INDUCTION OF CHROMOSOMAL AND NONCHROMOSOMAL MUTATIONS IN *CHLAMYDOMONAS REINHARDI* WITH N-METHYL-N′-NITRO-N-NITROSOGUANIDINE

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IN addition to a normal, chromosomal system of inheritance, the heterothallic, unicellular, green alga *Chlamydomonas reinhardi* possesses a nonchromosomal system of inheritance. The latter system was recognized 11 years ago by SAGER (1954), who found that mutants resistant to 500 μg per ml of streptomycin (*sr-2* mutations) exhibited uniparental inheritance. Since that time, mutations to streptomycin dependence (*sd*), acetate requirement (ac^-), and mutations with unidentified growth requirements have been discovered which show the same pattern of inheritance (SAGER and RAMANIS 1963). All of these mutations possess several properties in common in addition to uniparental inheritance. First, nonchromosomal mutations are stable in vegetatively reproducing cultures of cells. Second, although over 90% of the zygotes arising from reciprocal crosses between cells carrying different nonchromosomal markers produce progeny resembling the mating type plus (mt^+) parent, a small fraction of "exceptional zygotes" is found in every cross. In these exceptional zygotes nonchromosomal markers from the mating type minus (mt^-) as well as the mt^+ parent are transmitted to the meiotic progeny. Third, segregation of chromosomal markers is normal among the progeny of exceptional zygotes, but segregation of nonchromosomal markers is completely irregular and often takes place during vegetative division of the haploid progeny of meiosis (GILLHAM 1963; SAGER and RAMANIS 1963). Nevertheless, once the segregation process has been completed, the progeny cells contain a stable combination of the input nonchromosomal markers; some of the cells possess parental combinations of markers and others recombinant combinations (SAGER and RAMANIS 1963; GILLHAM unpublished). Fourth, the antibiotic streptomycin induces nonchromosomal mutations (e.g. *sr-2 sd*, and ac^- mutations), but there is no evidence suggesting that the antibiotic induces chromosomal mutations (SAGER 1960, 1962; GILLHAM and LEVINE 1962). All of these observations save the last suggest that nonchromosomal mutations may be comparable to chromosomal gene mutations except that their patterns of transmission and segregation do not obey classical Mendelian laws.

Within the past year, we have begun an extensive screening program the aim of which is the isolation and characterization of new nonchromosomal mutations. Thus far this program has been directed towards the isolation of nonchromosomal mutants resistant to basic antibiotics related to streptomycin. The present com-

munication describes two new nonchromosomal mutations which are resistant to and dependent upon the antibiotic neamine and discusses the induction of both chromosomal and nonchromosomal mutations in *C. reinhardi* with N-methyl-N'-nitro-N-nitrosoguanidine (nitrosoguanidine).

MATERIALS AND METHODS

Strains: The wild-type strain (137c) used in this work and the chromosomal mutants derived from it have been described in previous publications (LEVINE and EBERSOLD 1960; EBERSOLD, LEVINE, LEVINE, and OLMSTED 1962) with the exception of a new arginine-requiring mutant (*arg-7*) which was isolated after treatment of a wild-type *mt+* stock with nitrosoguanidine. This mutant is located in linkage group I between two other arginine-requiring mutants (*arg-1* and *arg-2*). The new mutant grows on arginine, but not ornithine or citrulline.

Media: The media used in these experiments and their abbreviations are as follows: MA = basal minimal medium, of LEVINE and EBERSOLD (1958) plus 2 g sodium acetate and 15 g Bacto Agar (Difco) per liter; MS50 = MA medium plus 50 mg streptomycin sulfate (Lilly) per liter; MS500 = MA medium plus 500 mg streptomycin sulfate per liter; MN100 = MA medium plus 100 mg neamine base per liter; Y = MA medium plus 4 g yeast extract (Difco) per liter; and HSA = the liquid high salt medium of SUEOKA (1960) supplemented with 2 g sodium acetate per liter. Streptomycin and neamine were sterilized by filtration and added to the appropriate media after the media had been autoclaved and allowed to cool.

Genetic methods: All of the pertinent methods including the making of crosses and the dissection and analysis of tetrads have been described by EBERSOLD and LEVINE (1959).

Induction of mutations with nitrosoguanidine: Wild-type cells were suspended from Y medium in 6.7×10^{-3} M potassium phosphate buffer, pH 6.8 for several hours to disperse them, or were grown to stationary phase in HSA medium and then washed once with 0.1 M citrate buffer, pH 5.0 and resuspended in citrate buffer to concentrations of 4 to 7×10^6 viable cells per ml. A filter-sterilized stock solution of nitrosoguanidine in citrate buffer was then added to the cell suspension to a final concentration of 50 μg per ml. The suspension was incubated in a flask at room temperature for 15 minutes with mechanical agitation, after which the cells were washed twice by centrifugation with citrate buffer, and plated on the appropriate medium. Cell survival following treatment ranged between 3.8 and 7.5%.

RESULTS

Induction of chromosomal and nonchromosomal mutations with nitrosoguanidine: In order to determine whether nitrosoguanidine could alter the frequency of both chromosomal and nonchromosomal mutations in *C. reinhardi*, cells of a streptomycin sensitive, *mt-* strain were treated with the mutagen and immediately plated on MS50 medium. After colonies of streptomycin resistant cells had appeared, they were replica-plated to MS500 medium. The MS50 plates supported growth of both *sr-2* mutants and chromosomal mutations to streptomycin resistance (*sr-1*), but the MS500 plates supported only the growth of *sr-2* mutants, since *sr-1* mutants are resistant to a miximum of 100 μg streptomycin per ml (SAGER 1960). Hence, a comparison of the two sets of plates made it possible to measure the frequencies of both *sr-1* and *sr-2* mutations in the same experiment.

Nitrosoguanidine was found to increase the frequency of both *sr-1* and *sr-2* mutations (Table 1). This effect was most striking in the case of the *sr-2* mutations. Although no *sr-2* mutants were recovered among the untreated cells in these experiments because the sample plated was not large enough, an extensive

TABLE 1

Frequency of sr-1 *and* sr-2 *mutants among wild-type cells plated on MS50 medium immediately following treatment with N-methyl-N'-nitro-N-nitrosoguanidine*

Experiment	Survival	Phenotype of mutation	Frequency per 10^7 viable wild-type cells	
			Before treatment	After treatment
65	7.4%	*sr-1*	2.22	18.7
		sr-2	0	43.7
66	3.8%	*sr-1*	2.73	124.0
		sr-2	0	210.0

Conditions of treatment with the mutagen are described in MATERIALS AND METHODS.

series of experiments by GILLHAM and LEVINE (1962) has shown that the frequency of *sr-2* mutants in untreated cultures of cells is about 4×10^{-8}. If this figure is used in estimating the effectiveness of the mutagen, it is seen that nitrosoguanidine causes a more than 100-fold increase in the frequency of *sr-2* mutants.

Since there is no evidence to suggest that chromosomal mutations are induced by streptomycin in *C. reinhardi* (GILLHAM and LEVINE 1962), it seems very probable that the increase in frequency of *sr-1* mutants following treatment with nitrosoguanidine is a direct result of the mutagenic effect of this chemical. In the case of nonchromosomal mutants such as *sr-2*, on the other hand, streptomycin itself is mutagenic and so it was necessary to determine whether nitrosoguanidine caused an increase in the frequency of *sr-2* mutants because it temporarily made sensitive cells susceptible to streptomycin mutagenesis or whether nitrosoguanidine itself was mutagenic. The experimental approach used to differentiate between temporary susceptibility to streptomycin mutagenesis and actual induction of *sr-2* mutations by nitrosoguanidine was an adaptation of the fluctuation test (LURIA and DELBRÜCK 1943).

Sensitive cells were treated with nitrosoguanidine, washed, and diluted into HSA medium. Aliquots of the diluted cell suspension were then dispensed to a series of tubes which were incubated with illumination until the cells in them had reached stationary phase. An aliquot from each culture was then dispensed to a single plate of MS50 medium. The frequency of *sr-2* mutants was estimated as before by replica-plating the MS50 plates to MS500 medium; viable cell counts made on MA medium at the beginning and end of the experiment served to estimate the amount of growth which had taken place. Control platings made prior to treatment of the sensitive cells with nitrosoguanidine permitted determination of the frequency of *sr-2* mutants in the untreated population of cells.

If nitrosoguanidine causes a temporary susceptibility of sensitive cells to streptomycin mutagenesis, one would expect a random distribution of *sr-2* mutants among the aliquots plated at the end of a fluctuation test since every cell should have the same probability of being mutated by streptomycin. If, on the other hand, nitrosoguanidine is itself mutagenic some aliquots will contain a number of mutant cells whereas others will contain none and the distribution of *sr-2* mutants among the cultures will be nonrandom. This situation will obtain because certain of the cultures will have received induced mutations at the begin-

TABLE 2

Frequency of sr-2 *and* sd *mutants in similar cultures of wild-type cells following treatment with nitrosoguanidine*

	Experiment 1		Experiment 2	
Number of cultures	29		23	
Volume of cultures	0.4 ml		1 ml	
Volume of samples	entire culture		0.2 ml	
Mutation frequency prior to nitrosoguanidine treatment	$0/1.8 \times 10^7$		$0/7.03 \times 10^6$	
Cells per ml at start of experiment	1.09×10^6		1.0×10^5	
Cells per ml at time of plating	8.82×10^6		ca. 8×10^6	
Mutant colonies obtained per culture	Number of cultures		Number of cultures	
	sr-2	*sd*	*sr-2*	*sd**
0	5	29	5	19
1	4	0	2	0
2	5	0	2	0
3	5	0	3	0
4	4	0	0	0
5	2	0	2	2
6	4	0	2	0
7	0	0	3	0
8	0	0	0	1
9	0	0	1	0
10	0	0	0	0
14	0	0	1	0
17	0	0	1	0
21	0	0	1	0
Average mutant colonies per culture	2.72	0	5.17	0.82
Variance	3.98	0	29.45	4.73
Chi-square	42.4		131.0	126.9
Probability	>0.025		$<.005$	$<.005$

* Only 22 cultures were tested.
See text for experimental details.

ning of the experiment and the mutant cells will have multiplied during the growth period whereas other cultures will not have received mutant cells.

The results of two fluctuation tests strongly support the idea that nitrosoguanidine is capable of inducing *sr-2* mutations (Table 2). This is best illustrated in the second of the two tests shown in Table 2 in which *sd* mutants were recovered in addition to *sr-2* mutants. Here, the fluctuations in numbers of mutants per culture were very large for both classes of mutants. The absence of such striking fluctuations in the first experiment is probably explained by the fact that the cells underwent fewer doublings on the average in Experiment 1 than Experiment 2. Nevertheless, both experiments support the conclusion that nitrosoguanidine is effective in inducing *sr-2* mutations and *sd* mutations as well.

Sensitivity of C. reinhardi *to basic antibiotics:* The wild-type strain of *C. reinhardi* has been tested for its sensitivity to several different basic antibiotics

TABLE 3

Sensitivity of Chlamydomonas reinhardi *to different basic antibiotics*

Antibiotic	Structure*	Lowest concentration of antibiotic (μg/ml) inhibiting growth of wild-type cells
Neomycin B†	2,6-Diaminohexose-D-ribose-deoxystreptamine-2,6-diamino-D-glucose	No inhibition at concentrations as high as 200 μg/ml
Neomycin C†	2,6-Diamino-D-glucose-D-ribose-deoxystreptamine-2,6-diamino-D-glucose	No inhibition at concentrations as high as 500 μg/ml
Neamine	Deoxystreptamine-2,6-diamino-D-glucose	20
Paromomycin	2,6-Diamino-D-glucose-D-ribose-deoxystreptamine-2-aminoglucose	10
Zygomycin	2,6-Diaminohexose-D-ribose-deoxystreptamine-2-aminoglucose	10
Streptomycin	Streptidine-streptose-2-methyl-amino-L-glucose	20
Kanamycin	6-Aminoglucose-deoxystreptamine-‡ 3-aminoglucose	50
Erythromycin	Cladinose-erythronolide-3-dimethyl-amino-deoxyhexose	100
Spectinomycin	Three-ring structure with no amino-sugars	40

Tests were carried out by streaking loopfuls of a suspension of wild-type cells on MA medium supplemented with antibiotic. Each antibiotic was tested in this way at several different concentrations.

* After SOKOLSKI *et al.* (1962).

† Both neomycins were also tested in a basic medium buffered with 0.05 M Tris (Tris (hydroxymethyl) aminomethane, Sigma 121, Sigma Chemical Co.) at pH 7.5.

‡ 4,6-Di-substituted. All other deoxystreptamines except neamine are 4,5-di-substituted.

(Table 3). All of the antibiotics tested proved to be effective growth inhibitors except neomycins B and C. It is perhaps surprising that the latter two antibiotics showed no detectable inhibitory effect in view of their respective similarity to zygomycin and paromomycin, both of which are highly toxic. In each case, the primary difference between these pairs of antibiotics lies in the fact that both of the neomycins possess an amino group at the 6-position of the aminoglucose moiety in addition to the amino group present in all four antibiotics at the 2-position.

Isolation of mutations resistant to or dependent upon basic antibiotics: Mutations have been isolated that are resistant to each of the basic antibiotics lethal to wild-type cells, but only those mutants resistant to neamine and paromomycin have been studied carefully. Chromosomal mutations resistant to both neamine (*nr-1*) and paromomycin (*pr-1*) have been found, and crosses of these mutants to each other and to *sr-1* reveal that the *pr-1*, *nr-1*, and *sr-1* loci are all unlinked (Table 4). Furthermore, crosses between two independently isolated *nr-1* mutants and between two independently isolated *pr-1* mutants showed that they were allelic. In neither case has a study of resistance levels been made, but it can be stated that one of the *nr-1* mutants is resistant to at least 600 μg neamine per ml and the *pr-1* mutation to 10 μg paromomycin per ml.

TABLE 4

Results of crosses between sr-1, nr-1, *and* pr-1 *mutations. Each mutant has been found to segregate 1:1 in crosses to wild-type cells. The numbers in each column are the number of tetrads belonging to each class*

Cross	Tetrad type		
	Parental ditype	Nonparental ditype	Tetratype
sr-1 pr-1 mt+ × + + *mt−*	14	17	17
nr-1 + *mt+* × + *pr-1 mt−*	6	5	13
nr-1 + *mt−* × + *sr-1 mt+*	5	6	13
pr-1a + *mt+* × + *pr-1b mt−*	30	0	0
nr-1a + *mt+* × + *nr-1b mt−*	30	0	0

Nonchromosomal mutations resistant to neamine (*nr-2*) and dependent upon this antibiotic (*nd*) were also recovered following treatment with nitrosoguanidine, but no attempt was made to show rigorously that these mutations were induced by nitrosoguanidine. Nevertheless, *nd* and *nr-2* mutations have never been found among large samples of cells plated without nitrosoguanidine treatment on neamine-containing media (GILLHAM, unpublished).

The results of crosses between wild-type cells and the *nr-2* and *nd* mutants as well as one of the *sd* mutants isolated in the fluctuation tests reveal the characteristic pattern of uniparental inheritance (Table 5), although the data obtained for the dependent mutants are limited because the fertility of crosses involving these mutants has thus far been low.

TABLE 5

Segregation of nr-2, nd, *and* sd *mutants in crosses to sensitive cells*

Cross	Segregation of nonchromosomal marker	Number of tetrads analyzed
nr-2 pf-20 mt+ × *ns* + *mt−**	4 *nr-2*: 0 *ns*	29
	Other	0
nr-2 + *mt+* × *ns arg-7 mt−*	4 *nr-2*: 0 *ns*	17
	Other	0
nr-2 + *mt−* × *ns pf-20 mt+*	0 *nr-2*: 4 *ns*	34
	Other	0
nd-a + *mt+* × *ns arg-2 mt−*	4 *nd*: 0 *ns*	3
	Other	0
nd-b + *mt−* × *ns arg-7 mt+*	0 *nd*: 4 *ns*	4
	Other	0
nd-c + *mt−* × *ns arg-7 mt+*	0 *nd*: 4 *ns*	6
	Other	0
sd + *mt−* × *ss pf-15 mt+*	0 *sd*: 4 *ss*	18
	Other†	0

In each cross a chromosomal mutation either conferring the requirement for arginine (*arg-2* or *arg-7*) or producing paralyzed flagella (*pf-15* or *pf-20*) was segregating. In all tetrads save one*, segregation of the chromosomal marker was 1:1. The abbreviations *ns* and *ss* refer to neamine sensitivity and streptomycin sensitivity respectively.

* One tetrad segragated 3 *pf-20+*: 1 *pf-20*.

† In a considerable number of tetrads from this cross none of the meiotic products formed colonies (13) or only two of them formed colonies (18). However, there is no evidence to indicate that this tetrad abortion is related in any way to the segregation of *sd* and *ss*.

TABLE 6

Cross-resistance and -dependence between mutations resistant to or dependent upon streptomycin and neamine

Strain tested	Neamine concentration (μg per ml)					Streptomycin concentration (μg per ml)			
	0	10	25	50	100	25	50	100	600
Wild-type mt+	+	(+)	−	−	−	−	−	−	−
Wild-type mt−	+	(+)	−	−	−	−	−	−	−
nr-2 mt+	+	+	+	+	+	−	−	−	−
sr-2 mt+	+	+	+	(+)	−	+	+	+	+
nd-a mt−	−	+	+	+	+	−	−	(+)	(+)
nd-b mt−	−	+	+	+	+	−	−	(+)	+
sd mt−	−	+	+	+	+	+	+	+	+

Tests were carried out by streaking loopfuls of a suspension of mutant cells on MA medium supplemented with antibiotic. Growth responses: + = heavy growth; (+) = weak growth; − = no visible growth.

As yet, nonchromosomal mutations resistant to or dependent upon paromomycin have not been isolated despite several attempts to induce these mutations with nitrosoguanidine.

Cross-resistance and -dependence between nr-2, sr-2, nd, *and* sd *mutations:* Cross-resistance was detected in the case of the *sr-2* mutant tested, but not in the case of the *nr-2* mutant. (Table 6). The *sr-2* mutant grew on concentrations of neamine as high as 50 μg per ml, but the *nr-2* mutation was unable to grow on concentrations of streptomycin as low as 25 μg per ml, which is the lowest level of streptomycin sufficient to suppress completely the growth of wild type. Considerable cross-dependence was found for both *sd* and *nd* mutants, but at the same time clear differences existed between these mutants. The *sd* mutant examined grew on all concentrations of neamine which supported growth of the *nd* mutants, but the *nd* mutants grew only on high concentrations of streptomycin.

DISCUSSION

These results show that nitrosoguanidine is effective in inducing both chromosomal and nonchromosomal mutations in *Chlamydomonas reinhardi.* The agent was shown to cause nonchromosomal mutations to streptomycin resistance (*sr-2*) and dependence (*sd*); nonchromosomal mutations to neamine resistance (*nr-2*) and dependence (*nd*) were also obtained after treatment with the mutagen. Previously, the only agent known which would induce nonchromosomal mutations in *C. reinhardi* was streptomycin, and there was no evidence suggesting that this mutagen also induced chromosomal mutations (SAGER 1962). Thus, the results reported here constitute the first demonstration that a mutagen capable of inducing chromosomal mutations in *C. reinhardi* can also induce nonchromosomal mutations in this organism.

Limited studies of cross-resistance and -dependence with the nonchromosomal mutants isolated in these experiments have established two things. First, the *sr-2*

mutant is able to grow on concentrations of neamine considerably higher than those which suppress the growth of wild-type cells, but the *nr-2* mutant is no more resistant to streptomycin than wild type. Second, although the *nd* and *sd* mutants can utilize either streptomycin or neamine as metabolites, differences do exist between the two kinds of mutants. The *sd* mutant is able to grow well on all concentrations of neamine and streptomycin tested, but the *nd* mutants can only grow on high concentrations of streptomycin. These observations show that both the *sr-2* and *sd* mutations are more tolerant of neamine than the *nd* and *nr-2* mutants are of streptomycin.

The cross-dependence phenomena reported here have also been seen in bacteria (SOKOLSKI, YEAGER and CHIDESTER 1962). There too it was found that at suitable concentrations streptomycin-dependent bacteria could utilize neamine and neamine-dependent bacteria could metabolize streptomycin. Furthermore, that work also revealed a broader range of cross-dependence than has so far been detected in *C. reinhardi*. For example, it was found that at suitable concentrations paromomycin would support the growth of a neamine-dependent strain of *Staphylococcus aureus* and streptomycin-dependent strains of *Salmonella paratyphi* and *Escherichia coli*, whereas no similar stimulation of growth by paromomycin has been observed for either the *nd* or *sd* mutants of *C. reinhardi*.

Finally, there is the question of the genetic relationship between the nonchromosomal mutations to antibiotic resistance and dependence. Here the data are fragmentary, but there are few facts which may be significant. First, in crosses between *sr-2* and *sd* cells an occasional zygote produces some streptomycin sensitive (*ss*) cells, whereas *ss* cells are never found in crosses between strains carrying the same *sd* or *sr-2* marker (SAGER and RAMANIS 1964). Second, crosses between a neamine-resistant, streptomycin-sensitive (*nr-2 ss*) mt^+ strain and a neamine-sensitive streptomycin-resistant (*ns sr-2*) mt^- strain yield two kinds of exceptional zygotes (GILLHAM, unpublished). The majority class gives rise to progeny with nonchromosomal markers in parental combinations (i.e. *nr-2 ss* or *ns sr-2*), but the minority class produces a small percentage of *nr-2 sr-2* cells as well. The doubly resistant cells are not easily attributed to mutation, because cells of this type have not been recovered from crosses between strains carrying the same *nr-2* or *sr-2* markers. Third, in crosses between an *ss* ac^- mt^- strain and an *sd* ac^+ mt^+ strain, exceptional zygotes were found to produce the four possible nonchromosomal marker combinations (i.e. *ss* ac^-, *ss* ac^+, *sd* ac^-, and *sd* ac^+) in approximately equal frequency (SAGER and RAMANIS 1963). Together, these results suggest that *sr-2*, *sd*, and *nr-2* may reside in a single determinant which behaves independently of a second determinant in which the *ac* marker is located. Obviously, many more crosses will be necessary to test this possibility adequately, but the preliminary findings do point towards the conclusion that nonchromosomal mutations resistant to and dependent upon neamine and streptomycin are closely related at both the physiological and genetic level.

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SUMMARY

The heterothallic, unicellular, green alga *Chlamydomonas reinhardi* possesses a nonchromosomal system of inheritance in addition to a normal, chromosomal system of inheritance. Mutations belonging to the nonchromosomal system characteristically exhibit uniparental inheritance.

N-methyl-N′-nitro-N-nitrosoguanidine induces both chromosomal (*sr-1*) and nonchromosomal (*sr-2*) mutations to streptomycin resistance. Furthermore, nonchromosomal mutations dependent upon streptomycin (*sd*) and resistant to (*nr-2*) or dependent upon (*nd*) the closely related antibiotic neamine have also been isolated with nitrosoguanidine. The *sr-2* mutant shows cross-resistance to neamine, but the *nr-2* mutant shows no cross-resistance to streptomycin. The *nd* and *sd* mutants can utilize either streptomycin or neamine as metabolites; nevertheless, clear differences exist between the two classes of mutants in this respect.

LITERATURE CITED

EBERSOLD, W. T., and R. P. LEVINE, 1959 A genetic analysis of linkage group I of *Chlamydomonas reinhardi*. Z. Vererb. **90**: 74–82.

EBERSOLD, W. T., R. P. LEVINE, E. E. LEVINE, and M. A. OLMSTED, 1962 Linkage maps in *Chlamydomonas reinhardi*. Genetics **47**: 531–543.

GILLHAM, N. W., 1963 The nature of exceptions to the pattern of uniparental inheritance for high level streptomycin-resistance in *Chlamydomonas reinhardi*. Genetics **48**: 431–439.

GILLHAM, N. W., and R. P. LEVINE, 1962 Studies on the origin of streptomycin resistant mutants in *Chlamydomonas reinhardi*. Genetics **47**: 1465–1474.

LEVINE, R. P., and W. T. EBERSOLD, 1958 The relation of calcium and magnesium to crossing over in *Chlamydomonas reinhardi*. Z. Vererb. **89**: 631–635. —— 1960 The genetics and cytology of Chlamydomonas. Ann. Rev. Microbiol. **14**: 197–216.

LURIA, S. E., and M. DELBRÜCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**: 491–511.

SAGER, R., 1954 Mendelian and non-Mendelian inheritance of streptomycin resistance in *Chlamydomonas reinhardi*. Proc. Natl. Acad. Sci. U.S. **40**: 356–363. —— 1960 Genetic systems in Chlamydomonas. Science **132**: 1459–1465. —— 1962 Streptomycin as a mutagen for nonchromosomal genes. Proc. Natl. Acad. Sci. U.S. **48**: 2018–2026.

SAGER, R., and Z. RAMANIS, 1963 The particulate nature of nonchromosomal genes in Chlamydomonas. Proc. Natl. Acad. Sci. U.S. **50**: 260–268. —— 1964 Recombination of nonchromosomal genes in Chlamydomonas. (Abstr.) Genetics **50**: 282.

SUEOKA, N., 1960 Mitotic replication of deoxyribonucleic acid in *Chlamydomonas reinhardi*. Proc. Natl. Acad. Sci. U.S. **46**: 83–91.

SOKOLSKI, W. T., R. L. YEAGER, and L. G. CHIDESTER, 1962 Cross-dependence between neamine and other basic antibiotics. Nature **196**: 776–777.