BIPARENTAL INHERITANCE OF NONCHROMOSOMAL GENES
INDUCED BY ULTRAVIOLET IRRADIATION

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The identification of nonchromosomal (NC) genes has been associated classically
with a pattern of inheritance called maternal, cytoplasmic, or non-Mendelian. In
the higher plants where NC genes were discovered1 and where the bulk of reported
instances of nonchromosomal heredity have been found2 NC genes are typically
transmitted from the female parent to all progeny and are not transmitted at all
from the male parent. In exceptional cases, a small amount of male transmission
has been observed and attributed to penetration into the egg of cytoplasm from the
pollen during fertilization. However, this explanation, though widely accepted, has
not been directly demonstrated experimentally.

In Chlamydomonas we identify NC genes on the basis of a similar phenomenon,
polarity of transmission in crosses. (The sexual, or mating type, difference is de-
termined by a single chromosomal gene, mt.) By analogy with higher forms, we
have called the mt+ parent, which transmits its NC genes to the progeny, the female,
and the mt− parent the male, and the process maternal inheritance. However, since
the two sexes are of equal size and fuse completely in zygote formation, the basis
of maternal inheritance in Chlamydomonas is clearly not prezygotic exclusion of pa-
ternal cytoplasm.

Maternal inheritance effectively abolishes the possibility of genetic recombi-
ation of NC genes. While this limitation may be advantageous to the organism
as judged by its persistence and pervasiveness in nature, it is highly disadvan-
tageous to the investigator. Because of maternal inheritance, geneticists have not
been able to employ their most powerful tool, recombination analysis. Thus it
is not even known whether the many NC genes described in higher plants3 are
linked in linear array or exist in some entirely different pattern of organization.
In Chlamydomonas, we found the first evidence of recombination in rare sponta-
neously occurring exceptional (biparental) zygotes.3 4

This paper reports a method of converting the standard pattern of uniparental
transmission of NC genes by the female parent to a biparental pattern in which NC
genes from both parents are transmitted to the progeny. The method is based upon
ultraviolet irradiation of the female parent immediately before mating.

We previously reported3 4 the presence in all crosses studied of rare (0.01–0.1%) excep-
tional zygotes transmitting NC genes from both parents to the progeny. We
now find that UV irradiation raises the frequency of exceptional zygotes dramati-
cally to values approaching 100 per cent of all zygotes formed.

The results reported here have led us to propose that in Chlamydomonas mating
induces the formation in the female gamete of a substance responsible for the loss of
the NC genetic material of the male. The susceptibility of male, but not of female,
NC genes to this substance might depend on protection of the female genes by bind-
ing to protein or by secondary structure. The effectiveness of low doses of UV and
the reversibility of the effect by visible light suggest that the UV target is a nucleic acid that controls induction of this substance in the female gamete. Higher UV doses lead to a second effect, the loss of the female NC genes, and this effect is also reversible by visible light, supporting our previous evidence\(^4\) that the NC genes themselves are composed of nucleic acids.

**Materials and Methods.**—Methods of growth, mating, and genetic analysis were previously described,\(^5\)\(^\text{4}\) as were the markers, both chromosomal and nonchromosomal. The three pairs of unlinked chromosomal genes, actidione resistance and sensitivity, methionine sulfoximine resistance and sensitivity, and mating type, allow an unambiguous classification of the four products of meiosis. Two sets of NC genes were used, \(ac_1\) and \(ac_2\) (leaky and stringent acetate requirements); and the streptomycin genes \(sr\) (streptomycin resistance), \(sd\) (streptomycin dependence), and \(nr\) (neamine resistance) (obtained from N. W. Gillham). Neamine resistance is linked to streptomycin resistance.\(^5\)\(^\text{6}\)

The parental strains were grown separately on a limiting concentration of \(\text{NH}_4\text{NO}_3\) to induce gamete formation\(^3\) and mixed under conditions of rapid mating and over 90 per cent zygote formation. UV irradiation and subsequent plating were carried out in dim yellow light. Time intervals between irradiation, mating, and exposure to white light varied with experimental procedures and are specified in the text. When photoreactivation was desired, zygotes were illuminated for 24 hours. Subsequently all zygotes were matured in the dark for about one week and germinated by exposure to light.

**Results.**—The use of UV irradiation to enrich the yield of exceptional zygotes was initially studied with the cross \(ac_1\ sr\ \times\ ac_2\ sd\). This cross had previously been extensively studied with spontaneously occurring exceptional zygotes.\(^5\)\(^\text{4}\) The effect of UV irradiation on the yield of exceptional zygotes in this cross is shown in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>UV dose (sec)</th>
<th>(\varphi)</th>
<th>(\sigma)</th>
<th>RS Zygote Colonies</th>
<th>R Zygote Colonies</th>
<th>Exceptional zygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>(1.2 \times 10^6)</td>
<td>(3.6 \times 10^3)</td>
<td>0.03</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0</td>
<td>(3.5 \times 10^6)</td>
<td>(3.4 \times 10^4)</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>0</td>
<td>(1.2 \times 10^4)</td>
<td>(1.1 \times 10^4)</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>30</td>
<td>(1.2 \times 10^6)</td>
<td>(1.1 \times 10^3)</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>60</td>
<td>(1.2 \times 10^6)</td>
<td>(1.0)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Gamete suspensions were irradiated, diluted 10-fold, and mated. When zygote formation was complete, zygotes were plated on RS agar for total counts and on R agar for exceptional zygotes.

Irradiation of the male parent before mating had no effect on the yield of exceptional zygotes while irradiation of the female parent increased the yield from 0.03 to almost 100 per cent of the zygotes formed.

Exceptional zygotes from this experiment were analyzed in the usual manner by subculturing and classifying progeny (zoospore clones) to determine whether the segregation of chromosomal and nonchromosomal genes was comparable to that observed in previous experiments with spontaneous exceptional zygotes. First, with respect to chromosomal genes, no effect of UV irradiation was observed in this or in any of our subsequent crosses. This finding is not surprising in view of the low doses
of irradiation employed and the evidence that the replication of chromosomal DNA does not occur until the time of zygote germination. The effect of UV irradiation upon the detailed pattern of segregation and recombination of NC genes will be the subject of a separate report. For present purposes, it is sufficient to note that after UV irradiation most zoospores of the best studied cross, ac sd X ac2 sr, are hybrid and exhibit segregation patterns very similar to those shown by the progeny of spontaneous exceptional zygotes. Further studies of the UV effect were carried out with crosses involving the markers sr and nr to facilitate scoring. Figure 1 shows survival curves of the mt' parent

![Effect of UV on survival of o gametes](image1)

**Fig. 1.**—Effect of UV on survival of o gametes. Gametes were irradiated, plated at dilution, and incubated in dark (curve D), in light immediately after plating (curve B), or in light after 2 hr in dark (curve A).

![Effect of UV on conversion of maternal to exceptional zygotes](image2)

**Fig. 2.**—Effect of UV on conversion of maternal to exceptional zygotes. Female gametes were irradiated, mated with unirradiated males, and kept in dark until zygote formation was completed. Zygotes were plated at dilution, incubated in dark (D), or in light (A). In curve B, mating gametes were exposed to light and all subsequent plating done in light. After 24 hr in light, A and B series were placed in dark with D series for one week, then all plates were exposed to light for germination of zygotes.

irradiated before mating, with and without photoreactivation. Curve D shows the inactivation rate of gametes without photoreactivation. Curve B refers to gametes photoreactivated immediately after the UV treatment and curve A to gametes kept in the dark for two hours before photoreactivation, for comparison with zygotes treated similarly.

The dark survival curve D is very similar to that obtained with many other related strains of Chlamydomonas, both as vegetative cells in the log phase of growth and as gametes. Photoreactivation has not been previously studied in this system, but the slope of the dose-reduction curve (Fig. 3, curve 1) for immediate photoreactivation is in line with that recorded for other organisms. With a two-hour dark period between exposure to UV and to visible light, there is less photoreactivation and there is a break in the dose-reduction curve (Fig. 3, curve 2).
The dose reduction curve is a means of evaluating the effectiveness of photoreactivation by plotting the UV dose with photoreactivation against the dose without photoreactivation which would give the same number of survivors. This plot gives a straight line which extrapolates through the origin and has a slope $q$. The higher the value of $q$ the less effective the photoreactivation, that is, the more similar is the UV dose giving the same number of survivors with or without photoreactivation.

Figure 2 shows the effect of UV dose and of photoreactivation on maternal inheritance in the cross $ss ns \times sr nr$. In the absence of photoreactivation, maternal zygotes were converted to exceptional ones at the rate shown in curve D. Curve A shows the effect of photoreactivation after mating; in curve B gametes were photoreactivated before mating.

The dose-reduction curves for zygotes are compared to those for gametes in Figure 3. When photoreactivation was carried out before mating (curve 3), the dose reduction value $q$ was 0.25, comparable with the value 0.31 obtained with gametes (curve 1). When photoreactivation occurred after mating, $q$ for zygotes was 0.4 (curve 4). This is lower than the value obtained for gametes kept a comparable length of time in the dark before photoreactivation (curve 2).

The significance of the results with zygotes became apparent only after subculture and classification of progeny appearing at various doses of UV. We found two classes of exceptional zygotes: those transmitting NC gene markers from both parents and those transmitting NC genes exclusively from the male parent. In the future, zygotes transmitting NC genes from both parents will be called biparental, and those with exclusively male progeny will be referred to as paternal zygotes; both classes are exceptional because they transmit male NC genes.

An example of the comparative effect of UV and visible light on the yield of maternal, paternal, and biparental zygotes is shown in Figure 4. The yield of paternal zygotes increases steadily with UV dose reaching 100 per cent by 60 seconds, but is readily reversible by visible light. Indeed, when exposure to visible light precedes mating, no paternal zygotes are found. The yield of biparental zygotes (Fig. 4A), on the other hand, is markedly decreased by the appearance of paternal zygotes at higher UV doses in the absence of photoreactivation (curve D).
With photoreactivation, the yield both of biparental and of maternal zygotes is increased. Apparently two effects result from UV irradiation: (1) the preservation of the NC genes of the male parent in the progeny, and (2) the loss of NC genes from the irradiated female parent. Both of these effects are reversible by visible light.

In the absence of photoreactivation, the yield of biparental zygotes shows a sharp optimum which varies somewhat with the UV dose from one cross to another. Both the preservation of male NC genes and the loss of female NC genes influence the yield. Photoreactivation optimizes the recovery of biparental zygotes by converting paternal zygotes into biparental ones at a higher rate than it converts biparental to maternal ones. Similar results have been obtained with a number of different crosses involving other combinations of markers. Forty to 50 seconds of UV irradiation followed by photoreactivation after mating has provided the best yield of biparental zygotes in most crosses studied thus far.

An important factor in alleviating UV damage to the zygote is the repair which occurs after mating irradiated females to unirradiated males. Table 2 shows the effect of irradiating the zygotes immediately after mating. Zygotes are evidently very susceptible to killing by UV irradiation and to photoreactivation of the UV damage, leading to survival.

Discussion.—This paper reports that UV irradiation of the female parent before mating leads to a dramatic rise in the frequency of exceptional zygotes, that is, zygotes transmitting NC genes from the male parent. This discovery is of great practical value in the analysis of nonchromosomal heredity because it provides a ready source of biparental zygotes for analysis. In the past it was necessary to use

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>Per Cent Survival With PR</th>
<th>Per Cent Survival Without PR</th>
<th>Per Cent Maternal Zygotes With PR</th>
<th>Per Cent Maternal Zygotes Without PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>97.4</td>
<td>99.4</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>1.5</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>30</td>
<td>100</td>
<td>&lt;1.0</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>&lt;0.1</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>80</td>
<td>&lt;0.1</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>25.7</td>
<td>&lt;0.1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>7.5</td>
<td>&lt;0.1</td>
<td>72</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 4.—Effect of UV on yield of biparental and paternal zygotes. Exceptional zygotes were classified as biparental (A) or paternal (B) and the data plotted to show the per cent of each resulting from the three treatments D, B, and A described in Figure 2.
special combinations of markers to select biparental zygotes since the spontaneous frequency was in the range of 0.1–0.01 per cent. The UV method provides freedom from selection; for the first time we can analyze crosses involving any combination of NC genes. This method has also made it possible to analyze the detailed segregation behavior of NC genes immediately after meiosis.6

The detailed mechanism of the UV effect is as yet unexplored and no molecular interpretation will be presented here. However, it seems appropriate to enumerate the observations to be accounted for in any working hypothesis:

(1) A process originating in the female parent is responsible for the loss of the male NC genes.

(2) This process is blocked by UV irradiation of the female before mating. Consequently the male NC genes survive, leading to the appearance of biparental zygotes with NC genes from both parents.

(3) This UV effect can be reversed by visible light with approximately the same efficiency as photoreactivation of the UV killing of gametes or vegetative cells. Thus photoreactivation restores the maternal pattern of NC gene transmission. The restoration is much more effective if photoreactivation occurs before mating than afterwards.

(4) At higher doses there is a second UV effect leading to the loss of the female NC genes and the consequent appearance of paternal zygotes transmitting NC genes from the male parent only. This effect is also reversible by visible light, leading to the restoration both of biparental and of maternal zygotes. Here, too, photoreactivation before mating restores maternal zygotes with a high efficiency, while photoreactivation after mating produces principally biparental zygotes. Apparently the preservation of the male NC genes is fixed to some extent by the mating process.

One simple working hypothesis is that at the time of mating the female gametes normally form a gene product, possibly an enzyme, required for a loss of the male NC genes. The first UV effect would then be to block the synthesis of this gene product (or to inactivate it). This process might be analogous to the UV induction of phage, resulting from a block in the synthesis of the phage repressor substance. The second UV effect would be a more direct interference with the replication of the female NC genes.

The fact that photoreactivation can convert UV irradiation-induced paternal zygotes to biparental or maternal ones is good evidence that NC genes are nucleic acid in composition. These results are in harmony with our previous finding that NC genes undergo recombination and therefore behave like nucleic acids.4 Although photoreactivation has been demonstrated only with DNA, recent reports10 indicate that pyrimidine dimers in RNA may have properties similar to those in DNA. Consequently we cannot critically distinguish DNA from RNA on the basis of our findings.

Finally, it should be noted that UV irradiation may provide a technique for investigating NC genes and maternal inheritance in other organisms. It may also provide a means to find out whether maternal inheritance in higher organisms is determined exclusively by anisogamy or whether it also has an enzymatic basis.

Summary.—The maternal pattern of NC gene transmission can be drastically altered by UV irradiation of the female gametes just before mating. The yield of
exceptional zygotes increases with UV dose from 0.01 to 100 per cent. Two classes of exceptional zygotes are recovered: biparental and paternal. Photoreactivation converts paternal to biparental and, less effectively, biparental to maternal. The results are interpreted to show (1) the nucleic acid nature of NC genes, and (2) the presence of a gene product in female gametes induced by mating that is responsible for loss of the male NC genome. It is hoped that these findings may also provide a new means of studying NC heredity in other organisms.

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5 Gillham, N. W., these PROCEEDINGS, 54, 1560 (1965).
8 Sager, R., and Z. Ramanis, manuscript in preparation.