CHLOROPLAST GENETICS OF CHLAMYDOMONAS. III. CLOSING THE CIRCLE

BURT SINGER, RUTH SAGER,[1] AND ZENTA RAMANIS[2]

Columbia University, New York, N.Y. 10025
and
Hunter College of the City University of New York, N.Y. 10021

Manuscript received October 20, 1975
Revised copy received January 27, 1976

ABSTRACT

A novel mapping procedure is presented for organelle genes or any other genetic system exhibiting a measurable frequency of exchanges occurring at a constant rate over a measurable time interval. For a set of markers in a multiply-marked cross, the exchange rates measure relative map distances from a centromere-like attachment point.

With this method, we present mapping data and a linear map of genes in the chloroplast genome of Chlamydomonas. The data are plotted as log (percent remaining heterozygotes) against time and map distances are taken as proportional to slope.

A statistical method which is an adaptation of jackknife methodology to a regression problem was developed to estimate slope values. A single line is fitted to pooled data for each marker from several crosses, and then lines are re-fit to a series of pooled data sets in each of which the observations from a single cross have been omitted. From these data sets a final summary slope is computed as well as a statement of its variability. The relative positions of new markers present in single crosses can then be estimated utilizing data from many crosses.

The method does not distinguish between one-armed and two-armed linear or circular maps. However, evaluation of this map in conjunction with cosegregation frequency data (SAGER and RAMANIS 1976b) provides unambiguous evidence of the genetic circularity of the Chlamydomonas chloroplast genome.

THIS paper presents a novel mapping procedure designed to establish relative map distances for chloroplast genes from a postulated centromere-like region (attachment point, *ap*) in chloroplast DNA (SAGER and RAMANIS 1970, 1976a; SAGER 1972). The ordering of genes relative to an attachment point does not distinguish between one-armed and two-armed linear maps or between linear and circular maps. However, this ordering is an essential piece of evidence which, when combined with co-segregation frequencies (SAGER and RAMANIS 1976b), establishes a unique gene arrangement and provides unambiguous evidence of genetic circularity of the Chlamydomonas chloroplast genome.

[1] Present address: Sidney Farber Cancer Center, Harvard Medical School, 35 Binney Street, Boston, Massachusetts 02115.
[2] Prsent address: Rockefeller University, 66th and York Avenue, New York, N.Y. 10021.

In previous studies (SAGER and RAMANIS 1968, 1970, 1976a, 1976b), we demonstrated that when zygotes from a genetically marked cross germinate, they give rise to zoospores which are initially heterozygous for each of the chloroplast markers in the cross. During growth of zoospore clones the markers segregate and recombine at a rapid rate so that after about 10 doublings, virtually all cells are homozygous for all markers. Sampling the population at prescribed points in time and plotting log (percentage of surviving hets) *versus* time reveals that:

(i) there is a linear relationship between log (percent surviving hets) and time, and

(ii) each gene can be associated with a line of different slope, which represents the segregation rate for that gene pair.

The basic interpretation of (i) and (ii) for mapping purposes is that the larger the absolute value of the slope associated with a particular gene, the further the gene is from the attachment point.

In numerous experiments of the kind described above, we found that the relative gene order remained constant from cross to cross but that differences were seen in slope values for the same genes in different crosses. This cross-to-cross variability arises from two principal sources:

1) The shift from synchronous doubling time to random doubling time as cell division proceeds. This shift is important because we use absolute time as as measure of biological (doubling) time.

2) Uncontrolled environmental and genetic variability between crosses.

These sources of variability were reduced experimentally by maintaining constant growth rates within and between experiments; and by choosing relatively isogenic parental strains (SAGER and RAMANIS 1976b).

A statistical method was then developed not only to assess the residual variability but also to provide the following information:

1) A summary slope for each of the markers present in several crosses.

2) An adjusted slope for each marker in each cross which is based not only on the observations in that cross but also on the variability intrinsic to the full data set.

3) A means to utilize data on the markers present in several crosses for positioning new markers present in single crosses.

The model underlying our interpretation of slope data has been presented previously (SAGER 1972; SAGER and RAMANIS 1976a, 1976b). We have described two modes of segregation of heterozygous markers in vegetative clones: Type II, giving rise to one *het* and one segregated offspring; and Type III, giving rise to two segregated offspring, each homozygous for one of the parental alleles. As previously reported (SAGER and RAMANIS 1976b) Type II events occur with equal frequency for each of the 15 genes examined; whereas Type III events occur with a different and characteristic frequency for each gene. We have proposed that the relative frequencies of Type III events measure the relative distances of each gene from a centromere-like attachment site, much as gene-centromere distances are estimated in the ordered asci of Neurospora. In the Chlamydomonas

chloroplast, however, the mode of distribution is presumed to be mitotic-like since the majority of progeny remain *hets* and reduction to homozygosity requires an exchange.

MATERIALS AND METHODS

The stocks used in these experiments and the crosses investigated are listed in Table 1; they are a subset of the crosses reported in a previous paper (SAGER and RAMANIS 1976a). Media and methods including the crossing procedure are as previously described (SAGER and RAMANIS 1976a).

For kinetic analysis of segregation, zygote plates containing about $2–4 \times 10^4$ mature zygotes were treated with chloroform vapor for 30 sec to kill residual vegetative cells 5–6 days after mating, and 24–48 hours later, zygotes were germinated by exposure to light for about 18 hours. Cells were checked under the microscope to determine when germination was complete. As soon as zygotes had divided, the germinated zoospores were washed off into acetate containing medium by adding 3 ml medium to each plate, and rubbing gently with a glass spreader. Only zoospores are released by this procedure; zygotes and partly germinated zoospores remain tightly bound to the agar.

Zoospore cell concentrations were adjusted to about 1×10^5 cells/ml, in acetate medium, and aliquots were plated once per 60 or 120 minutes. Cells were plated on acetate-agar for replica plating, and on minimal medium to score for acetate requiring mutants. Plates were replica-plated after 5–6 days incubation in light and were scored for acetate requirement after 6 days.

Colonies were scored for drug resistance by replica plating from the acetate plates to acetate medium with and without the drug. Acetate mutants were scored by inspection of minimal

TABLE 1

Crosses used in this study

Cross	P_1 (*mt+*)		P_2 (*mt-1*)
7	*ac1 ac2+ sm2-s ery-s spc-r car-s tm-r* 11,932–4	×	*ac1+ ac2 sm2-r ery-r spc-s car-r tm-s* 11,927–1
8	*ac1 ac2+ sm2-s ery-s cle-s spc-r tm-r* 11,932–4	×	*ac1+ ac2 sm2-r ery-r cle-r spc-s tm-s* 11,907–3
9	*ac1+ ac2 sm2-r ery-r ole-r spc-s tm-s* 11,968–6	×	*ac1 ac2+ sm2-s ery-s ole-s spc-r tm-r* 11,909–3
10	*ac1+ ac2 sm2-r ery-r spi-r spc-s tm-s* 11,978–3	×	*ac1 ac2+ sm2-s ery-s spi-s spc-r tm-r* 11,909–3
11	*ac1 ac2+ sm2-r ery-r car-r spc-s tm-s* 11,925.5	×	*ac1+ ac2 sm2-s ery-s car-s spc-r tm-r* 11,927–2
12	*ac1+ ac2 sm2-r ery-s spc-s tm-s* 13,363–4	×	*ac1 ac2+ sm2-s ery-r spc-r tm-r* 13,363–7
13	*ac2+ sm2-s spc-s mr1-r tm-r* 12,632–6	×	*ac2 sm2-r spc-r mr1-s tm-s* 13,181–6
14	*ac2+ sm2-s spc-s mr2-r tm-r mt+* 12,674–1	×	*ac2 sm2-r spc-r mr2-s tm-s mt—* 13,181–6
15	*ac2+ ac3 sm2-s ery-s spc-s tm-r* 7788–12	×	*ac2 ac3+ sm2-r ery-r spc-r tm-s* 13,181–6
16	*ac4 sm2-r ery-s spc-s tm-s* 7860–19	×	*ac4+ sm2-s ery-r spc-r tm-r* 11,932–4
17	*ac1 sm2-r ery-r kan-s tm-s* 11,925–5	×	*ac1+ sm2-s ery-s kan-r tm-r* 11,482–4

plates: *ac1* mutants give small light green colonies, *ac2* mutants give pin-point colonies, and *ac1/ac2* heterozygotes give medium sized, medium green colonies that are readily distinguished from *ac1* homozygotes and from rare wild types.

Zero-time samples were expected to consist 100% of heterozygotes, but in fact some parental-type colonies were always found. They may have resulted from incomplete killing of residual gametes by chloroform, as well as premature germination and zygotic segregation. All homozygotes present at zero-time were disregarded in the computations, by normalizing the values to 100% heterozygotes. By this means, only segregations occurring after zoospore germination were included in the reported results.

RESULTS

1. *The Statistical Method*

The statistical analysis was developed to provide a rationally based method for:

(a) fitting straight lines to the experimental data for each marker in each cross,
(b) providing a reliable summary line for each marker,
(c) assessing the variability between crosses.

The primary difficulty in the data was the small number of time points in each cross (typically 5 points) as well as the variable environment from cross to cross.

The procedure developed below may be viewed as an adaptation of jackknife methodology to a regression problem. The basis of the jackknife, in the present instance, is that taking one marker at a time we fit a single line to the pooled data from all the crosses and then re-fit lines to a series of pooled data sets in each of which the observations from a single cross have been omitted. These calculations provide the ingredients for our final summary slope, a statement of its variability, and an adjusted slope for each cross which is based not only on the five observations in that cross but also on the variability intrinsic to the full data set.

For each marker in each cross we require the computation of three numbers:

(i) the sum of (log {percent surviving hets} × (observation time))
(ii) the sum of observation times
(iii) the sum of (observation time)^2.

Then we compute the value

$$b_{all} = \frac{\text{sum over all crosses of (i)} - 2 \times \text{sum over all crosses of (ii)}}{\text{sum over all crosses of (iii)}}$$

which is simply the slope of a least squares line fitted to the pooled data from all crosses. This is *not*, however, our final summary slope. For the final slope value, we first prepare slope estimates b_j, where $j = 1,2, \ldots N_c$ (N_c = total number of crosses involving the marker under consideration), computed using the same recipe as in b_{all} except that cross j is omitted. Then we compute the adjusted slope $\bar{b}_j = N_c\, b_{all} - (N_c - 1)\, b_j$, to be associated with cross j. The virtue of this estimate over a simple least squares estimate, based only on the small amount

of data in a single cross, is such that much of the bias in a simple least squares estimate which can arise due to excessively small sample sizes, has been removed. A final summary slope is then defined as the average of these less biased estimates, i.e.

$$b_{final} = \frac{1}{N_c} \sum_{j=1}^{N_c} \bar{b}_j$$

The jackknife estimate, b_{final}, is our best slope estimate for each marker. An estimate of its variance is given by

$$s^2 = \frac{\sum_{j=1}^{N_c} \bar{b}_j^2 - \frac{1}{N_c}\left(\sum_{j=1}^{N_c} \bar{b}_j\right)^2}{N_c\ (N_c - 1)}$$

and a 95% confidence interval for b_{final}, interpreted as a measure of internal variability in the data, is

$$b_{final} \pm t_{(N_c-1)}{}^{S}$$

where $t_{(N_c-1)}$ is the .975 percentage point from student's t-distribution with $(N_c - 1)$ degrees of freedom. For a detailed discussion of the jackknife and other illustrations of its use, the reader should consult MOSTELLER and TUKEY (1968) and MILLER (1974).

2. *Growth Rates*

An important factor in comparability of segregation rates from one cross to another is the growth rate of the culture. Under our conditions (see MATERIALS and METHODS) zygotes germinate synchronously as judged by miscoscopic inspection, and randomization of cell division time occurs early in zoospore multiplication. As a result, not all cells sampled at a given time after germination will be members of the same cell generation. Nonetheless, if the growth rate remains constant during the sampling period and relatively constant from one cross to another, we can use absolute time as an adequate measure of growth for kinetic analysis of segregation. The growth rates listed in Table 2 show that the rates were very similar for all the crosses reported in this paper.

3. *Slope Data*

The results for the five principal markers are given in Table 3 and shown graphically in Figure 1. It is of some interest to note that the variability in the data shows a gene-specific effect. In particular, the variability of the slope value for *spc* is almost double that for the next variable gene *ac*, actually pooled data for *ac1* and *ac2*. The gene *ery* is intermediate and the genes *tm1* and *sm2* show the lowest variability. We may assume that the lowest variability is a maximum estimate of the noise level generated by factors such as non-synchrony and experimental factors discussed above. Variability above that level must then be associated with specific genes or regions of the DNA. In the case of *ac*, variability might result from the fact that data on *ac1* and *ac2* were pooled, although the genes show about 1% recombination (SAGER, unpublished). We have no explana-

TABLE 2

Growth rates of germinating zygote populations sampled in this study

Cross	1/k*
7	3.53
8	3.46
9	3.29
10	4.08
11	3.16
12	3.70
13	3.16
14	3.11
15	3.97
16	2.75
17	2.90
22	3.70
23	4.07
24	5.39
25	4.12
Average	3.513±0.45

* 1/k = time in hours for population to double.

tion for the high variability of *spc*, except to suggest a relationship with the variability in co-segregation of certain gene pairs involving *spc* reported in the previous paper (SAGER and RAMANIS 1976b). The same process may also affect *ery*.

New genes, other than the five principal markers, were usually introduced only in single crosses. In this situation our only recourse to obtain a slope estimate was simply to fit a least squares line to the observed values. Such a least-squares estimate could then be compared with the adjusted jackknife estimates for the five principal markers corresponding to the particular cross in question, as well as single-cross least squares estimates for these markers. For an illustration of the assessment of gene location which can be made on the basis

TABLE 3

Summary slopes and variability assessment for principal markers

Gene	Slope ± variability*	Number of crosses†
tm1	—0.01204 ± .0021	15
ac	—0.01709 ± .0084	13
sm2	—0.02459 ± .0027	13
spc	—0.02513 ± .0138	14
ery	—0.04512 ± .0060	10

* See text for methods of computation.

† Crosses 7 and 8 were done twice; cross 12 three times; all others listed in Table 1 once each. *Ac* segregation was not scored in cross 8A or cross 16. *Sm2* was not scored in cross 7A and 7B; *spc* was not scored in cross 17; *ery* was not scored in crosses 7A, 8A, 13, 14 and 15.

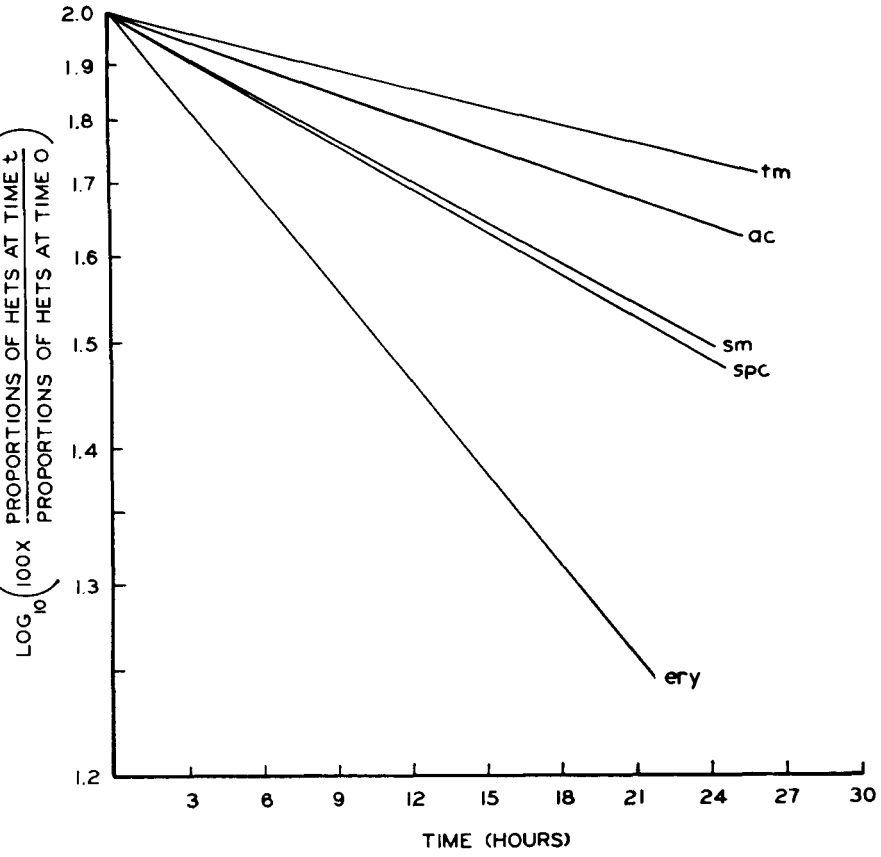


FIGURE 1.—Segregation rates of principal genes. Based on data of Table 3. See text for details.

TABLE 4

Slopes of markers present in single crosses

Gene	Slope*	Cross
car†	—0.02398	7B
cle	—0.01809	8A
	—0.02453	8B
ole	—0.03063	9
spi	—0.02138	10
car†	—0.04367	11
mr1	—0.00991	13
mr2	—0.01788	14
kan	—0.02670	17

* Least squares computation.

† In both crosses 7B and 11 the slope for *car* was similar to the least squares slope for *spc*, and less than that for *ery*.

of this kind of information, consider the marker *mr1* in cross 13 (see Table 4). Its least squares slope value is −0.0099. In the same cross, the marker *tm1* had at least squares value of −0.01099 and an adjusted jackknife (pseudo-value) of −0.01133 which is still greater than the summary slope of −0.01204. This suggests that the marker *tm1* is actually closer to *mr1* than would appear from a comparison of the summary slope value for *tm1* with the single-cross least squares slope for *mr1*. Similarly, the gene *ac* shows a higher adjusted slope for cross 13 than its summary slope. This suggests that *mr1* is very close to at least one of these two genes: further discrimination is impossible using this method alone because of the inability to distinguish between arms in a linear arrangement or between linear and circular arrangements. However, the above information together with co-segregation frequencies (SAGER and RAMANIS 1976b) derived from pedigree data place *mr1* close to *tm* and on the opposite side of *ap* from *ac* in a circular arrangement. The least squares slopes for other genes incorporated in a single cross are listed in Table 4. An assessment analogous to that described above was used to establish their location relative to *ap* as well as to the nearest principal markers.

4. *Establishing Circularity*

There are two geometrical models which have been viewed as candidates to describe the arrangement of genes in the Chlamydomonas chloroplast genome:

(i) Two linear arrangements (arms) connected by an attachment point.

(ii) One circle including an attachment point.

In using pedigree and kinetic segregation data to establish relative distances between genes in either model as well as to discriminate between them, we employ the following principles:

(a) (pedigrees) If one pair (resp. triplet, quadruplet) of genes co-segregates more frequently than a second pair (resp. triplet, quadruplet) of genes, then the first pair (triplet, etc.) is closer together than the second pair (triplet, etc.)

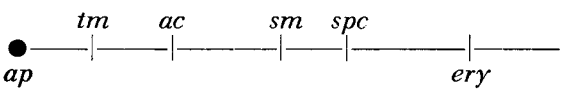
(b) (pedigrees) if one gene undergoes Type III segregation more frequently than a second gene, then the first gene is further away from the attachment point than the second gene.

(c) (kinetic data) the greater the absolute value of the slope of a line associated with a particular gene (as in section 3), the further is the gene from the attachment point.

Note: (b) and (c) provide complementary evidence in support of gene order relative to an attachment point.

The combination of evidence from the pedigree and kinetic experiments, described in (i)-(iv) below, establish the five principal markers in the circular arrangement shown in Figure 2. Similar evidence from single crosses supports the full circular arrangement shown in Figure 3.

(i) The summary slopes establish the positions relative to *ap*.



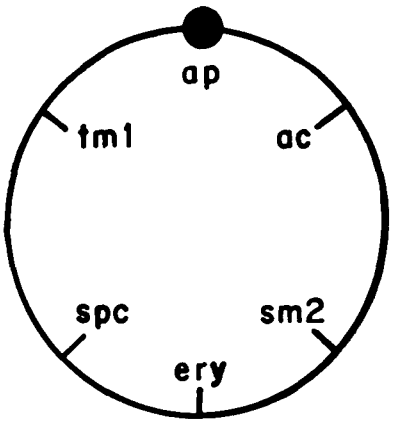


FIGURE 2.—Relative map positions of 5 genes present in several crosses. Based on data of Table 3 and of a previous report (SAGER and RAMANIS 1976b).

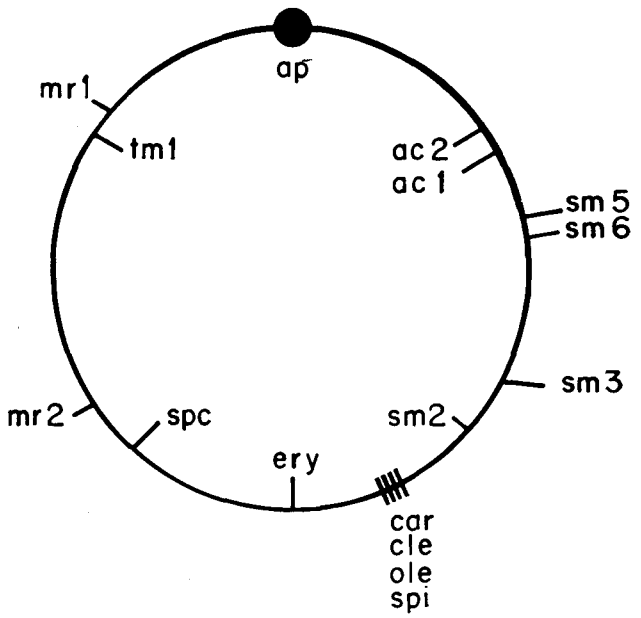


FIGURE 3.—Relative map positions of 15 genes. Based on data of this paper as well as previous reports (SAGER and RAMANIS 1970, 1976b).

(ii) The co-segregation frequency for (*spc-sm*) is less than the corresponding frequencies for (*spc-ery*) and (*sm-ery*). In addition the frequencies for (*spc-ery*) and (*sm-ery*) are roughly the same. This establishes *sm* and *spc* on opposite sides of *ery*.

(iii) The rarity of (*tm-ac*) co-segregation establishes *tm* and *ac* on opposite sides of *ap*.

(iv) The following relationships implied by the slopes,

(distance from *ap* to *sm*) $\approx$ (distance from *ap* to *spc*) is less than (distance from *ap* to *ery*)

together with the following relationships implied by co-segregation frequencies

(distance from *spc* to *sm*) is greater than

(distance from *spc* to *ery*) $\approx$ (distance from *sm* to *ery*)

are consistent only with the circular arrangement in Figure 2. Placing *ery* at the end of either arm of a linear arrangement would contradict the last inequality.

The full set of co-segregation frequencies (SAGER and RAMANIS 1976b) represents complementary evidence all consistent with the order established by (i)-(iv) above. A methodology for refining the above picture and establishing map distances using co-segregation and recombination information simultaneously will be the subject of a future publication.

DISCUSSION

This paper presents the first genetic evidence of circularity of an organelle genome, namely the chloroplast DNA of Chlamydomonas. Evidence that the genome under investigation is located in chloroplast DNA has been summarized previously (SAGER 1972, 1975; GILLHAM 1974). The circularity of chloroplast DNA has been demonstrated in higher plants (HERRMANN *et al.* 1975; KOLODNER and TEWARI 1975; MANNING, WOLSTENHOLME and RICHARDS 1972; SAGER and SCHLANGER (review) 1976), and in Euglena (MANNING *et al* 1971) but not in Chlamydomonas nor in the giant alga Acetabularia (GREEN *et al.* 1975). Circularity of mitochondrial DNA was first demonstrated with animal cells (reviewed in BORST and KROON 1969; BORST 1972) and subsequently with greater difficulty because of its large size in yeast (HOLLENBERG *et al.* 1969). At present, mitochondrial DNAs from all organisms that have been carefully examined with the exception of Tetrahymena have been shown to be circular by extraction from cells and examination in the electron microscope (BORST 1972). Despite intensive genetic studies in several laboratories (BOLOTIN *et al.* 1971; DUJON, SLONIMSKI and WEILL 1974; LINNANE, HOWELL and LUKINS 1974; WILKIE and THOMAS 1973), genetic evidence of circularity of mitochondrial DNA has not yet been reported.[1]

Genetic evidence of circularity of other DNAs began with the demonstration that the linkage map of *E. coli* was circular, as shown by the use of different *Hfr* strains which allowed recombination frequencies to be measured all around the map (JACOB and WOLLMAN 1958); and a similar result was later obtained by

[1] Since writing this paper, the first evidence of circularity based upon deletion mapping of yeast mitochondrial DNA has been reported (MOLLOY, P. L., A. W. LINNANE and H. B. LUKINS, 1975, J. Bact. **122**: 7–18).

transduction analysis with *Salmonella typhimurium* (SANDERSON and DEMEREC 1964). Circularity of the linkage map of phage T_4 was shown by the fact that the map as a whole had no ends. (STREISINGER, EDGAR and DENHARDT 1964). A circular linkage map of *Streptomyces coelicolor* was established by HOPWOOD (1965) on the basis of observed frequencies of unselected markers recovered following selection of particular recombinant classes in suitably marked crosses. In developing a theory of formal phage genetics for circular maps, STAHL and STEINBERG (1964) point out that if every recombination that is scored involves an even number of exchanges, then a linear map will appear to be circular. HOPWOOD states this possibility cannot be ruled out by his genetic data.

The evidence of genetic circularity in the chloroplast genome of Chlamydomonas is based upon the reconciliation of two kinds of measurement: co-segregation frequencies (SAGER and RAMANIS 1976b) and kinetic analysis of the rate of transition from heterozygosity to homozygosity for each of a series of linked markers, described in this paper. According to our model, Type III events require an even number of exchanges, since we recover all progeny without selection and lethality is minimal. The physical basis of the observed genetic circularity needs to be investigated by a method that is independent of exchange events.

Both methods of mapping need to be considered in relation to the patterns of segregation of chloroplast markers and the models that have been proposed to explain these patterns. After zygote germination, the resulting four zoospore products of meiosis, each haploid for the nuclear genome, divide mitotically to form four zoospore clones, each derived from one of the four meiotic products. When crosses are made involving chloroplast markers located all around the map, it can be seen that a small amount of segregation from heterozygosity occurs in the zygote, but that after germination most zoospores are still fully heterozygous, while a minority have become homozygous for one or two markers. Most of the segregations to homozygosity occur during the first few cell divisions in the growth of zoospore clones.

Our present understanding of the patterns of segregation and recombination that occur in zygotes and in zoospore clones has been developed on the basis of genetic analysis over the past several years (SAGER and RAMANIS 1970, 1976a, 1976b), involving twenty-five multifactorial crosses. These crosses or sub-sets of them were analyzed by two methods: pedigree analysis (SAGER and RAMANIS 1970, 1976a, 1976b) and the kinetic analysis described in this paper.

Pedigree analysis, in which each of the four zoospore clones is classified for all segregating markers after two mitotic doublings, makes it possible to distinguish between reciprocal and non reciprocal exchanges, and to distinguish events occurring in zygotes from those occurring at each of the first two post-meiotic mitoses. By means of pedigree analysis, we have been able to show that the chloroplast genome, as represented by the markers we have studied, is diploid, and that in vegetative cells exchanges occur at a four-strand stage after replication of the chloroplast DNA and before cell division.

In a detailed investigation of allelic segregation ratios (SAGER and RAMANIS 1976a), we found that some genes gave segregation ratios close to 1:1 under all

conditions examined, whereas other linked genes in the same cross showed preferential segregation of the allele carried by the *mt+* parent, and that this preference was allele-specific. Since these segregations are the consequence of Type II (nonreciprocal) events, we have suggested that allele-specific deviations from 1:1 result from preferential heteroduplex repair, similar to that seen in gene conversion in other organisms (FOGEL and MORTIMER 1972). In a comparison of progeny from zygotes mated after 0, 15″, 30″, and 50″ UV irradiation of the *mt+* gametes, we demonstrated the absence of an effect of UV upon allelic segregation ratios.

The same pedigree data were examined in a subsequent paper (SAGER and RAMANIS 1976b) that utilized the frequencies of co-segregation of sets of genes to generate an unambiguous linear map of 15 chloroplast genes. The same gene order was shown by the data, whether Type II segregation frequencies were used exclusively, or whether the frequencies of Type II, Type III, and zygotic segregations were pooled. Pairwise co-segregation frequencies alone provided unambiguous data for gene order, and the order was confirmed by co-segregation runs of three or more genes. Apparent lengths of co-segregation runs, as fractions of the total map, indicated much longer stretches of conversion-like events than have been reported for other genetic systems. Comparisons of co-segregation frequencies after 15″, 30″ and 50″ UV irradiation showed little if any consistent effect of UV on exchange frequencies.

The linear map generated from co-segregation frequency data gave the gene order *ac-sm2-ery-spc-tm1* for the principal markers each present in several crosses. In the present paper, in which relative distances of each gene from the centromere-like attachment point, *ap*, were measured, the order for these genes was *ap-(ac,tm1)-(sm2,-spc)-ery*. The two arrangements can be reconciled by assuming that the map is circular with the order *ap-ac-sm2-ery-spc-tm1-ap*.

The logic for establishing circularity is outlined in the text. The essential elements are (1) that the co-segregation frequencies for (*sm2-ery*) and (*spc-ery*) are roughly the same, and both are higher than the co-segregation frequency for (*sm2-spc*); (2) that the co-segregation frequencies for (*spc-tm1*) and for (*ac-sm2*) are higher than those for (*ac-spc*) and (*sm2-tm1*); (3) that from the kinetic method, the relative distances between markers implied by the slope values are consistent only with the circular arrangement. The positions of the other markers, each present in only one or two crosses, support and are fully consistent with circularity.

The principal advantages of the co-segregation mapping method are: (1) the detailed pattern of exchange events is revealed; (2) no selection occurs; (3) lethality of zoospores can be detected; and (4) the data can also be used for recombination analysis. The principal advantage of the kinetic method is that a very large sample size can be scored to obtain more accurate estimates of gene-attachment point distances than can be secured from pedigrees. In addition, the method is technically easy and rapid, and the approximate location of new markers can be readily obtained.

The kinetic method of mapping, presented in this paper, can be applied to any other genetic systems in which exchange events occur over a measurable time interval, preferably at a constant rate. The method was applied by CALLEN (1974) to four mitochondrial genes in *Saccharomyces cerevisiae*. Unfortunately, CALLEN sampled cells only at 0, 8, 24 and 48 hours after mating, whereas most of the segregation occurred during the first 8 hours. Nonetheless the results suggest that sampling during the period of active segregation would provide instructive data for mapping purposes.

In our papers (SAGER and RAMANIS 1976a,b; this paper), we have refrained from proposing a metric for map distance, principally because it is not yet evident which of several possible metrics would be most suitable. Studies now in progress of mapping by recombination frequencies should contribute important data for a quantitative comparison of map distances based on the three methods: co-segregation, kinetics, and recombination; and will thereby provide criteria for choosing the most appropriate metric.

This work was supported by grants from the National Institute of Health (U.S. Public Health Service #GM-13970), from the American Cancer Society, and from the City University of New York.

LITERATURE CITED

BOLOTIN, M., D. COEN, J. DEUTSCH, B. DUJON, P. NETTER, E. PETROCHILO and P. P. SLONIMSKI, 1971 La recombinaison des mitochondries chez *Saccharomyces cerevisiae*. Bulletin de l'Institut Pasteur **69**: 215–239.

BORST, P., 1972 Mitochondrial nucleic acids. Ann. Rev. Biochem. **41**: 333.

BORST, P. and A. M. KROON, 1969 Mitochondrial DNA: Physicochemical properties, replication, and genetic function. Int'l. Review of Cytology **26**: 107–190.

CALLEN, D. F., 1974 Recombination and segregation of mitochondrial genes in *Saccharomyces cerevisiae*. Molec. gen. Genetics **134**: 49–63.

DUJON, B., P. P. SLONIMSKI and L. WEILL, 1974 Mitochondrial genetics IX: A model for recombination and segregation of mitochondrial genomes in *Saccharomyces cerevisiae*. Genetics **78**: 415–437.

FOGEL, S. and R. K. MORTIMER, 1971 Recombination in yeast. pp. 219–236. In: *Ann. Rev. of Genetics*, Vol. **5**. Edited by H. L. ROMAN, A. CAMPBELL and L. M. SANDLER, Annual Reviews Inc., Palo Alto, California.

GILLHAM, N. W., 1974 Genetic analysis of the chloroplast and mitochondrial genomes. pp. 347–391. In: *Ann. Rev. of Genetics*, Vol. **8**. Edited by H. L. ROMAN, A. CAMPBELL and L. M. SANDLER, Annual Reviews Inc., Palo Alto, California.

GREEN, B. R., H. BURTON, V. HEILPORN and S. LIMBOSCH, 1975 The cytoplasmic DNA's of *Acetabularia mediterranea*: Their structure and biological properties. pp. 35–60. In: *The Biology of Acetabularia*. Edited by J. Brachet and S. Bonotto, Academic Press, New York.

HERRMANN, R. G., H. J. BOHNERT, K. V. KOWALLIK and J. M. SCHMITT, 1975 Size, conformation and purity of chloroplast DNA of some higher plants. Biochim. Biophys. Acta **378**: 305–317.

HOLLENBERG, C. P., P. BORST, R. W. J. THURING and E. F. J. VAN BRUGGEN, 1969 Size, structure and genetic complexity of yeast mitochondrial DNA. Biochim. Biophys. Acta **186**: 417–419.

HOPWOOD, D. A., 1965 A circular linkage map in the Actinomycete, *Streptomyces coelicolor*. J. Mol. Biol. **12**: 514–516.

JACOB, F. and E. WOLLMAN, 1958 Genetic and physical determinations of chromosomal segments in *Escherichia Coli*. Symp. Soc. Exp. Biol. **12**: 75–92.

KOLODNER, R. and K. K. TEWARI, 1975 The molecular size and conformation of chloroplast DNA of higher plants. Biochim. Biophys. Acta **402**: 372–390.

LINNANE, A. W., N. HOWELL, H. B. LUKINS, 1974 Mitochondrial genetics. pp. 193–213. In: *The Biogenesis of Mitochondria*. Edited by A. M. KROON and C. SACCONE, Academic Press Inc., New York.

MANNING, J. E., D. R. WOLSTENHOLME, R. S. RYAN, J. A. HUNTER and O. C. RICHARDS, 1971 Circular chloroplast DNA from *Euglena gracilis*. Proc. Natl. Acad. Sci. U.S. **68**: 1169–1173.

MANNING, J. E., D. R. WOLSTENHOLME and O. C. RICHARDS, 1972 Circular DNA molecules associated with chloroplasts of spinach, *Spinacia oleracea*. J. Cell Biol. **53**: 594–601.

MILLER, RUPERT G., 1974 The jackknife—A review. Biometrika, **61**: 1–15.

MOSTELLER, F. and J. W. TUKEY, 1968 Data Analysis, Including Statistics, pp. 80–203. In: G. LINDZEY and E. ARONSON, *The Handbook of Social Psychology*, Vol. II, 2nd edition, Addison-Wesley Publishing Co., Reading, Mass.

SAGER, RUTH, 1972 *Cytoplasmic Genes and Organelles*, Academic Press, New York. ——, 1975 Patterns of inheritance of organelle genomes: Molecular basis and evolutionary significance. pp. 252–267. In: *Genetics and Biogenesis of Mitochondria and Chloroplasts*, Edited by C. W. BIRKY, P. S. PERLMAN and T. J. BYERS, Ohio State University Press, Columbus, Ohio.

SAGER, R. and Z. RAMANIS, 1968 The pattern of segregation of cytoplasmic genes in Chlamydomonas. Proc. Natl. Acad. Sci. U.S. **61**: 324–331. ——, 1970 A genetic map of non-Mendelian genes in *Chalmydomonas*. Proc. Natl. Acad. Sci. U.S. **65**: 593–600. ——, 1976a Chloroplast genetics of Chlamydomonas: I. Allelic segregation ratios. Genetics **83**: 303–321. ——, 1976b Chloroplast genetics of Chlamydomonas: II. Mapping by cosegregation frequency analysis. Genetics **83**: 323–340.

SAGER, R. and G. SCHLANGER, 1976 Chloroplast DNA: Physical and genetic studies. In: *Handbook of Genetics*, Vol. **5**, edited by B. C. KING (in press).

SANDERSON, K. E. and M. DEMEREC, 1964 Microbial Genet. Bulletin **20**: 11.

STAHL, F. W. and C. M. STEINBERG, 1964 The theory of formal phage genetics for circular maps. Genetics **50**: 531–538.

STREISINGER, G., R. S. EDGAR and G. H. DENHARDT, 1964 Chromosome structure in phage T4: I. Circularity of the linkage map. Proc. Natl. Acad. Sci. U.S. **51**: 775–779.

WILKIE, D., D. T. THOMAS, 1973 Mitochondrial genetic analysis by zygote cell lineages in *Saccharomyces cerevisiae*. Genetics **73**: 368–377.

Corresponding editor: N. W. GILLHAM