cells lack any structures resembling basal bodies (Goodenough and St. Clair, 1975). We isolated DNA from the *bald2* mutant strains and examined the Gulliver hybridization pattern (Fig. 5). In this experiment we included a lane containing DNA from the diploid A parent. The reduced hy-

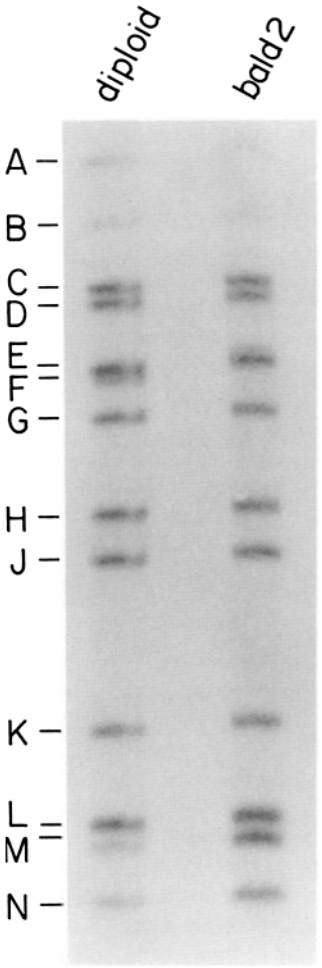

Figure 5. Hybridization pattern of Gulliver elements in *bald2* strains. DNA was isolated from the diploid A parent and a strain carrying the *bald2* mutation. The Gulliver hybridization patterns were determined as described in the Fig. 2 legend.

bridization intensities of Gulliver elements M and N relative to Gulliver element L in the diploid A lane demonstrate that a twofold decrease in hybridization intensity would have been easily detectable under the conditions used. We found that the hybridization intensity of Gulliver elements G and H was the same in *bald2* mutant strains as in wild-type strains. Therefore, we conclude that the copy number of linkage group XIX is unchanged in this mutant strain, despite the absence of basal bodies in >98% of the cells.

We also examined the relative copy number of linkage group XIX in strains that carry the *vfl2* mutation. The *vfl* phenotype is characterized by cells carrying a variable number of flagella per cell. Therefore, these cells carry a variable number of basal bodies as well. Populations of cells that carry the *vfl2* mutation carry from zero to six flagella per cell, although <15% of the cells in a population carry more than two flagella (Kuchka and Jarvik, 1982). We found no change in the relative hybridization intensities of Gulliver elements G and H in DNA isolated from cells with the *vfl2* mutation (data not shown). Because most cells in a population have zero, one, or two flagella, however, the average number of flagella per cell using the distributions reported by Kuchka and Jarvik (1982) is between one and two per cell. Thus, the result obtained with DNA isolated from cells carrying the *vfl2* mutation do not distinguish between a basal body location for linkage group XIX or a nuclear location.

Discussion

To understand the physical basis for some of the unusual genetic properties of linkage group XIX in *Chlamydomonas reinhardtii*, we have initiated a molecular analysis of this chromosome. We report the isolation of DNA sequences from this linkage group and their use in determining its copy number in the cell. By carefully determining its copy number relative to nuclear linkage groups, we can make inferences about its cellular location. The simplest explanation for our data is that linkage group XIX corresponds to a nuclear chromosome. This is consistent with our finding that its copy number is the same as nuclear linkage groups in both haploid and diploid cells. In addition, a nuclear location for this linkage group agrees with our observation that the copy number of linkage group XIX does not change in cells that lack basal bodies.

An alternative model, which is not excluded by our data, is that linkage group XIX normally localizes to only one of the two basal bodies in haploid cells. In diploid cells, which also contain two basal bodies, our data do not distinguish between a model in which two copies of linkage group XIX reside in one basal body, or a model in which one copy of linkage group XIX would be found in each basal body. In cells lacking basal bodies, such as those carrying the *bald2* mutation, linkage group XIX must localize elsewhere in the cell. In this model, the means by which linkage group XIX is segregated would be markedly different in haploid cells, diploid cells, and in cells carrying the *bald2* mutation.

We consider this model for localizing linkage group XIX to the basal bodies unsatisfactory for several reasons. First, there is no evidence that linkage group XIX localizes to only one of the basal bodies in either haploid or diploid cells. Second, if both copies reside in one basal body, then it is unclear how two copies of a chromosome of this size are packed in the volume available within one basal body. The difficulty in packing even one copy of a chromosome of this size into the interior of a basal body has been discussed previously (Hall et al., 1989; Johnson and Rosenbaum, 1990). Alternatively, if linkage group XIX is present in both basal bodies in diploid cells, then its properties must be much different in this cell type than in haploid cells, where only one of the two basal bodies could contain a copy.

Our conclusion that linkage group XIX resides in the nucleus differs dramatically from that of Hall et al. (1989), who reported that linkage group XIX is a basal body chromosome present in two or more copies per haploid cell. They base their conclusion on two sets of experiments. First, they found that linkage group XIX was present in two or more copies per haploid cell based on the result of genomic Southern blots. Second, they observed labeling over both basal bodies in in situ hybridization experiments using DNA probes derived from linkage group XIX. For both experiments it is likely that inadequately controlled experimental conditions are responsible for their interpretations.

In the determination of the copy number of linkage group XIX (called the ULG in their study), Hall et al. (1989) compared the signals obtained from a calculated amount of genomic DNA equivalents to signals observed against dilutions of a calculated amount of a reference DNA. This method is clearly less accurate than the one used in the present study, in which hybridization signals were compared within the same lane using the same probe. The method of Hall et al. (1989) is unsatisfactory because errors could be made in calculating the absolute genome size, calculating the number of genome equivalents present in the reference DNA, or in signal variation arising from comparisons of DNA samples in different lanes. These sources of error occur twice, because two different blots are analyzed, one with a probe from linkage group XIX and one with a probe from another linkage group. These errors could have combined to produce a twofold error in their determination of linkage group XIX copy number.

The in situ hybridization data of Hall et al. (1989) are more difficult to reconcile with our data. They observed labeling over each of the two basal bodies with probes that contain DNA sequences from linkage group XIX. We offer several possible explanations for why our data conflict. The simplest explanation for their in situ results is that the hybridization observed is due to artifactual staining and is not specific for DNA. This can be seen upon close inspection of a cell hybridized with a probe named λ19 (Fig. 8, row *C* in Hall et al., 1989). Strong DAPI staining was observed in this cell to both the nucleus and the basal bodies. The results of an in situ hybridization experiment with the λ19 probe to the same cell showed a signal only in the region of the basal bodies. The absence of hybridization to the nucleus is surprising, because this probe contained repeated sequences dispersed throughout the genome, and the DAPI image showed clearly that DNA was present in the nucleus. Given that this probe was unable to hybridize to nuclear sequences under conditions in which basal body hybridization was quite strong, it seems imprudent to conclude that the basal body signal represents hybridization to DNA. This interpretation is consistent with the results of Johnson and Rosenbaum (1990), who find no evidence for the presence of DNA in *Chlamydomonas reinhardtii* basal bodies.

An alternative explanation for the in situ hybridization results is that the repetitive sequences within the probes hybridized to a specific basal body-associated nucleic acid that does not correspond to linkage group XIX. Two of the three probes used in the in situ hybridization experiments contained repetitive DNA sequences. The one single copy sequence used did not display a hybridization signal over the basal bodies. The repetitive sequences in the other probes may have hybridized to some as yet unidentified nucleic acid. We argue above, however, that such a nucleic acid is unlikely to be DNA. It is possible that this nucleic acid is RNA. Heidemann et al. (1975) have presented evidence that the ability of *Chlamydomonas reinhardtii* basal bodies to nucleate aster formation in unfertilized *Xenopus* eggs is sensitive to RNase. Hall and co-workers (1989), however, report that their in situ hybridization signals are insensitive to treatments with RNase A and RNaseH. If the signal represents hybridization to RNA, this RNA species must be resistant to these enzymes under their experimental conditions.

A third way in which the in situ hybridization results could be reconciled with our data would be if the signals arose from nuclear DNA sequences collapsed around the basal bodies. A mechanism for this is suggested by the work of Salisbury et al. (1987). They observed that if *Chlamydomonas reinhardtii* cells are subjected to mechanical shear, pH shock, or exposed to calcium, the nucleus contracts to the anterior end of the cell into the vicinity of the basal bodies. If the extremely harsh treatments used to obtain basal body staining in the work of Hall et al. resulted in a similar displacement of the nucleus towards the basal bodies, then it is possible that the hybridization observed over the basal bodies actually reflects hybridization to nuclear DNA.

Properly performed in situ hybridization experiments will allow the location of linkage group XIX to be addressed directly. At the present time, however, this approach lacks the necessary sensitivity. Published procedures show it is possible to localize nuclear ribosomal RNA genes to the nucleus (Hall et al., 1989), but these genes occur in several hundred tandem copies per cell (Howell, 1972). Single copy nuclear sequences have not been localized in *Chlamydomonas reinhardtii*. Until this positive control is performed, it is premature to rule out a nuclear location for linkage group XIX based on the inability to observe nuclear staining in in situ hybridization experiments. When those conditions are in hand, we predict that in situ hybridization experiments will demonstrate that linkage group XIX localizes to the nucleus.

There are two additional ways to reconcile our results with the in situ hybridization data. First, one may imagine that haploid strains carry two copies of each nuclear linkage group. There is no evidence, however, to propose that cells that appear haploid by both genetic and cytological criteria are actually 2n. The observed mutation rates in *Chlamydomonas reinhardtii* haploid strains are consistent with one copy of each chromosome in a cell (Luck et al., 1977; Dutcher and Gibbons, 1988). Cytological studies of *Chlamydomonas reinhardtii* chromosomes yield chromosome numbers approximately equal to the number of linkage groups (Loppes et al., 1972; Storms and Hastings, 1977; Dutcher et al., 1991). A final possibility is that the copy number of linkage group XIX is actually 1/2n, with each basal body carrying a single strand of DNA. We consider this idea untenable. No evidence exists for such a single-stranded DNA in *Chlamydomonas reinhardtii*. The extremely gentle lysis procedures used in isolating intact chromosomal DNA for pulsed-field gel electrophoresis suggest linkage group XIX has the mobility of a 6–9 megabase double-stranded DNA molecule (Hall et al., 1989).

Linkage group XIX remains an enigmatic linkage group, but the results reported here suggest that in at least two respects it is similar to other linkage groups in *Chlamydomonas reinhardtii*. First, we find linkage group XIX exists in the same copy number as other linkage groups in both haploid and diploid cells. Thus, no unusual models need be proposed to explain how a genetically haploid linkage group corresponds to a physically diploid chromosome, which would be the case if this chromosome resided in the basal bodies (Hall et al., 1989). Second, the presence of transposons, along with other dispersed repetitive sequences (Hall et al., 1989), on linkage group XIX demonstrates that this linkage group contains sequences other than those affecting microtubule-based processes. Moreover, we have recently identified two loci affecting tryptophan metabolism that map to linkage group XIX (Galloway, R. E., and S. K. Dutcher, unpublished observations). These loci provide the first examples of linkage group XIX mutations that do not affect microtubule function. Further work will determine if linkage group XIX is indeed significantly different from other linkage groups in *Chlamydomonas reinhardtii*. In any event, in the absence of any compelling evidence for a basal body location, its genetic properties should be evaluated in the context of a nuclear location.

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