Perturbation of Chloroplast Gene Transmission in Diploid and Triploid Zygotes of Chlamydomonas reinhardi by 5-Fluorodeoxyuridine

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Summary. Haploid cells or diploid cells homozygous (mt⁺/mt⁺ or mt⁻/mt⁻) or heterozygous (mt⁺/mt⁻ phenotypically mt⁻) for the mating-type locus and homoplasmic for a chloroplast marker conferring resistance to an antibiotic were crossed with haploid cells of opposite mating-type and carrying another chloroplast marker. Before mating, one or both of the parental strains were grown for 8 days on agar containing 1 mM 5-fluorodeoxyuridine (FUdR), which selectively reduces the amount of chloroplast DNA in Chlamydomonas. In all cases, the chloroplast allele of the treated parent was less frequently transmitted to the meiotic progeny of the zygote than in the corresponding control cross. The effect of FUdR was more pronounced on haploid cells than on diploid cells which initially contained a two-fold higher amount of chloroplast DNA.

The results are discussed in relation to current models for uniparental inheritance of non-Mendelian genes.

Key words: Chlamydomonas — Chloroplast genetics — Triploid zygotes — 5-fluorodeoxyuridine

Introduction

In Chlamydomonas reinhardi, the thymidine analogue, 5-fluorodeoxyuridine (FUdR), interferes selectively with the rate of chloroplast (chl) DNA replication: after several days of growth in the presence of the drug, the amount of chl DNA and the number of chl nucleoids per cell is considerably reduced, without any significant modification of nuclear DNA content and vegetative growth rate (Wurtz et al. 1977; Matagne and Hermesse 1981). The specific action of FUdR on chl DNA can be due to its phosphorylation by thymidine kinase — an enzyme specific of the chloroplast (Swinton et al. 1974) — into 5-fluorodeoxyuridylate which, in bacteria, binds to thymidylate synthetase and thus blocks DNA replication (Santi and McHenry 1972).

When mating-type plus (mt⁺) cells are treated with FUdR and mated with untreated mating-type minus (mt⁻) cells, the normal maternal transmission of chl genes is perturbed: the proportion of exceptional zygotes transmitting chl alleles from the paternal (mt⁻) parent to their meiotic progeny dramatically increased (Wurtz et al. 1977). As proposed by Wurtz et al. (1977), this finding strongly suggests that FUdR decreases the number of DNA molecules present in the chloroplast and thus deeply modifies transmission of alleles residing in these molecules. A second conclusion drawn by Wurtz et al. (1977) is that the results are not easily explained by the modification-restriction mechanism proposed by Sager and Ramanis (1973): FUdR should have no effect on nuclear genes postulated to control modification of mt⁺ chl DNA and restriction of mt⁻ chl DNA; hence, “restriction of paternal chloroplast DNA should still occur despite the fact that FUdR has reduced the input of maternal chloroplast DNA and the normal, maternal pattern of inheritance of chloroplast genes should not be altered by FUdR treatment”.

Recent data (Burton et al. 1979) showing that in the young zygotes maternal chl DNA is extensively methylated and conserved whereas paternal chl DNA is largely degraded, support the hypothesis of modification-restriction. For these authors, FUdR would have “additional effects on enzymes of DNA synthesis, processing and degradation”.

We recently showed that when cells of Chlamydomonas were artificially fused with polyethylene glycol, one of the two parental strains having been treated with
FUDR prior to fusion, the chl allele of the non treated parent was preferentially transmitted to the diploid progeny of the fusion products (Matagne and Hermesse 1981). Since, in the artificially fused cells, the elimination of chl alleles from one or the other parent is normally bidirectional (Matagne 1981), no modification-restriction mechanism is suspected to act in these diploid cells; consequently the bias observed in the transmission of chl genes after treatment of one of the parental strains by FUDR is most easily explained by the reduction of chl DNA copies.

We here describe the patterns of chl gene transmission observed in crosses between haploid and diploid (mt+/mt+, mt−/mt− or mt+/mt−) gametes, one or both parents having been pregrown in the presence of FUDR. The results are discussed in relation to the number of chl DNA copies contributed by each parent and to the modification-restriction mechanism proposed to act in the zygotes.

**Material and Methods**

**Strains and Culture Conditions.** Homozygous (mt+/mt+ or mt−/mt−) diploid strains homoplasmic for a chloroplast marker conferring the resistance for spectinomycin were constructed by polyethylene glycol induced fusion as previously described (Matagne and Mathieu 1983). The heterozygous (mt+/mt−) diploid strain resistant to spectinomycin was isolated from a sexual cross.

The transmission of chloroplast genes has been analyzed in the following crosses:

- haploid x haploid: sr mt+ x spr mt−
- haploid x diploid: sr mt+ x CW4 arg-7-2 spr mt−/ CW15 arg-7+ spr mt−
- sr mt+ x CW4 arg-7-2 spr mt+ / CW15 arg-7 spr mt−
- spr mt− x CW15 arg-7-2 spr mt+ / CW15 arg-7 spr mt−

CW15 and CW4 are complementary nuclear markers causing a cell-wall-less phenotype; arg-7-2 and arg-7 are complementary closely linked nuclear markers resulting in auxotrophy for arginine; sr and spr are chloroplast markers conferring resistance to streptomycin and spectinomycin respectively.

Two agar solidified (15 g/l Bacto-agar Difco) media were used: the minimal M medium and the M−N+YE (M medium lacking NH4Cl and enriched with 4 g/l yeast extract) medium (Loppes et al. 1972). 5-fluorodeoxyuridine (FUDR) was passed through Millipore filter (0.22 μ) and added to partially cooled agar medium after autoclaving.

Analysis of Chloroplast Gene Transmission in the Zygotes. Suspensions of opposite mating-type were mixed for 0.5-1 h and the suspensions containing zygotes and unmated cells were plated onto M−N+YE agar medium (200–300 zygotes per plate). After maturation of the zygotes and elimination of the unmated vegetative cells (Matagne and Mathieu 1983), the plates were exposed under continuous light to induce meiosis of the zygospores and subsequent mitotic divisions of the meiotic products. The colonies formed, each issued from the meiotic progeny of one zygospore, were transferred onto fresh M−N+YE agar medium and after subsequent growth, replicated onto M−N+YE+spectinomycin (500 mg/l) and M−N+YE+streptomycin (100 mg/l). The colonies resistant to one antibiotic only were classified as derived from uniparental maternal (MZ) or paternal (PZ) zygospores, while those able to grow on both antibiotic media were classified as derived from biparental (BPZ) zygospores.

**Results**

Results obtained in control (untreated) crosses (Table 1A) fully confirm those previously obtained (Matagne and Mathieu 1983). In crosses mt+/mt− and mt+/mt− x mt−, the transmission of chl genes is almost exclusively maternal. In crosses between haploid mt+ and diploid mt− and diploid mt− and mt+ and diploid mt− x mt+mt−, the transmission is preferentially paternal depending upon whether the diploid is heterozygous mt+/mt− or homozgyous mt+/mt− respectively. As previously discussed, these results indicate that 1) the presence of the mt+ allele in the heterozygous diploid gametes and 2) the increased amount of chl DNA in the diploid gametes favor the transmission of the chl allele contributed by these gametes.

When the maternal parent alone is treated with FUDR, the percentages of maternal zygotes (MZ) decrease and in parallel, the proportions of exceptional zygotes (BPZ + PZ) increase (Table 1B). However, the exact nature of the perturbation differs according to the cross: FUDR treatment of the mt+ parent in a standard haploid x haploid cross leads to a significant increase in the frequency of PZ (Table 1B and Wurtz et al. 1977) whereas the transmission remains preferentially maternal (70%) when the treated parent is an mt−/mt− diploid. Similar crosses with other strains gave similar results (not shown). When the paternal (untreated) parent is diploid, the transmission is almost exclusively paternal.

When only the paternal parent has been pregrown on FUDR, the transmission is most often (97%) or exclusively (100%) maternal in three of the four crosses (Table 1C). In the fourth cross (mt+ x mt+/mt−), the transmission, preferentially paternal in the control (57%), becomes preferentially maternal (64%). Hence, the effect of FUDR on the paternal parent is visible only when the frequency of PZ in control crosses is sufficiently high (i.e. in crosses mt+ x mt−/mt− and mt+ x mt+/mt−) to allow detection of a decrease after FUDR treatment.