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CURRENT PROBLEMS IN RESEARCH

Genetic Systems in Chlamydomonas

Both chromosomal and nonchromosomal systems of genetic determinants are being analyzed in this alga.

Ruth Sager

It is the plan of this article to describe some studies of *Chlamydomonas*, a unicellular alga that is an admirable microorganism for genetical and physiological investigations. Both previously published and current unpublished findings are discussed within the framework of the general working hypothesis. Preliminary results and speculations are included, leaving a more detailed presentation of the data for publication elsewhere (*1*).

The focus of interest in this research has been the investigation of nonchromosomal heredity. Attention has been centered principally upon two systems: chloroplast formation, known from studies with higher plants (*2*) to be influenced by nonchromosomal as well as by chromosomal genes, and streptomycin resistance. My co-workers and I have studied chromosomal heredity in *Chlamydomonas*. In addition, we have studied a nonchromosomal genetic system exhibiting uniparental transmission and conferring streptomycin resistance, and we have preliminary evidence about a different genetic system involving the chloroplast.

Before discussing these systems, let us take a quick look at the organism itself (*3*). Normal green cells each contain one cup-shaped chloroplast which occupies about 50 percent of the cell volume and contains all of the chlorophyll, carotenoids, and the enzymes of photosynthesis and of the CO_2-to-starch pathway. The schematic diagram in Fig. 1 summarizes the principal structural elements of the organism as seen in light and electron microscopy. Electron micrographs of thin sections of normal green and dark-grown yellow mutant cells show the similarities and differences in chloroplast structure which have resulted from the absence of chlorophyll in the yellow mutant, y_1^-. Although the total chloroplast volume remains unchanged, and starch is stored as usual, no organized lamellar membranes form in the absence of chlorophyll. When such yellow cells are placed in the light, chlorophyll synthesis begins at once, and within 24 hours they are indistinguishable from normal green cells. Thus, the ability to make a normal chloroplast has not been irreversibly lost in this yellow mutant. I return to this point below in discussing the inheritance of the y_1 factor.

The simple life cycle of *Chlamydomonas* (Fig. 2) involves fusion of haploid cells of opposite mating type to form the diploid zygote, which does not divide mitotically but undergoes meiotic reduction with the production of four haploid progeny. Tetrad analysis, the genetic analysis of all four products of individual meioses, can be readily carried out with this material. Gilbert Smith first obtained the complete life cycle of *Chlamydomonas reinhardi* on defined media, and Smith and Regnery showed that mating type was inherited as a unit factor difference (*4*). Subsequently, the mating type gene was located about 35 map units from its centromere, and a number of other genes were mapped (*5*). Through tetrad analysis it was shown that regular 1:1 segregation of alleles was the rule, and no abnormalities were apparent in the segregation of a number of genes affecting drug resistance and pigment formation (*5*). Similar regularities have been reported in the segregation of nutritional mutants (*6*). In the results discussed here, tetrad analysis was employed and centromere distances were computed as in previous publications (*5*, *7*).

A Genetic System Involving Uniparental Transmission

When wild-type *Chlamydomonas* is plated on streptomycin-agar (100 micrograms per milliliter), most cells die, and the colonies which appear consist of streptomycin-resistant (*sr*) mutants. Under standardized conditions, two classes of *sr* mutants are found: *sr*-100, resistant to 100 μg of streptomycin per milliliter on agar, and arising with a frequency of about 10^{-6}, and *sr*-500 (previously called *sr*-2), resistant to 500 μg/ml and arising with a frequency of about 10^{-7} under conditions discussed below. Fifty-seven *sr*-100 mutants of independent origin have been crossed to wild type: all segregate 1:1, and the *sr*-100 factor in one of the strains has been located by tetrad analysis about 20 map units from its centromere.

With the *sr*-500 mutants, streptomycin resistance segregates in an entirely different manner at meiosis. Twenty-seven independently isolated *sr*-500 strains, phenotypically indistinguishable in resistance level, and derived from both mating types, have

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been crossed. All of them show the same pattern of inheritance of resistance as the *sr*-2 strain initially described (*7*). As shown in Fig. 3, the *sr* determinant in this strain exhibits only uniparental transmission, all progeny corresponding to the resistance level of the parent of mating type *plus*. The demonstration that this pattern of segregation does not result from multifactorial inheritance, from chromosome aberration, or from a lag in expression of the progeny phenotypes has been previously reported and discussed (*7*).

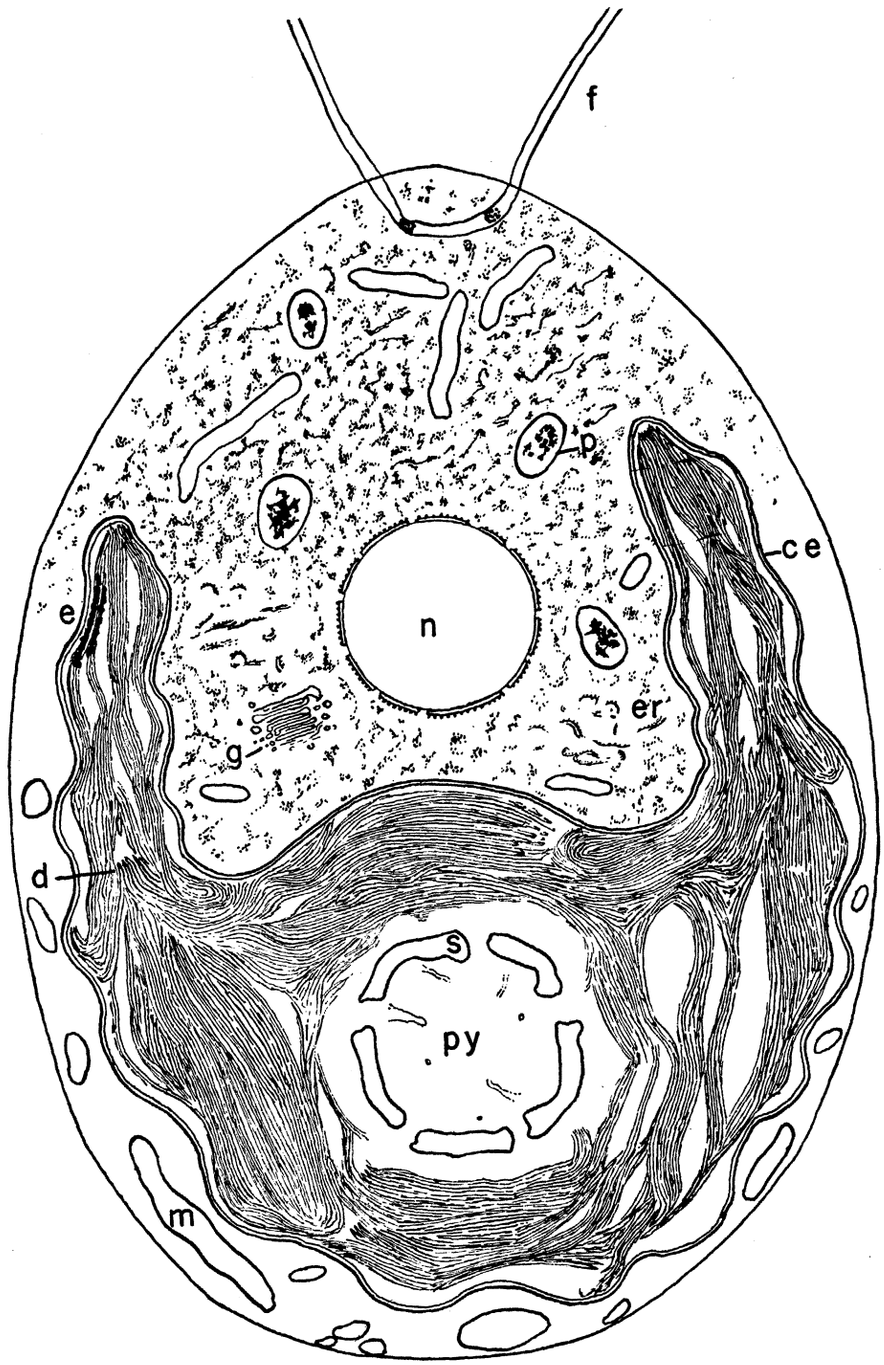

Fig. 1. Diagrammatic sketch of normal green *Chlamydomonas* as seen at low magnification in the electron microscope. The chloroplast is shown surrounded by the double chloroplast envelope (*ce*) within which the eye-spot (*e*), pyrenoid (*py*), and starch plates (*s*) are located, as well as the paired lamellar membranes arranged as discs. At low magnification the disc arrangement is clearly seen only in occasional well-oriented regions. The cytoplasm also contains other systems of organelles, including mitochondria (*m*), Golgi material (*g*), endoplasmic reticulum (*er*) consisting of membranes and ribonucleic acid-containing granules, and vacuoles containing metaphosphate (*p*). The nucleus (*n*) is surrounded by a double membrane with pores and a dense coating of ribonucleic acid-containing granules on its outer surface.

The *sr*-500 determinant is noteworthy for its stability. No reversions to sensitivity have been found over a period of several years, or after a variety of treatments designed to inactivate it or to dilute it out. All of our evidence to date indicates that the *sr*-500 factor is at least as stable and as well integrated in the cell as are chromosomal genes. Electron micrographs of *sr*-500 cells likewise have revealed no differences from the morphology of wild-type sensitive cells.

Two other phenotypic traits, one being streptomycin dependence (*sd*) and the other being resistance to 1500 μg of streptomycin per milliliter (*sr*-1500), have been found to exhibit the same pattern of uniparental transmission as does resistance to 500 μg/ml (*8*). Only one streptomycin-dependent strain has been recovered in many searches for mutants. It grows best with 100 μg/ml but survives well with 500 μg/ml. In the absence of streptomycin, vegetative cells multiply slowly for a few divisions and then stop growing, but do not die. The new clones arising after a cross, however, have an absolute streptomycin requirement and die very quickly if it is not met. The pattern of inheritance of *sd* is shown in Fig. 4; all progeny resemble the parent of mating type *plus*. It would be interesting to obtain a double *sd sr*-500 mutant, but as yet this has not been accomplished because of the uniparental pattern of transmission. As previously reported (*7*), occasional zygotes transmit *sr*-500 from the parent of mating type *minus*, and experiments are in progress to screen for such exceptions in crosses with *sd*.

A number of *sr*-1500 strains were isolated after subculturing an *sr*-500 mating type *minus* strain on streptomycin. They may be either double mutants (*sr*-500 plus *sr-x*) or mutated *sr*-500's. Whichever is the case, the resistance is lost in crosses to wild-type cells (*ss*) of mating type *plus*. Some of these strains subsequently were found to be unstable, reverting back to the 500-μg/ml level of resistance.

Recently a chromosomal gene, *A*, has been found which amplifies the resistance level both of the chromosomal *sr*-100 and of the non-chromosomal *sr*-500, although it confers no resistance itself upon *ss* strains (*8*). Thus, strains of *ss A* and *ss a* are phenotypically indistinguishable, but

strains of *sr*-100 *A* and *sr*-500 *A* are each resistant to over 2 mg/ml (Table 1). The presence of *A* does not alter the pattern of inheritance of *sr*-500, as shown in Fig. 5, despite the evident physiological interaction between the two determinants. *A* resembles *sr*-100 (with which it is unlinked) in not influencing the inheritance pattern of *sr*-500, and in segregating independently from it. It has also been found that neither *A* nor *sr*-100 influence the segregation of *sd*.

We have employed a number of these genotypic combinations in preliminary studies of the physiology of streptomycin resistance. Using streptomycin-C^{14} (*9*) we examined the uptake of the drug by the following strains: *ss a*, *ss A*, *sr*-100 *a*, *sr*-100 *A*, *sr*-500 *a*, and *sr*-500 *A* (*10*). On exposure to 50 μg/ml, they all bind streptomycin to the same extent, about 10^8 molecules per cell, as determined by radioactivity counts of water-washed cells. This binding remains constant for more than 24 hours. If cells are washed with growth medium before counting, most of the radioactivity comes out, leaving a residue of 5 to 10 percent which increases slowly with continued exposure to the drug. These experiments provide no evidence of permeability differences between the strains, in contrast to observations reported with *Escherichia coli* (*11*). Further studies of the kinetics of uptake are in progress.

In our system, a more sensitive indicator of streptomycin uptake is its effect upon chlorophyll synthesis. Sublethal concentrations of the drug interfere with the synthesis of chlorophyll in the dark, a step which can be performed by wild-type *Chlamydomonas*. The sensitive step is blocked reversibly by streptomycin in *ss* and *sr*-100 strains, but not in strains containing *sr*-500 or *sd* (Table 1). Apparently, the site at which streptomycin blocks chlorophyll synthesis is more available in cells with *sr*-100 than in cells containing *sr*-500. The presence of *A* does not alter the picture. These results indicate that a difference exists in the mode of action of *sr*-100 and *sr*-500, despite the ability of each to interact with *A*.

Thus we have four nonchromosomal determinants, or one determinant with four alternative forms (*ss*, *sr*-500, *sr*-1500, *sd*), which all exhibit the same pattern of inheritance. In our view, these determinants are permanent cell constituents, ranking with chromosomal genes as part of the hereditary repertory of the organism. They are well integrated both in physiological and in replication mechanisms, and their pattern of inheritance is independent of their state of expression.

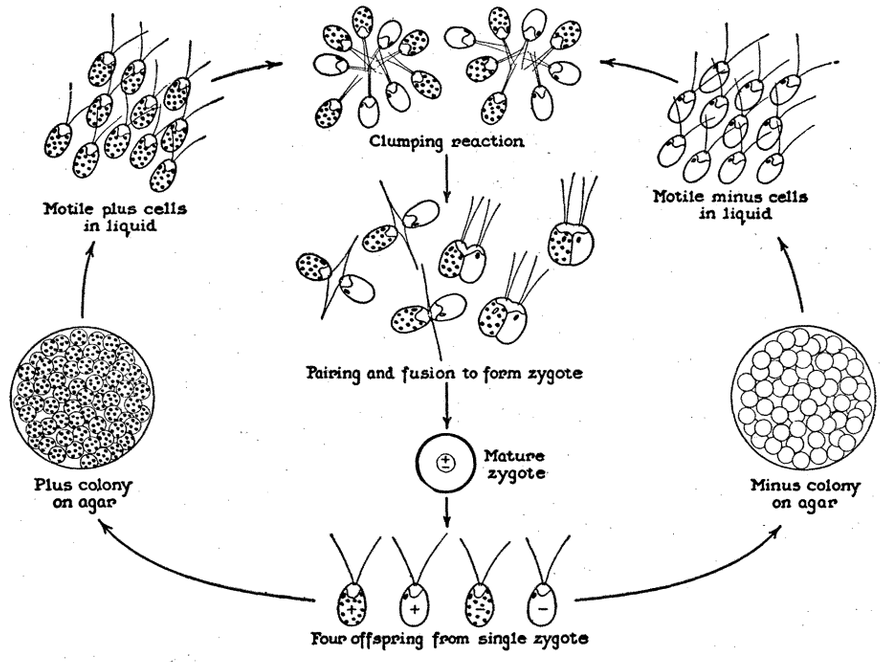

Fig. 2. The life cycle of *Chlamydomonas reinhardi*, showing the segregation of mating type, denoted by plus and minus signs, and of the marker y_1 (the dotted cells are y_1^+, the undotted are y_1^-).

What Are Nonchromosomal Determinants?

Stable hereditary determinants exhibiting nonchromosomal patterns of segregation in meiosis have been reported since the early days of Mendelian genetics, but in no instance has either their chemical constitution or their location within the cell been established. Despite this paucity of information, there has been no dearth of hypotheses about them. Before turning to further experimental evidence, it might be useful, for purposes of orientation, to consider briefly what some of these hypotheses have been.

On the gene hypothesis, these determinants are permanent cell constituents which replicate and segregate in a regular manner, well integrated with cell division. They are genes without chromosomes. Their stability does not depend upon whether they are latent or expressed; their nonappearance as spontaneous or radiation-induced mutants may result from their presence in the cell as a number of replicates. If so, their mutation or loss might require some sort of directed, not random, event. This consideration led us to study the conditions under which *sr*-500 mutants arise, as discussed below.

The episome hypothesis of Jacob and Wollman (*12*) is an extrapolation from the finding in *Escherichia coli* of two kinds of genetic particles (prophage and *F*) which can exist either localized on a chromosome and well integrated in the cell or not localized on a chromosome, poorly integrated, and infectious. If the ability of genetic elements to attach to and detach from

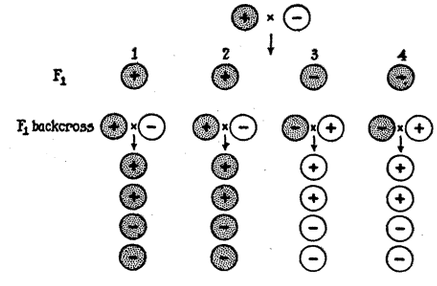

Fig. 3. Inheritance of streptomycin resistance of strain *sr*-2. Plus and minus signs refer to mating type. The initial cross, *sr* $mt^+ \times$ *ss* mt^-, gave rise exclusively to *sr* offspring, which segregated 2:2 for the markers *mt* and y_1. F_1 clones of plus mating type backcrossed to *ss* mt^- produced all *sr* offspring (4:0), but F_1's of minus mating type backcrossed to *ss* mt^+ produced only sensitive progeny (0:4). Stippled, streptomycin-resistant; unstippled, streptomycin-sensitive.

chromosomes is a general feature, then the same elements may be chromosomal at one time and nonchromosomal at another. Evidence to support this hypothesis would require identifying for a particular determinant both a mapable state, showing linkage with other genes, and an unmapable state; as yet this has not been found except in *E. coli*.

The steady-state hypothesis [as formulated, for example, by Delbruck (*13*) and by Pollock (*14*)] proposes that a sudden change in some environmental condition may alter a particular reaction rate and that this in turn may alter others, in such a way that a new steady state of interlocking reaction rates becomes established, conferring a new phenotype upon the cell, and thereby mimicking the effect of a mutation. The new phenotype would be expressed and transmitted to progeny, until a suitable change in the environment intervened. One cannot predict the stability of such systems a priori, but it seems unlikely that they would persist for years in the absence of the inducing agent, as streptomycin resistance does in the absence of streptomycin. Catcheside has referred to the "conditional permanence of a steady-state system" (*15*) to set it off from the unconditional stability of a gene.

Virtually nothing is known about the inheritance of cell structures, but cytologists have, for the past hundred years, been describing the origin of some organelles from pre-existing structures of the same kind. Such a process would require the presence of supertemplates concerned with the arrangement of aggregates of molecules. If supertemplates exist, they represent a class of hereditary determinants. Some of the nonchromosomal chloroplast mutations which have been described in higher plants may be of this type.

Returning now to the *sr* mutants, we favor the first hypothesis—that nonchromosomal determinants are genes—as being closest to the experimental observations so far available, for the following reasons: (i) The absence of segregation in crosses and the uniparental transmission through the zygote show that the determinant is not on a chromosome during meiosis. It seems very unlikely that it leaves the chromosome during meiosis, when segregation is so critical, and becomes reintegrated with the chromosome at other times, but this possibility has not been excluded. (ii) The identification of four different phenotypes with respect to streptomycin, all showing the same pattern of uniparental heredity, suggests that they may represent mutational alternatives (or different alleles) of the same determinant. (iii) The unconditional stability of the *sr*-500 factor under a great variety of environmental conditions and growth rates, and after five generations of outcrossing, all in the absence of streptomycin, argues against the application of the steady-state hypothesis to this system. (iv) The fact that *sr*-500 interacts with the chromosomal gene *A* in conferring a higher resistance level upon the cell, without altering the *sr*-500 pattern of inheritance, is further evidence of the separation of physiological expression from hereditary transmission. Such a separation is one of the principal features of genetic material, in contrast to steady-state systems. (v) Evidence of the nonchromosomal nature of the material comes also from studies of its mutational origin, discussed below.

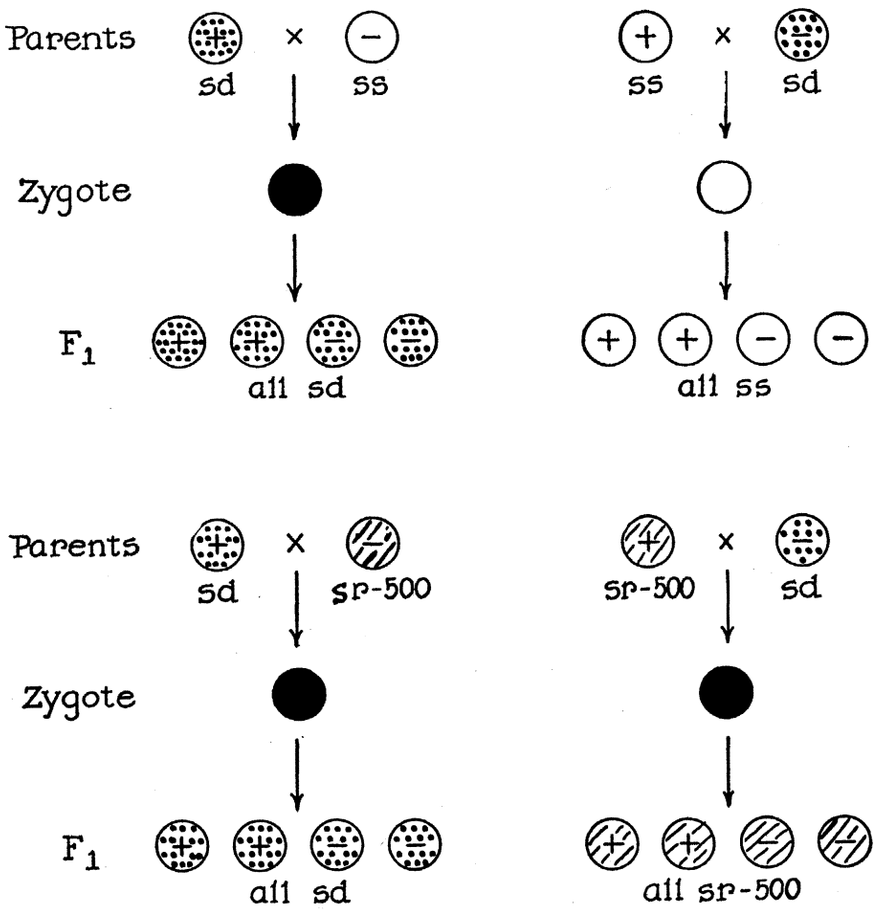

Fig. 4. Inheritance of streptomycin dependence. In reciprocal crosses of *sd* with *ss*, all progeny resemble the parent of mating type *plus* in their response to streptomycin. The same is true in reciprocal crosses of *sd* with *sr*-500. Dotted, *sd*; hatched, *sr*-500; white, *ss*; black, not *ss* but may be either *sd* or *sr*-500.

Streptomycin as a Mutagen of Nonchromosomal Elements

A long-term investigation has been in progress to analyze the conditions required for mutation from *ss* to *sr*-500 (*10*). Initially, the plan was simply to find out whether or not *sr*-500 mutants appeared at random in populations of *ss* cells before treatment with streptomycin. Technical difficulties in the system arose from the very low frequency of *sr*-500 mutants recovered and from the lethality of streptomycin; both of these properties complicate the analysis. As a result, it has not been possible to obtain a fully unequivocal answer, but a number of lines of evidence support the view that mutations to *sr*-500 occur only in the presence of streptomycin.

1) The frequency of *sr*-500 mutants appearing on streptomycin-agar plates is a function of the survival time of *ss* cells on the plates. This time can be varied within wide limits by varying the medium used. In general, no *sr*-500 mutants are recovered on minimal

medium under conditions in which *ss* cells die rapidly in contact with the drug. Addition of acetate to the medium (at the same *p*H), which greatly increases survival time but allows only one or two doublings of cells, results in the appearance of *sr*-500 mutants, in yields at least 100 times that of controls without acetate. The increase in number of mutations is not proportional to the increase in population size but is a function of survival time in the presence of streptomycin.

2) Even with a favorable medium, *sr*-500 mutants are found only at a low streptomycin concentration (100 μg/ml), although immediately after recovery, these mutants are fully resistant to 500 μg/ml. Once established, *sr*-500 mutants show no lag in growth with a concentration of 500 μg/ml, even after years of subculture in the absence of the drug. Thus, it seems that the *sr*-500 mutants develop resistance gradually, in contrast to the behavior of chromosomal mutants, but that, once established, the resistance level is maintained.

3) In fluctuation analysis, no accumulation of pre-existing *sr*-500 mutants has been found; in these experiments *sr*-500 mutants arise only as plate mutants, despite the screening of large populations of cells. On the other hand, mutations to the chromosomal *sr*-100 occur in the tubes with the expected high variance of spontaneous mutations, and at a rate consistent with the frequencies observed in other experiments.

These experiments clearly demonstrate the difference in mutational response of the genetic elements *sr*-100 and *sr*-500, and the results are consistent with the hypothesis that *sr*-500 mutations are induced by streptomycin. (Possible mechanisms of this induction include the intracellular selection of particles which may have mutated independently.) The alternative possibility, that there is a special requirement for *expression* of spontaneous *sr*-500 mutations, rather than for the mutation process itself, has not been fully excluded.

Support for the induction hypothesis has come from another line of work: the mutagenic effect of streptomycin upon chlorophyll-forming ability. It may be recalled that some years ago streptomycin was reported to inhibit the greening process in young seedlings of higher plants (*16*) and in the green flagellate *Euglena* (*17*). In both systems, under some conditions, the loss of greening ability was permanent. These results are very interesting, for they suggest that streptomycin has induced a mutation in a genetic determinant.

Table 1. Phenotypic properties of various streptomycin-resistant and streptomycin-dependent strains. *S*, streptomycin.

Genotype	Streptomycin-resistance level on agar (μg/ml)	Chlorophyll-forming ability in dark	
		Without S	With S
y_1^+ *ss a*	20	Green	Yellow
y_1^+ *ss A*	20	Green	Yellow
y_1^+ *sr*-100 *a*	100	Green	Yellow
y_1^+ *sr*-100 *A*	2000	Green	Yellow
y_1^+ *sr*-500 *a*	500	Green	Green
y_1^+ *sr*-500 *A*	2000	Green	Green
y_1^+ *sd a*	500	Green	Green
y_1^+ *sd A*	2000	Green	Green

In *Chlamydomonas* we have found that streptomycin interferes specifically with formation of chlorophyll in the dark (*18*). When wild-type cells, which are killed by 20 μg of streptomycin per milliliter, are treated with sublethal concentrations of the drug, the synthesis of chlorophyll in the dark is blocked, but the effect is reversible. No permanent effects of streptomycin have been observed at these low concentrations. With the use of streptomycin-resistant strains, it has been possible to obtain yellow mutants in which the ability to form chlorophyll in the dark has been permanently lost. To achieve this result, cells must be grown with a high concentration of streptomycin for some time. Experiments have been carried out on agar, rather than in liquid, to make sure that all cells of the initial population are being observed, not just a selected group of pre-existing mutants.

In preliminary experiments we found that cells containing the *sr*-500 factor could be grown on 1500 μg of streptomycin per milliliter in the dark, with about 50 percent viability. Most of the colonies developing under these conditions gave rise to some permanently yellow progeny upon subculture in the absence of streptomycin. However, colonies formed very slowly, and the possibility of selection occurring within clones led us to experiment with lower concentrations.

Cells grown with 1000 μg/ml in the dark showed 100 percent viability, although on streptomycin they were pale green in color. Since streptomycin does not destroy existing chlorophyll but only interferes with new synthesis, it was necessary to subculture treated cells to determine the effects of the drug. In these experiments, both streptomycin-treated and control colonies were subcultured with and without streptomycin, and marked differences were observed. The results are summarized very briefly here.

Essentially we found that one treatment of cells by growth in the dark with streptomycin had induced a reversible susceptibility of the chlorophyll-forming system to a second treatment of the same kind. Thus, if cells from the first streptomycin-agar plates

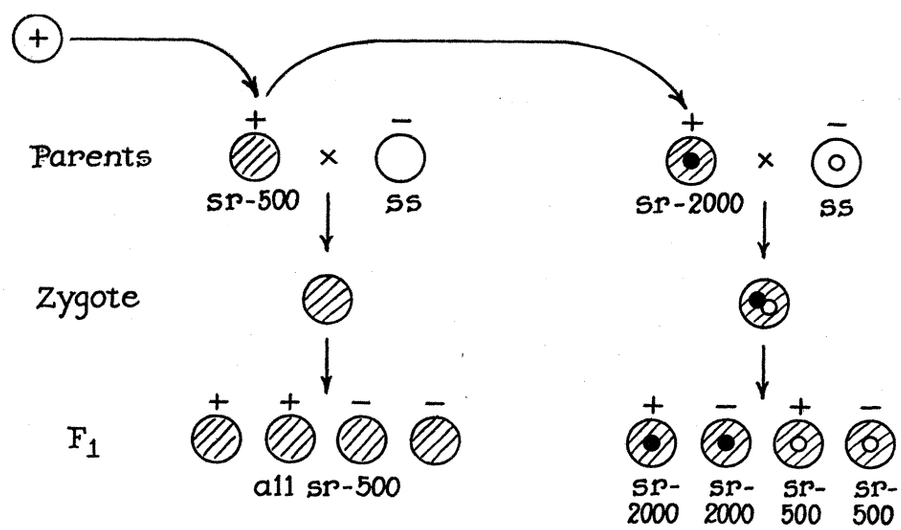

Fig. 5. Inheritance of resistance to 2000 μg of streptomycin per milliliter. Strains of mating type *plus*, carrying *sr*-500, segregate 4:0 for streptomycin resistance in crosses with *ss*; strains carrying both *sr*-500 and the chromosomal gene *A* crossed with *ss a* segregate 2:2 for resistance to 500 μg/ml and 2000 mg/ml. Thus the 2:2 segregation of *A*/*a* is superimposed on the 4:0 segregation of *sr*-500. Hatched, *sr*-500; white, *ss*; black nucleus, *A*; white nucleus, *a*.

were subcultured in the absence of streptomycin, they produced as many green progeny as did the controls, but the same clones subcultured with streptomycin gave rise to a high percent of yellow colonies, which were subsequently yellow in the absence of streptomycin. Approximately 22 percent of yellow colonies were obtained, in contrast with 0.1 percent in the controls given one treatment with streptomycin and about 0.01 percent in the completely untreated initial controls. The observed frequency of yellow colonies represents an underestimate of the number of mutations which occurred, because some of the induced yellow strains were unstable and were characterized by a constant tendency to revert from yellow to green, this mutability itself being a heritable property transmitted by the yellow cells in mitosis and also in meiosis (*18*).

At least half of the yellow colonies obtained—that is, clones representing about 10 percent of all initially treated cells—remained stable yellow upon further subculture and in crosses with other strains. This number itself represents a 1000-fold increase over the controls. That these strains result from induced mutation, not from selection, is shown by the viability of both green and yellow cells under the conditions of the experiment.

We have not yet investigated the mutagenicity of streptomycin towards other cell traits, because it seems important first to define the cellular changes which correlate with the induced effects already observed. If the mechanism of mutagenic action of streptomycin can be clarified at all, the information acquired thereby should provide a less empirical approach to the recovery of other kinds of mutants.

Genetic Analysis of Streptomycin-Induced Yellow Mutants

In previous studies of mutations affecting pigment formation (*5*) it was seen that spontaneous mutations involving the loss of chlorophyll-forming ability in the dark segregated 1:1 in crosses with wild-type green strains. One of these yellow mutants provided the y_1^- genetic factor subsequently included in many crosses. By now, more than 800 tetrads have been analyzed in which a number of genetic pairs, including y_1^-/y_1^+, were segregating. In all of these crosses, the y_1 pair showed only first-division segregation.

This datum can be interpreted either as complete linkage with a centromere or as evidence of a nonchromosomal location. In the absence of any crossing over with the centromere, the demonstration of recombination with some known chromosomal factor is necessary to establish a chromosomal location for y_1. To date we have eliminated five chromosomes, of the eight to ten occurring in this species, as carriers of y_1, and we are in the midst of testing the others. Since this organism has but one chloroplast per cell, the 1:1 segregation might conceivably be that of a unit cytoplasmic organelle segregating in an oriented manner at the first meiotic division, rather than that of a chromosome.

Although the hypothesis that y_1 is nonchromosomal has been under consideration for some time, we have not had markers on all the chromosomes to provide a critical test. Our recent findings concerning the streptomycin-induced yellow mutants have provided new support for this hypothesis from a different point of view. Ten independently arising streptomycin-induced yellow mutants have been crossed with our standard spontaneous y_1^-, and no green recombinants were recovered, with thousands of zygotes scored in each cross. Evidently, then, the spontaneous and the induced yellow mutants result from mutation of the same determinant, and the linkage analysis carried out with the original y_1^- applies as well to the new y_1^- factors.

Without representing critical evidence, the inducibility of the y_1 mutations supports the view that they are nonchromosomal, because of the weight of evidence that chromosomal gene mutations cannot be specifically induced. The action of chemical mutagens such as nitrous acid, 2-aminopurine, and 5-bromo-uracil seems to be directed against individual nucleotides or short sequences rather than against the large functional unit as a whole, and the frequency of induced mutation of any particular gene is very low. The action of streptomycin, on the other hand, seems to be directed specifically against y_1, in the sense that such a great number of the treated cells give rise to mutants. Perhaps the y_1 factor is much more accessible to the drug than a chromosomal gene would be. This speculation is supported by the evidence that *sr*-500 determinants, which are clearly nonchromosomal, are also induced by streptomycin. In our view, the working hypothesis that streptomycin is a mutagen of nonchromosomal genes seems to accord best with the evidence so far available.

Conclusion

In this article I have discussed two genetic systems which appear, for different reasons, to be nonchromosomal. In neither system is the evidence complete, for the determinants themselves have not been identified. It is already clear, however, from their patterns of inheritance, that the two systems are different, for the chloroplast system is independent of mating type in its transmission, while the streptomycin-resistance system is transmitted only by one mating type. From the cytologist's viewpoint (Fig. 1), there are many organelles and membrane systems with a precision and complexity of organization suggesting genetic control. It is likely that different organelles are duplicated and transmitted through the zygote in contrasting ways. If so, our *sr* and y_1 determinants may actually represent markers, reflecting these differences in the mode of inheritance of different cytoplasmic structures.

In the past, it has been a great convenience for geneticists to think exclusively in terms of a single genetic system carried on chromosomes, particularly since chromosomal genes have provided the determinants most accessible to random change. In the modern analysis of cell heredity and gene action, however, it is of the utmost importance to identify all classes of genetic determinants. In attempting to carry out such a program with *Chlamydomonas*, streptomycin has provided a means for the recovery of two classes of genetic factors which differ in a number of properties from chromosomal genes. It is our hope that further analysis of these two systems will lead to their specific identification, and beyond that, to a more informed approach to the search for further genetic systems (*19*).

References and Notes

1. In some aspects of this work I have enjoyed the collaboration of Dr. Yoshihiro Tsubo and the assistance, at various times, of Sydell Fleischer, Charlotte Marfey, Ann Shiffer, Christine Thomas, and Fran Yablonsky. It has been my good fortune to collaborate with Dr. George E. Palade in the electron microscopy. The generosity and interest of Prof. Francis J. Ryan in providing laboratory space for this work and his assistance in other ways is gratefully acknowledged.
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Radiation Environment in Space

Satellites and space probes are revealing the kinds and amounts of radiation men will encounter in space.

Homer E. Newell and John E. Naugle

That vast region beyond the earth's atmosphere often referred to as the void of outer space is not really empty. Through interplanetary space there stream electromagnetic radiations of all wavelengths, electrons, protons, and other nuclei, including cosmic rays, and aggregate particles called micrometeorites. In fact, many of the fundamental particles and quantum radiations have already been observed, and, doubtless, all will be eventually.

Within the solar system, the sun is the primary source of both electromagnetic radiations and particle radiations. In addition to the visible wavelengths which pour forth continuously from the sun, there are ultraviolet and x-ray radiations of variable intensity. At times of great solar activity clouds of electrons and protons are spewed forth and sweep through the regions of interplanetary space. It even appears likely that the sun may contribute to the cosmic radiation.

Before May 1958, radiation was not considered a serious hazard to space travel. Little was feared from the electromagnetic radiations to be encountered. Most of the wavelengths would be in the visible regions, and it was expected that the ultraviolet and x-ray intensities would be low enough to cause no concern. Rocket observations bore out this conclusion. Moreover, the available experimental data indicated that the only particle radiations in space would be cosmic rays, and that the radiation level due to cosmic rays would be negligible for most space missions.

However, on 1 May 1958, James A. Van Allen announced the discovery of the great radiation belts around the earth. The radiation levels in these belts are not negligible. A second phenomenon, the so-called solar proton beams, or solar cosmic rays, was discovered shortly thereafter. Thus, in the course of a few months radiation changed from an unimportant factor in space travel to a major factor affecting the choice of trajectories and determining the size and weight of the spacecraft and their physical configuration.

In this article we summarize some of the information on radiations in space obtained by means of satellites and space probes. The physical nature of these radiations is discussed, together with the mechanism by which the radiation interacts with matter. Dosage levels are defined. The salient factors in the choice of shielding are given. Finally, an attempt is made to assess the importance of radiations in space to various space missions, such as Project Mercury, circumlunar flights, and the operation of unmanned satellites and space probes.

Electromagnetic Radiations

As stated above, the electromagnetic radiations encountered in interplanetary space are primarily solar in origin. At a distance of one astronomical unit from the sun, the total energy flux in this solar radiation amounts to about 2 calories per square centimeter per minute, which is equivalent to 0.14 watt per square centimeter. The radiation is mostly in the visible wavelengths. About 7 percent of the total energy flux lies in the ultraviolet regions between 2000 and 4000 angstroms; in still shorter wavelengths in the vicinity of the Lyman-alpha line of hydrogen, 1216 A, the total intensity is down by many orders of magnitude, averaging about 6×10^{-7} watt/cm^2. In the soft x-ray wavelengths intensities fall off another order of magnitude or more. Occasionally harder x-rays are observed at the time of the solar flares. Gamma radiation is normally of negligibly small intensity.

Rocket observations by the Naval Research Laboratory group have revealed ultraviolet fluxes from distant astronomical sources. Moreover, the hydrogen in interstellar and interplanetary space is a source of some radiation in the Lyman-alpha wavelengths.

The intensity of the solar radiations

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