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The Mechanism of Maternal Inheritance in *Chlamydomonas*: Biochemical and Genetic Studies[1]

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Summary. The cytoplasmic linkage group of *Chlamydomonas* shows maternal inheritance, i.e. preferential transmission of cytogenes from the female ($m\ t^+$) parent and loss of the corresponding male ($m\ t^-$) genome in sexual crosses. The mechanism of this process is postulated to be enzymatic modification of chloroplast DNA of the female to protect it from a restriction enzyme which degrades the chloroplast DNA of the male parent in the zygote soon after fusion. Genetic, biochemical and physical data bearing on this hypothesis are summarized and discussed.

This paper will discuss studies of the molecular mechanisms regulating maternal inheritance of cytoplasmic genes (cytogenes) in *Chlamydomonas*. Maternal (or more generally uniparental) inheritance is the hallmark of cytoplasmic inheritance and the original criterion by which cytogenes were first identified; it is still the simplest means of distinguishing cytoplasmic from nuclear genes in most organisms (Sager 1972). Yet the mechanism of this fundamental process is not understood and has hardly been studied.

The molecular basis of maternal inheritance is also of interest in a wider context, namely the phenomenon of chromosome elimination. In the normal life cycle of many invertebrates especially the insects (Rhoades 1961), elimination of particular chromosomes is a characteristic feature of somatic development. In mammalian cells, preferential chromosome elimination is a regular occurrence following somatic cell fusion during the growth of interspecies hybrids (Weiss and Green 1967). Not only nuclear chromosomes, but also the mitochondrial DNA (Attardi and Attardi 1972, Clayton and Teplitz 1971) of one of the parental cells, is regularly excluded during growth of hybrid clones. Understanding the mechanism of chromosome elimination would be of great utility in controlling the process experimentally and thereby determining the chromosome composition of hybrid clones.

An analogous system is modification-restriction in bacteria, in which foreign (unmodified) DNA's are preferentially attacked and digested while the native (modified) DNA's within the same cell are fully protected (Arber and Linn 1969, Boyer 1971). Our present understanding of maternal inheritance in *Chlamydomonas*, as will be presented in this paper, strongly suggests that the mechanism is a kind of modification-restriction system. The evidence will be summarized and discussed on the basis of a model to be presented below.

Why is it that maternal inheritance is such a reliable criterion for the identification of cytogenes? The short answer is that transmission of cytogenes from one parent, rather than from both, seems to be a general property of cytoplasmic genetic systems. The evidence from studies of numerous organisms has recently been extensively discussed (Sager 1972).

Briefly stated, in higher plants and in eukaryotic microbes (e.g. *Chlamydomonas*, yeast, *Neurospora*), maternal transmission in meiosis coupled with exclusion of cytogenes from the male, is the rule. Superficially, the mechanisms of male exclusion appear to differ in organisms e.g. higher plants and fungi in which the male contributes very little cytoplasm, from those like *Chlamydomonas* and yeast, in which the fusing gametes are genetically different but of equal size.

In the latter class, the exlusion mechanism must be enzymatic, since genes from the two parents co-exist after fertilization within the same cell. In the former class, simple physical exclusion has long been postulated as the sole basis of maternal inheritance. The recent studies of Tilney-Bassett (1970) (see Kirk and Tilney-Bassett 1967) confirming and extending the classical original observations by Baur (1909) of varying amounts of paternal transmission of cytoplasmic genes in the flowering plant *Pelargonium* have demonstrated that physical exclusion of male cytoplasm is not a sufficient explanation of the complex patterns of cytoplasmic transmission seen in this plant, and have focussed on the probable existence of an enzymatic mechanism, and the need for a mole-

[1] This paper is dedicated, with great admiration and affection, to Professor Marcus M. Rhoades, whose enthusiasm and curiosity contributed so much to my own scientific development, whose openness to biochemical and molecular interpretations of genetic data, played an exemplary role in focussing my own approach to research and whose fundamental studies of cytoplasmic inheritance in maize (Rhoades 1933, 1946) were directly responsible for my determination to tackle this difficult and perplexing area of research.

cular interpretation. A report of paternal inheritance in *Neurospora* (Srb 1966), (discussed in Sager 1972), increases the likelihood that analogous mechanisms of preferential transmission and exclusion of cytogenes are also at work in the fungi.

The plan of this paper is first to review published evidence on the mechanism of maternal inheritance in *Chlamydomonas*, especially the effects of UV irradiation (Sager and Ramanis 1967) and the different fates of chloroplast DNA's from male (*mt*−) and female (*mt*+) gametes in the sexual cycle (Sager and Lane 1972). These findings set the direction of more recent studies to be presented, which include the effects of various chemical treatments and of a new mutation upon the polarity of transmission of cytogenes from the two parents in the meiotic phase of the life cycle.

Effects of UV Irradiation upon Maternal Inheritance

Chlamydomonas reinhardi is heterothallic with two mating types, determined by the nuclear alleles *mt*+ and *mt*−; and isogamous, the *mt*+ and *mt*− gametes being of equal size. Thus, the discovery (Sager 1954) that cytogenes exhibited polarity of transmission in meiosis (i.e. maternal inheritance) immediately raised the question of mechanism. The first insight into the mechanism came in the same study (Sager 1954), with the recognition that some spontaneously occurring exceptions to the rule of maternal inheritance were also present. It was felt that the key to understanding the mechanism might well lie in investigating these exceptions. Indeed, the existence of spontaneous exceptions, not resulting from mutations and with a frequency of about 10^{-3}, already suggested a biochemical or enzymatic control mechanism.

The next important clue came with the discovery (Sager and Ramanis 1967) that UV irradiation of the female (*mt*+) but not of the male (*mt*−) parent immediately before mating, resulted in almost total inhibition of the maternal pattern of transmission, and gave rise instead to exceptional zygotes with a biparental or paternal transmission pattern. (Exceptional zygotes are defined as those that transmit cytogenes from the male parent (Sager 1954), including *biparental* zygotes transmitting cytogenes from both parents and *paternal* zygotes transmitting *only* cytogenes from the male.) The precise proportions of maternal, biparental, and paternal zygotes depended on the UV dose, and the whole range (even 100% paternal) could be recovered with UV exposures giving little loss of zygote viability. The effective UV doses were sufficient to kill all but ca. 1% of the unmated gametes (plated as controls) but they were rescued by mating to unirradiated males. Irradiation of the male gametes before mating had no effect on maternal inheritance, and irradiation of zygotes after fusion was highly lethal.

The ratio of biparental to paternal zygotes was found to be UV dose dependent, as was the disappearance of maternal zygotes. The UV effect was shown to be photoreactivable. When gametes which had been exposed to UV were subsequently exposed to visible light, either before or during mating, the proportions of the three classes were shifted back towards the maternal. However, the efficiency of photoreactivation was such that the conversion from paternal to biparental was far more effective than from either class of exceptional zygotes back to maternal.

In practice this situation was very useful, permitting us to collect the largest number of biparental zygotes which were utilized for further genetic analysis. At the level of mechanism, this result suggested the existence of two different UV-sensitive and photoreactivable reactions, the first concerned with the inhibition of maternal inheritance, and the second, requiring a higher UV dose, concerned with survival of the irradiated genome. The fact that both are photoreactivable shows that both targets are DNA but does not further identify them.

We postulated that the second reaction might be involved primarily with replication of chloroplast DNA (the probable carrier of the cytogenes under observation; see below), whereas the first reaction, might be rather directly involved in the regulation of maternal inheritance, and therefore of particular interest in the present context. The studies with ethidium bromide, various antibiotics and other chemicals to be presented below, stem directly from these considerations, and represent attempts to pinpoint more directly the site of UV action. Before turning to these studies, however, it is necessary to review briefly what is known about the behaviour of chloroplast DNA's from the two parents during the meiotic phase of the life cycle.

Behaviour of Chloroplast DNA's in the Sexual Life Cycle

The identification of the cytogenes being investigated in *Chlamydomonas* with chloroplast DNA is based on a number of separate lines of evidence. Genetic analysis revealed the occurrence of regular patterns of segregation and recombination characteristic of DNA; the UV effect and photoreactivation described above pointed to a DNA target; the only well-defined cytoplasmic DNA known in *Chlamydomonas* is chloroplast DNA (Sager 1972, Sager and Ishida 1963, Sueoka *et al.* 1967). Many additional observations have further supported the correlation of the known cytogenes with chloroplast DNA (reviewed in Sager 1972) but recently two independent lines of experimentation have greatly strengthened the evidence. One line of evidence is the correlation between an alteration affecting chloroplast ribosome function and a cytogene mutation mapping in the

cytoplasmic linkage group of *Chlamydomonas* (Schlanger *et al.* 1972, Sager and Ramanis 1970). This correlation confirms and extends the findings of Gillham *et al.* (1970) that chloroplast ribosomes from some cytoplasmic mutants of *Chlamydomonas* were 66 S rather than 70 S, indicating some unspecified alteration; and of Mets and Bogorad (1971) that chloroplast ribosomes from a cytoplasmically inherited erythromycin resistant mutant were impaired in their erythromycin-binding ability compared with the wild type. In our study (Schlanger *et al.* 1972) mutation to carbomycin resistance conferred resistance at the ribosome level as assayed in an *in vitro* amino acid incorporating system. Thus, known cytogenes of *Chlamydomonas* influence the structure and function of chloroplast ribosomes, strongly supporting the hypothesis that they are located in chloroplast DNA.

The second recent line of evidence involves the study of the different fates of chloroplast DNA's from male and female gametes during zygote maturation, showing that the chloroplast DNA from the female parent is preserved and replicated, while that from the male is degraded soon after zygote formation (Sager and Lane 1972). This pattern of replication and destruction is precisely the same as the transmission pattern of cytogenes observed in crosses with suitable markers.

The studies of chloroplast DNA in the sexual cycle were performed by prelabelling the DNA's of male and female parents during growth of the gamete cultures with ^{15}N or ^{14}N, and then making crosses: ^{14}N (female) $\times$ ^{15}N (male) and the reciprocal (Sager and Lane 1972). (In another series of experiments, as yet unpublished, radioisotope labels (^{14}C- and ^{3}H-adenine) were also used.) Two remarkable events occurring soon after zygote formation were discovered. (1) Chloroplast DNA from the female parent undergoes a density shift within six hours after zygote formation, as seen in CsCl density gradients, shifting from the bouyant density of 1.695 gm/cm^3, characteristic of vegetative cells and of gametes, to the lighter density of 1.690 gm/cm^3 in zygotes. (2) Chloroplast DNA from the male parent disappears from the gradients by about 6 hours after zygote formation, no longer being detectable as a high molecular weight component.

Gradients from samples of 24-hour old zygotes are virtually indistinguishable from the 6-hour samples, and no further changes are seen in these two features of the DNA until the first round of replication several days later, i.e. the chloroplast DNA from the female parent remains at the shifted density, and that of the male does not reappear. Thus it seems highly probable that the observed events represent the molecular basis of maternal inheritance, the loss of genetic markers from the male resulting directly from the observed disappearance of the male chloroplast DNA. We have furthermore interpreted the accompanying density shift in the female chloroplast DNA as evidence of a biochemical modification in that DNA, serving to protect it from enzymatic degradation.

Our detailed studies of later times in zygote maturation when replication of chloroplast DNA occurs, have not yet been published. In brief, they confirm the published findings: a density difference in the surviving DNA, depending on the polarity of the cross, is retained and can be seen through at least one round of replication.

In the interpretation of these results, the timing of cellular events after mating needs to be recalled. Light microscope observations (Sager, unpublished), later confirmed by electron microscopy (Friedmann *et al.* 1968, Cavalier-Smith 1970) show that after zygote formation, a period of a few hours elapses before the fusion of chloroplasts and of nuclei. The time of chloroplast fusion, of importance in the present context, was estimated at approximately six hours. This is the same interval chosen on technical grounds, in our physical studies (Sager and Lane 1972 and in preparation) for taking the first sample, by which time the early events affecting chloroplast DNA had already occurred. Thus the biochemical and physical changes in chloroplast DNA occur before or near the time of chloroplast fusion.

As mentioned earlier, neither the density shift nor the degradation of male chloroplast DNA can be detected in the gametes before mating occurs. We do not know whether the enzymes required for these events are already present in an inactive form or not. If so, their activity is not seen until after zygote formation. The possibility that the enzymes are presynthesized is strengthened by the likelihood that very little if any protein synthesis occurs during the first 24 hours after zygote formation. The conversion of vegetative cells into gametes, capable of mating, requires extensive nitrogen-deprivation, (Sager and Granick 1954) and during this period of gametogenesis, ribosomes are degraded (Siersma and Chiang 1971). After mating occurs, under our procedure, no nitrogen source is added to the medium during the first 24 hours, and only then NH_4NO_3 is added to fulfill the nitrogen requirement for maturation of zygotes. Thus the changes in chloroplast DNA occur at a time of minimal availability of either ribosomes or a nitrogen source for protein synthesis.

Effects of Antibiotics and Other Chemicals upon Maternal Inheritance

These studies were initiated to find a chemical substitute for the UV treatment that would block maternal inheritance specifically. Inhibitors of protein synthesis were examined on the hypothesis that proteins concerned with maternal inheritance and zygote formation might be the only ones synthesized in this period of ribosome breakdown. Rifamycin and cyclic AMP were tested to see if transcription was involved, and ethidium bromide was tested as

Table 1. *Increase of exceptional zygotes after treatment of gametes*

Treatment	Female (mt^+) treated		Male (mt^-) treated	
	% exceptional zygotes	relative increase	% exceptional zygotes	relative increase
UV irradiation				
mated 2 hrs. after UV	68.	200	1.0	0.25
mated 6 hrs. after UV	40.	120	4.5	14.
Ethidium bromide				
10 μg/ml, 6 hrs.	12.	70.	5.0	24.
20 μg/ml, 6 hrs.	12.	70.	25.*	12.
Cycloheximide				
10 μg/ml, 6 hrs.	0.05	d	5.8	7.5
20 μg/ml, 6 hrs.	1.5	2.6	4.6	8.1
Erythromycin				
500 μg/ml, 6 hrs.	1.2	4.0	6.8	20.
Spiramycin				
200 μg/ml, 6 hrs.	2.0	6.5	10.	30.
Rifamycin SV				
20 μg/ml, 6 hrs.	1.2	5.2	5.0	21.
Butyryl cyclic AMP				
500 μg/ml, 6 hrs.	0.5	d	5.0	6.0
1000 μg/ml, 6 hrs.	0.7	1.0	4.0	5.0
DL-ethionine				
200 μg/ml, 6 hrs.	3.0	9.0	1.0	3.0

* High value owing to decreased viability of maternal zygotes d = decrease in treated sample relative to untreated control.

Cells were grown as previously described (Sager and Ramanis 1967) on agar with 1/5 the usual NH_4NO_3 for four days, washed off plates into minimal medium without NH_4NO_3 (N-free medium) and kept in dim light overnight. Cells were then gametic: i. e. capable of fusion within an hour after mixing the two mating types. Gametes were treated with inhibitors in suspension in dim light (table top) then washed and mated. Relative increase is computed as the percent exceptional zygotes in treated/untreated cultures.

UV irradiation corresponded to dose giving about 10% surviving gametes, if plated for survival without photoreactivation, but approximately 100% surviving zygotes, as a result of (1) fusion between irradiated and unirradiated cells, and (2) photoreactivation.

Table 2. *Increase of exceptional zygotes after treatment during gametogenesis*

Treatment	Female (mt^+) treated		Male (mt^-) treated	
	% exceptional zygotes	relative increase	% exceptional zygotes	relative increase
UV irradiation				
18 hrs. in dim light	13.	40.	0.9	3.
18 hrs. in dark	25.	80.	1.0	3.
Ethidium bromide				
10 μg/ml	8.	45.	0.004	d
Cycloheximide				
5 μg/ml	0.3	d	33.*	45.*
10 μg/ml	0.7	1.0	25.*	34.*
20 μg/ml	0.2	d	60.*	82.*
Erythromycin				
500 μg/ml	3.0*	10.*	7.	20
Spiromycin				
100 μg/ml	3.4	10.	5.	16.
Rifamycin SV				
10 μg/ml	4.8	15.	2.5	8.
25 μg/ml	5.0	15.	1.6	5.
50 μg/ml	4.3	13.5	2.2	7.
DL-ethionine				
10	2.0	3.0	0.3	1.0
50	4.0	7.0	1.0	2.0
100	5.0	9.0	0.3	1.0
100	4.0	5.0	1.2	1.5
200	6.0	6.5	1.0	1.2
200	10.0	30.0	1.0	3.0

* High values owing to low viability of all zygotes and preferential survival of exceptional zygotes.

d = decrease in treated sample relative to untreated control.

Cells were grown as in experiments of Table 1. After four days, cells were washed off plates, resuspended in N-free medium and incubated in dim light overnight for gamete formation in the presence of the inhibitor. Gametes were then washed and mated. Results were computed as in Table 1. UV irradiation as in Table 1.

a potential replication block. (While this work was in progress, we found (Flechtner and Sager, in prep.) that ethidium bromide (EB) degraded chloroplast DNA preferentially in exponentially growing cells but this effect may not explain the action of EB in the mating system.)

The principal findings of these studies are summarized in Tables 1 and 2. In these experiments, the parental cultures were grown on agar under standard conditions for gametogenesis (Sager and Ramanis 1967). When growth ceased (after about 12—14 doublings) the cells were washed off the plates, resuspended in a nitrogen-free minimal medium, and incubated a few hours in the light until aliquots exhibited rapid zygote formation. The parental cultures were then considered fully differentiated gametes, and treated with antibiotics or other chemicals as noted in the table. For treatment during gametogenesis, the chemicals were added as soon as cells were washed off the plates and left for 18 hours in dim light, a condition that permitted slow gametogenesis. After treatment, the cells were washed, mixed with equal numbers of cells of the opposite mating type, and allowed to mate. When zygote formation was complete, aliquots were plated on a standard medium to determine total zygote viability (zygote colony formation) and on a selective medium to assay the number of zygotes receiving genes from the male parent. Results are expressed in two ways: the percent of exceptional zygotes in treated cultures; and

the relative increase in the percent of exceptional zygotes in the treated vs. untreated cultures.

The largest effects are seen after UV irradiation of the female parent, confirming the results first reported in 1967 (Sager and Ramanis 1967) and utilized continuously since that time in genetic studies. No chemical treatment approached the yield of exceptional zygotes obtained with UV. The phenanthridium dye, ethidium bromide, produced a similar effect to that of UV but less pronounced, when the female gametes were treated for 6 hours in dim light before mating. However, when the male gametes were treated, an effect of EB was also seen, amounting to 5% exceptional zygotes with 10 μg/ml EB, and 25% with 20 μg/ml. The latter figure is somewhat misleading, resulting in part from preferential survival of exceptional zygotes, rather than from a real increase.

Nonetheless, the small but real effect of EB on male gametes raised two questions. 1. Does the effect result from carryover of the drug into the zygotes, where its real target is some system coming from the female parent? This question concerns all chemical treatments in contrast to UV irradiation. 2. Is there a target in the male gamete which is also involved in maternal inheritance? The results of treatments with the other chemicals shown in Table 1 bear on both questions.

All the other compounds tested (except for DL-ethionine), namely cycloheximide, erythromycin, spiramycin, rifamycin SV, and butyryl-cyclic AMP clearly had a greater effect when males were treated than females. This finding shows that the effect on males cannot be attributed simply to carryover of the chemical, and that consequently the male gametes do play some independent role in the control of maternal inheritance.

Further support for this conclusion comes from the study of chemical treatments during gametogenesis, summarized in Table 2. Here again, UV irradiation and EB were the principal treatments effective on female gametes, with DL-ethionine showing a small effect; whereas some of the same compounds effective on male gametes were also effective when the cells were treated during gametogenesis.

The most dramatic effects were obtained with cycloheximide, with recoveries of 60% exceptional zygotes. However, extensive lethality occurred in these cultures, and the high yield of exceptional zygotes resulted primarily from their preferential survival.

Comparison of Tables 1 and 2 indicates that treatments during gametogenesis except for cycloheximide were generally less effective in influencing exceptional zygote formation than were treatments of mature gametes. This observation supports the impression from previous studies that the choice of alternative pathways, maternal vs. exceptional zygotes, is made during a very short interval near the time of zygote formation.

A Mutation, *mat*, Influencing Maternal Inheritance

A mutation of unknown origin appeared in an *mt*— stock culture carrying the cytoplasmic markers *spc* —*r* (spectinomycin resistance) and *sm2*—*r* (high level streptomycin resistance). The mutation was detected by its dramatic effect on zygote viability and on maternal inheritance. In a cross with an *mt*+ strain carrying the cytoplasmic marker *ery*—*r* (erythromycin resistance), only 1% of the expected zygotes germinated, and about 50% of them were exceptional, as shown by transmission of cytogene markers from the male parent. Of the exceptional zygotes recovered 2/3 were biparental and 1/3 were paternal. Each of the parental strains of this cross were then test- crossed, and the new phenotype was found associated with the male (*mt*—) parent. Less than 1% of the expected zygote germination occurred, and about 20% of the zygotes were exceptional.

Transmission of this trait to the F_2 generation was examined by choosing one exceptional zygote from the first cross, and independently crossing each of the 6 progeny from that zygote (2 *mt*— and 4 *mt*+). The two *mt*— F_1's showed the same phenotype: decreased zygote viability and 30% exceptional zygotes in one cross and 20% exceptional in the other. Test crosses of the female F_1's also showed low viability but gave frequencies of exceptional zygotes close to control values: 0.1%, 0.3%, 0.4%, and 2.0%.

These results suggested that the *mt*+ F_1's might be carrying the gene but not expressing it. To test this possibility, several male (*mt*—) progeny of the F_2 generation were back-crossed with a standard *mt*+ tester stock. The % exceptional zygotes in these crosses was higher than in the controls, but lower than the results of crosses in earlier generations. Thus, we do not yet know whether the gene is nuclear or cytoplasmic. Nonetheless, these preliminary results demonstrate the presence of a mutant gene called *mat*, responsible for the drastic reduction in viability of zygotes, coupled with a preferential survival of exceptional zygotes when carried in the male parent but not in the female. Its phenotypic effect is remarkably like that resulting from cycloheximide treatment of male (*mt*—) cultures during gametogenesis.

Discussion

This paper has summarized the available evidence bearing on the molecular basis of maternal inheritance in *Chlamydomonas*. Maternal inheritance is seen genetically as the transmission of cytogenes from the female parent to all progeny, and the concurrent disappearance of the corresponding cytogenome from the male. The principal known physical and biochemical events underlying these genetic observations are the following: (1) in zygotes, chloroplast DNA from the female parent undergoes a density shift, while that from the male parent disappears as a high

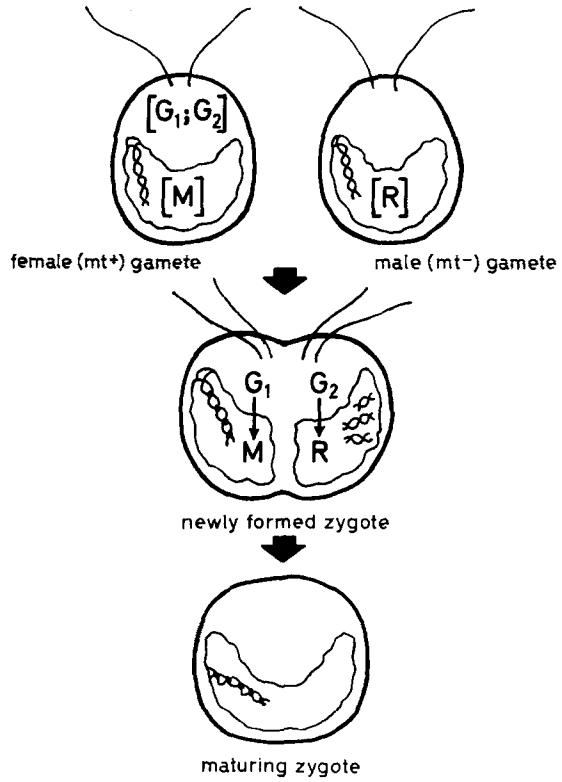

Fig. 1. Proposed mechanism of maternal inheritance of chloroplast DNA in *Chlamydomonas*

Female (mt^+) gamete contains inactive modification enzyme [M] in chloroplast, and two regulatory substances, G_1 and G_2, in the cell sap. The male (mt^-) gamete contains inactive restriction enzyme [R] in its chloroplast. After zygote formation and before fusion of chloroplasts, the modification enzyme is activated by G_1 to modify chloroplast DNA in the female chloroplast, and the restriction enzyme is activated by G_2 to degrade chloroplast DNA in the male chloroplast. The two chloroplasts then fuse, and only the chloroplast DNA from the female parent is available for replication

molecular weight component bandable in CsCl density gradients; (2) these effects on chloroplast DNA occur soon after mating, within the first few hours of zygote development; and (3) prior events occurring in both female and male gametes contribute to the process of maternal inheritance.

The model shown in Fig. 1 was developed as an aid in visualizing the steps in this process, including those revealed by experimental data and those postulated to occur. The features of this model will first be described and then discussed in relation to the data presented above.

A Proposed Molecular Mechanism of Maternal Inheritance

In this model, the female (*mt*+) parent contributes three essential components of the system: 1. a modification enzyme responsible for the density shift of female chloroplast DNA, 2. a regulator substance which activates the modification enzyme after zygote formation, and 3. a regulator substance which activates a restriction enzyme produced in the male (*mt*−) parent. The restriction enzyme responsible for degrading male chloroplast DNA is postulated to be synthesized in the male gamete, but activated by a regulator from the female parent some time after mating.

The time course is critical. The density shift in female chloroplast DNA occurs after mating, but the modification enzyme may be present in an inactive form well before mating, and the same is true of the restriction enzyme in the male. Both timing and compartmentalization must contribute to the (assumed) protection of the female DNA by modification before it can be attacked by the restriction enzyme; and conversely, the male DNA must be shielded from the modification enzyme so that it remains susceptible to degradation. Thus, the two postulated regulator substances play key roles in the timing.

The fact, noted above, that chloroplasts do not fuse for a few hours after mating, is of great importance. During this interval, chloroplast DNA from the female could be modified by an enzyme localized within the female chloroplast, and unavailable to male DNA. Similarly, the restriction enzyme could be localized with the male chloroplast, activated by a substance coming in from the mixed cytoplasm, and degrading the male DNA before chloroplast fusion. By the time the chloroplasts fuse, the female DNA would be fully protected and resistant to the restriction enzyme. Because of technical difficulties in obtaining samples of zygotes during the first 6 hours after mating, it has not been possible to find out experimentally whether the effects on male and female chloroplast DNA proceed simultaneously or sequentially. However, both are completed within about 6 hours.

The model in Figure 1 is based primarily on the bacterial modification-restriction systems (Arber and Linn 1969, Boyer 1971). The data so far available for the *Chlamydomonas* system seem to fit this model better than any other we have considered. Both the density shift of the female, and disappearance of the male chloroplast DNA's are predictable from the bacterial data. However, the density shift we have observed, amounting to a bouyant density of 0.005 gm/cm^3 in CsCl, is very high compared with the few methyl groups added to modify bacterial DNA's. If methylation is the chemical basis of the density shift, about 5% of the bases would have to be methylated.

Support for the view that methylation itself may be involved in the density shift comes from the observed effect of treating gametes (Table 1) and cells during gametogenesis (Table 2) with DL-ethionine, a competitive inhibitor of methionine, the usual methyl donor. In the bacterial system, methionine has been shown to be required for modification, and ethionine to inhibit the process (Arber and Linn 1969). The chemical basis of the density shift in *Chlamydo-*

monas is currently under investigation in our laboratory.

Specific evidence concerning each component of the model will now be considered. The synthesis of the modification enzyme in the female gamete is pure speculation, since no mutants affecting this step have been discovered. The fact that inhibitors of protein synthesis have a very small effect on maternal inheritance when female gametes are treated may indicate that the enzyme is synthesized during gametogenesis; this assumption is supported by the effects of rifamycin, erythromycin, and spiromycin treatment of the female during gametogenesis. The lethality associated with cycloheximide treatment makes it difficult to distinguish whether cytoplasmic protein synthesis is important in the female during gametogenesis, but the effects of the other antibiotics support the idea that a protein synthesized in the chloroplast of the female parent during gametogenesis is necessary for maternal inheritance. Whether this protein is the modification enzyme or some other component is not indicated by these data.

The fact that UV irradiation of the female gametes immediately before mating has such a profound effect on maternal inheritance, blocking it 100% without lethality of zygotes at optimal UV doses, indicates that some controlling event which can be blocked by UV, occurs very close to the time of mating. We postulate that this event is the release of a regulator substance that activates the restriction enzyme in the male chloroplast after mating. We must assume that the release of this substance is coupled to mating, since the UV effect decays with time, being less effective if cells are mated six hours after irradiation than two hours after irradiation, and much less effective if given earlier in gametogenesis. Also, the UV effect is photoreactivable, showing that the target is DNA. These results are reminiscent of UV induction of bacteriophage, a process known for 30 years but still not fully understood. In the lambda system, a repressor which normally holds lambda in check, is inactivated by UV leading to induction, but the mechanism by which UV irradiation inactivates the repressor is unknown (Ptashne 1971).

The assumption that the restriction enzyme is made in the male gamete is based primarily on the behaviour of the mutant strain *mat* and on the preferential survival of exceptional zygotes after cycloheximide treatment of males during gametogenesis. In both cases, the male parent determines the switch from maternal to exceptional zygotes, and in both cases, lethality of zygotes and preferential survival are involved. In addition, treatment of male gametes by compounds that interfere with transcription and translation (rifamycin, cyclic AMP, erythromycin, and spiramycin) was shown (Table 1) to increase the yield of exceptional zygotes substantially, while the corresponding treatment of the females had a much smaller effect.

We have concluded, from the data presented and discussed in this paper, that proteins synthesized in each of the parental strains, during gametogenesis or at the time of mating, are required for the process of maternal inheritance. The concept that a modification enzyme is produced in the female chloroplast and a restriction enzyme in the male chloroplast is proposed as a working model consistent with the available evidence. Further studies are of course required, and are in progress, to test the model.

Application to other Systems

Maternal, or uniparental, inheritance is such a pervasive feature of cytoplasmic inheritance in all known systems, that the mechanism is of fundamental interest. The application of the modification-restriction system of bacteria to maternal inheritance in *Chlamydomonas* raises the question of whether a similar mechanism may be operative in all organisms. Indeed, at present, modification-restriction is the only mechanism known for destroying some DNA's and not others co-existing within the same cell at the same time.

If one grants that the mechanism may be applicable to other organelle DNA's as well, which are naked DNA's like those of bacteria and viruses, one may then ask whether nuclear chromosomes, which are nucleoproteins, may also be regulated by a similar mechanism. As noted in the introduction, two kinds of nuclear systems deserve consideration in this light. One system is that of chromosome elimination or diminution as found in normal life-cycles of many invertebrates (Rhoades 1961) and the other is preferential chromosome elimination as it occurs in somatic cell hybrids (Weiss and Green 1967). In some interspecies hybrids, e.g. mouse and Chinese hamster, X- or γ-irradiation of one "parent" before fusion has been shown to predetermine which chromosomes will be preferentially lost (Pontecorvo 1971); and labeling with 5-bromodeoxyuridine (BUdR) to sensitize chromosomes to visible light has also been successful (Puck and Kao 1967). However, in human-mouse hybrids, the strong polarity of elimination of human chromosomes has been found not reversible by irradiation (Pontecorvo, personal communication).

Finally, it should be noted that restriction systems, heretofore identified only in bacteria, probably have a wide distribution in nature. Indeed, the usefulness of an elegant mechanism for distinguishing between different DNA's simultaneously present in the same cell has such broad possibilities and applications, that its existence must surely not be limited to the bacteria.

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Literature

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