for 5 minutes at 200*g* and incubated at 4°C for 2 to 18 hours. After incubation, the cells were gently resuspended and layered over Ficoll-Hypaque gradients. The SRBC rosette-forming cells (T cells) were found in the bottoms of the tubes after centrifugation. After lysis of the SRBC with tris-buffered ammonium chloride solution, the T cells were washed three times in HBSS.
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Cytoplasmic Reversion of *cms-S* in Maize: Association with a Transpositional Event

Abstract. *Spontaneous reversion to fertility in S male-sterile cytoplasm of maize is correlated with the disappearance of the mitochondrial plasmid-like DNA's, S-1 and S-2, and changes in the mitochondrial chromosomal DNA. Hybridization data indicate that one of the plasmid-like DNA's, S-2, is prominently involved in the mitochondrial DNA rearrangements.*

The S cytoplasm (*cms-S*) is one of several types of cytoplasmic male sterility in maize (*1*). *Cms-S* can be distinguished from other types on the bases of (i) certain nuclear genes called "restorers of fertility" (Rf) that suppress the expression of male sterility (*1*, *2*), (ii) electron microscopy and restriction endonuclease fragment analysis of mitochondrial DNA's (mtDNA's) (*3*, *4*), and (iii) the nature of mitochondrial protein products (*5*). In addition, two plasmid-like mtDNA's (S-1 and S-2) are uniquely associated with *cms-S* (*6*).

Certain *cms-S* strains undergo spontaneous reversion to male fertility (*2*, *7*). Genetic analysis of these revertants indicates that the underlying change can occur in either the cytoplasm or the nucleus. In this report we describe an analysis of the mtDNA's of seven cytoplasmically reverted strains. The results indicate that mitochondrial genes are involved in the cytoplasmic inheritance of male fertility. They also suggest that the unique plasmid-like DNA's identified in mitochondria of *cms-S* strains (*6*) are the physical manifestation of the fertility episome postulated earlier on the basis of genetic reversion analysis (*2*, *8*).

Concomitant with the reversion of the S type of male sterility to the fertile condition is the disappearance of the free plasmid-like DNA's, S-1 and S-2, and the appearance of new mtDNA restriction fragment patterns in the revertants. Significantly, we have demonstrated sequence homology between the plasmid-like DNA S-2, and some of the new restriction fragments. In other instances, S-2 homologies have been identified where new fragments were not visualized. It is clear that rearrangements have occurred in the mitochondrial chromosomal DNA and that sequences homologous to the S-2 DNA are prominently involved. It is tempting to speculate that the insertion of the plasmid-like sequences into the mitochondrial chromosome (or chromosomes) is associated with the reversion to male fertility.

Seven male-fertile revertant stocks were selected for study in which it was established by prior genetic analysis that the mutational event took place at the cytoplasmic level (*9*). The control strains, designated *cms-Vg* (M825/0h07), were derived from male-sterile siblings of revertants in families in which the male-sterile revertants were first identified. The standard *cms-S* control strain carried the nuclear background of inbred line B37.

Mitochondrial DNA's (*10*) from the standard *cms-S* source, a control *cms-Vg* (M825/0h07) strain, and two revertant strains, 285 and 369, were fractionated by agarose gel electrophoresis (Fig. 1). All cytoplasmic types exhibit an uppermost wide band containing the high-molecular-weight chromosomal mtDNA and a fast-migrating, low-molecular-weight (1.2×10^6) circular DNA (*6*). Two additional bands, designated S-1 and S-2, are present in mtDNA from the standard *cms-S* strain (Fig. 1A) and from

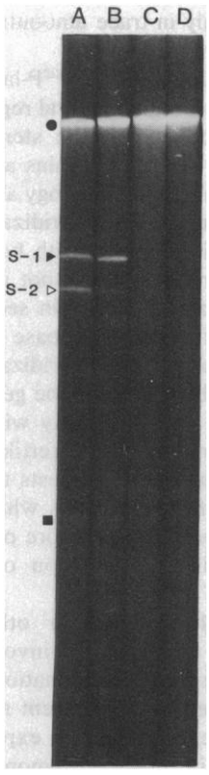

Fig. 1. Gel electrophoresis of mtDNA preparations from (A) *cms-S*, (B) *cms-Vg*, (C) revertant 285, and (D) revertant 369. Symbols: ●, high-molecular-weight chromosomal DNA; S-1 ▶, S-1 DNA; S-2 ▷, S-2 DNA; and ■, small-molecular-weight circular DNA.

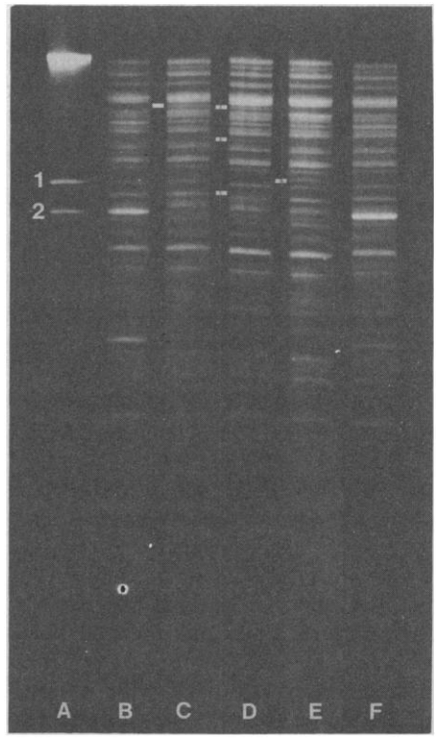

Fig. 2. Gel electrophoresis of *Xho*I digests of mtDNA from (B) *cms-S*, (C) revertant 251, (D) revertant 733, (E) revertant 369, and (F) *cms-Vg*; (A) is undigested mtDNA from *cms-S*, 1 is S-1 DNA, and 2 is S-2 DNA. Dashes indicate position of new bands in revertant strains which were absent in *cms-S* and *cms-Vg*.

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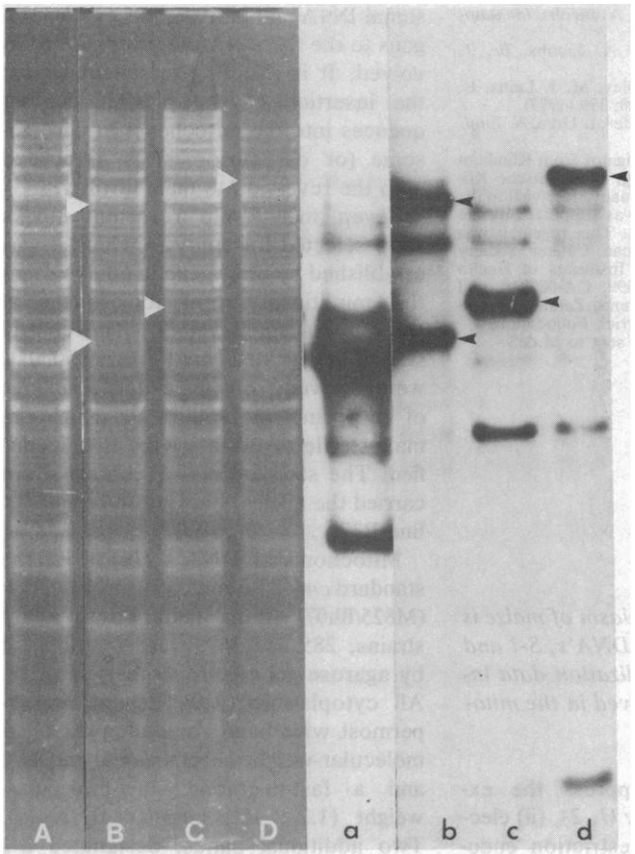

Fig. 3. Gel electrophoresis of *Xho*I digests of mtDNA's (A to D) and autoradiographs of corresponding Southern blots hybridized with ^{32}P-labeled S-2 DNA, respectively (a to d). The mtDNA's are from (A and a) *cms-Vg*, (B and b) revertant 733, (C and c) revertant 369, and (D and d) revertant 296. Arrows indicate hybridization coinciding with new bands in revertants.

the four control *cms-Vg* (M825/0h07) strains (represented by Fig. 1B). In *cms-S* (Fig. 1A), the S-1 and S-2 DNA's occur in equimolar amounts, but in the *cms-Vg* control strains (Fig. 1B), a marked reduction in amount of S-2 DNA is observed. In all *cms-S* strains previously studied (*6*) S-1 and S-2 were found in equimolar amounts; thus the *cms-Vg* strain is atypical. This discrepancy may be related to the unusually high reversion rate of this strain.

The more remarkable finding is that the S-1 and S-2 DNA's were no longer apparent in electrophoretograms of mtDNA prepared from cytoplasmically reverted strains. This is illustrated for revertant strains 285 and 369 (Fig. 1, C and D), and has been demonstrated (not shown) for five other revertant strains. Although there is evidence that some revertants may still carry trace amounts of the S-1 and S-2 plasmid-like DNA's, there is little doubt that the mutational step from cytoplasmic male sterility to male fertility is associated with the virtual disappearance of the plasmid-like DNA's in all seven revertants examined.

Preparations of mtDNA from the standard *cms-S*, *cms-Vg* (M825/0h07), and three revertant strains, 251, 369, and 733, were digested with restriction endonuclease *Xho*I and fractionated by gel electrophoresis (*11*). The fragment patterns of mtDNA from *cms-S* and *cms-Vg* (M825/0h07) were indistinguishable with respect to number and position of bands (Fig. 2, B and F). Cleavage fragments resulting from the digestion of the S-2 DNA present in mtDNA preparations of *cms-S* and *cms-Vg* differed slightly in intensity because of the reduced quantity of S-2 DNA found in *cms-Vg* (Fig. 2, B and F).

The three revertant strains exhibited new fragment bands that were not observed in the *cms-S* and *cms-Vg* (M825/0h07) control strains (Fig. 2, B to F). Moreover, each of the three revertants is distinguishable on the basis of these new bands (see dashes on Fig. 2). Similar analyses of mtDNA from the other revertant strains indicated that some, but not all, were distinguishable from each other by *Xho*I digestion.

The association of the disappearance of the plasmid-like DNA's with the appearance of altered mtDNA's suggested the possibility that revertant strains may have arisen by the insertion of S-1 or S-2 DNA's, or both, into the mitochondrial chromosomal DNA. We have tested this hypothesis by direct DNA-DNA hybridization using Southern blots (*12*) and labeled DNA probes. Mitochondrial DNA's prepared from the control and revertant strains were digested with restriction endonucleases, fractionated by gel electrophoresis, and transferred to nitrocellulose filters. Labeled probes were prepared by nick translation (*13*) of S-1 and S-2 DNA's that were isolated from the *cms-S* strains by preparative gel electrophoresis. Radioactively (^{32}P) labeled S-1 or S-2 DNA's were hybridized to mtDNA fragments on nitrocellulose filters (*14*) and hybrid bands were detected by autoradiography.

We found that α-^{32}P-labeled S-2 DNA hybridized to several of the new *Xho* restriction fragments which characterized the fertile revertant strains (Fig. 3). These results demonstrate that some of the new bands do indeed carry sequences that are homologous with the S-2 plasmid-like DNA. One new restriction fragment in strain 733 and one in strain 296 did not show homology with the S-2 probe. The hybridization also revealed several regions of homology common to the sterile control and fertile revertant strains. This important finding indicates that sequences homologous to the plasmid-like DNA, S-2, are present in the mitochondrial chromosomal DNA of both fertile revertant and sterile strains. Further distinctions were also noted; several S-2 homologies observed in the sterile control did not appear in the fertile revertant strains. Part of this difference is attributable to the fact that the free plasmid-like DNA, S-2, is absent or present only in trace amounts in the revertant strains.

Hybridization of α-^{32}P-labeled S-1 DNA also revealed several regions of homology common to the sterile control and fertile revertant strains as well as a few differences in homology among them (not shown). A few hybridization bands appeared in common with both the S-1 and S-2 probes. This is not unexpected since S-1 and S-2 contain sequence homology of about 1500 base pairs (*15*). Most important, the hybridization bands produced by the S-1 probe generally did not match unambiguously with the new *Xho*I fragments of the fertile revertant strains. This result suggests that the sequences of the S-2 DNA which are not common with S-1 are more prominently involved in the detection of the new bands.

Although mutation or other events cannot be ruled out, the involvement of nonhomologous recombination is strongly suggested by the present results. Especially relevant to this explanation is the diversity observed among the various revertants; strains 733, 369, and 296 each manifested different restriction fragment patterns as well as unique hybridization patterns with the plasmid-like DNA probes. These distinctions are in

accord with transpositional events taking place at different sites. The S-1 and S-2 molecules may be uniquely equipped for insertion since both DNA's contain terminal inverted repeats (*16*). Inverted repeats are often characteristic of transposable elements in prokaryotic systems [reviewed in (*17*)]. Finally, transposable elements, termed "controlling elements," have been recognized for years in maize where they have been shown to inhibit normal activities of a variety of different nuclear genes (*18*).

Common to the mtDNA of male-sterile and revertant cytoplasms are at least five *Xho*I cleavage fragments with homology to the plasmid-like DNA's. Although the significance of this finding is not clear, it may mean that the plasmid-like DNA's could have arisen from the mitochondrial genome by excision and that this particular event is associated with the origin of the S type of cytoplasmic male sterility. Studies with prokaryotic systems suggest that transposable elements are able to move from one site to another without leaving the original site (*19*). Therefore, the presence in the mtDNA of sequences homologous to the S-1 and S-2 DNA's after their excisions would be anticipated if excision events behave similarly in the two systems.

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9. The revertant stocks used were 251, 274, 285, 296, 352, 369, and 733. In all but the last strain, the male-sterile source materials from which these revertants arose can be traced to an initial cross of a *cms-Vg* version of inbred line M825 as female parent, with inbred line 0h07, carrying normal cytoplasm, as male parent; *cms-Vg* is one of a number of independently discovered male-sterile types that falls in the general category of *cms-S*. The source of revertant 733 can be traced to an original cross involving male-sterile inbred line WF9 with *cms-S*, as female parent, and inbred line M825 carrying normal cytoplasm as male parent.
10. Preparation of maize mtDNA and gel electrophoresis techniques were described in (*3, 4, 6*).
11. The endonuclease *Xho*I was purchased from New England Biolabs or Bethesda Research Laboratories, Inc. Digestions and electrophoresis were as previously described (*4*).
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14. Hybridization mixtures contained 5 × SSC, 4 × Denhardt solution (four times each of 0.02 percent Ficoll 400, 0.02 percent polyvinylpyrrolidone, 0.02 percent bovine serum albumin), 0.02*M* tris-HCl (*p*H 7.4), 50 percent spectrograde formamide, 0.05 percent sodium dodecyl sulfate, heat-denatured, sonicated calf thymus DNA (20 μg/ml) and usually more than 10×10^6 dpm of probe per membrane. Filter membranes were incubated for 48 hours at 37°C, with occasional shaking and then washed three times with 200 ml of hybridization mixture, without carrier DNA, for 30 minutes at 37°C, washed once with 200 ml of 2 × SSC, blotted, dried, and mounted for autoradiography with two sheets of DuPont Cronex 4 x-ray film and a Kodak X-omatic regular intensifying screen. The x-ray films were developed after 2 to 4 days of exposure at −90°C.
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Testosterone-Mediated Sexual Dimorphism of Mitochondria and Lysosomes in Mouse Kidney Proximal Tubules

Abstract. *In kidney proximal tubules of male mice the mitochondria are larger and more electron-lucent, autophagic vacuoles and lysosomes (predominantly myeloid bodies) more numerous and voluminous, and exocytosed intraluminal myeloid bodies more common than in females. Males also have higher kidney activities of mitochondrial cytochrome* c *oxidase and lysosomal hydrolases, and excrete larger quantities of hydrolases and protein in the urine. Orchiectomy evokes the feminine pattern whereas testosterone administration induces the male pattern. Endogenous testosterone modulates mitochondrial structure and function and enhances the activity of the lysosomal-vacuolar system in proximal tubule cells.*

The mouse kidney displays a complex response to testosterone administration which includes hypertrophy, augmented RNA and protein synthesis, and increases in β-glucuronidase and a number of other specific proteins (*1*). The testosterone-induced increase in kidney β-glucuronidase is restricted to the epithelial cells of proximal tubules (PT) (*2–4*), as is the cellular hypertrophy (*1, 5*), and large amounts of β-glucuronidase are excreted in the urine (*2–4, 6*) together with hexosaminidase (*4, 6*), β-galactosidase (*4, 6, 7*), and several other lysosomal enzymes (*6*). Testosterone administration in female mice induces an accumulation of lysosomes containing abundant layered, myelinlike membranes (cytoplasmic membranous bodies or myeloid bodies) in kidney PT cells, and enhances the exocytosis of these lysosomes into the tubule lumen (*6*). The extruded myeloid bodies and membrane-bound lysosomal enzymes are subsequently demonstrable in urine sediments. The testosterone-induced increment in urinary lysosomal enzymes reflects the hormone-mediated production and egestion of lysosomal myeloid bodies by PT cells (*6*). We now describe a testosterone-dependent sexual dimorphism in ultrastructure of PT cells involving the lysosomes and mitochondria and the tissue activities of several enzymes associated with these organelles. There is also a sex difference in the urinary excretion of lysosomal enzymes and protein.

Male and female mice of an inbred strain (A/J) were used for these experiments. Male mice were subjected to transcrotal orchiectomy under trichloroethylene inhalation anesthesia to study the effect of endogenous testosterone. Female mice received four subcutaneous injections of 1 mg of testosterone propionate (TP) in 0.05 ml of ethyl oleate

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