Identification of a Maize Nuclear Gene Which Influences the Size and Number of *cox2* Transcripts in Mitochondria of Perennial Teosintes

Pam Cooper, Ed Butler[1], and Kathleen J. Newton

Division of Biological Sciences, University of Missouri, Columbia Missouri 65211

Manuscript received February 20, 1990
Accepted for publication June 21, 1990

ABSTRACT

The involvement of nuclear genes in mitochondrial gene expression was investigated by identifying alterations in mitochondrial gene expression that occur when teosinte cytoplasms are introduced into certain maize inbred nuclear backgrounds. The cytoplasms from the teosintes *Zea perennis*, *Zea diploperennis*, and *Zea luxurians* were introduced into the maize A619 or W23 lines by recurrent backcrossing. Northern analysis revealed that the *Z. perennis* and *Z. diploperennis* mitochondrial *cox2* transcript patterns were dependent upon the maize nuclear genotype. In a W23 nuclear background, these teosinte mitochondria have two major transcripts of 1.9 and 1.7 kb, whereas in an A619 background, they have three major transcripts of 1.9, 1.5 and 1.3 kb. No effect of nuclear background on *cox2* transcripts was observed for plants possessing *Z. luxurians* cytoplasm. All teosinte-maize combinations possess larger, minor *cox2* transcripts of 3.9, 3.3 and 3.0 kb; nuclear background has no effect on these transcripts. Immunoblot analysis showed a threefold reduction of the COXII polypeptide in *Z. perennis*-A619 combinations compared to *Z. perennis*-W23 combinations. All the major and minor transcripts possess both *cox2* exons. The *cox2* intron is missing from all the major transcripts and is present only in the 3.9- and 3.0-kb minor transcripts. The 1.7- and 1.3-kb transcripts are missing untranslated regions 3′ to the *cox2* gene; therefore at least some of the size heterogeneity is due to differential termination or downstream processing. Genetic analyses indicate that a single nuclear gene is responsible for the observed differences in the major *cox2* transcripts, and that A619 carries the dominant allele. This gene, designated *Mct*, is specific for *cox2*, as no transcript size differences were observed for the other two mitochondrial *cox* genes.

SEVERAL chloroplast and mitochondrial enzyme complexes consist of subunits encoded by both the cytoplasmic and nuclear genomes. Thus proper organelle function requires a compatible interaction between gene products from two distinct genomes that occurs when those genomes have evolved under the same selective pressures. Nuclear cytoplasmic incompatibility may be found within a species, or as a result of intergeneric or interspecific hybridization, with its outward manifestation being a developmentally aberrant phenotype (GRUN 1976).

Cytoplasmic male sterility (CMS) is a frequently encountered phenotype in plants that is generally considered to result from disturbances in nuclear-mitochondrial interactions (DUVICK 1965; HANSON and CONDE 1985). CMS plants fail to shed functional pollen, and the trait is maternally inherited. CMS can arise following interspecific crosses between maize and certain teosintes (wild relatives of maize). Teosinte cytoplasm can be introduced into a maize nuclear background by using the maize line as the male parent in a program of recurrent backcrossing to the teosinte-derived female line. After several generations of backcrossing, the nuclear genome becomes essentially that of the maize inbred, while the maternally inherited cytoplasmic genomes remain those of the original teosinte. When cytoplasm from the perennial teosinte *Zea perennis* is present with the nuclear genotype of A619 or of other Oh43-derived maize inbreds, the resulting plants are male sterile (GRACEN and GROGAN 1974; LAUGHNAN and GABAY-LAUGHNAN 1983). Outcrosses of these *Z. perennis*-maize combinations to other maize lines restored fertility. *Zea diploperennis* in an A619 background also results in partial or full male sterility (J. KERMICLE, personal communication; K. J. NEWTON, unpublished observations).

A second trait attributed to an incompatibility between teosinte cytoplasmic and maize nuclear genomes is an abnormal kernel/plant phenotype designated "teosinte cytoplasm associated miniature" (tcm) (ALLEN, EMENHISER and KERMICLE 1989). This trait was originally identified in *Z. perennis* plants whose nuclear genome had been replaced by that of maize inbred W23 (KERMICLE and LONNQUIST 1973). Kernels are much smaller but structurally similar to normal kernels. They germinate more slowly and give rise to short-statured, pale-green, yet fertile plants. Normal kernel and plant development can be restored

[1] Present address: Arizona Cancer Center, University of Arizona, Tuscon Arizona 85724.

in these teosinte-maize combinations by outcrosses to maize lines, such as A619, that possess dominant nuclear rectifiers (*Rcm1*, *Rcm2*) of the kernel defect (ALLEN, EMENHISER and KERMICLE 1989). Teosintes more closely related to maize, *i.e.*, those classified along with maize in *Zea* Section Zea (DOEBLEY and ILTIS 1980) are not tcm susceptible (ALLEN, EMENHISER and KERMICLE 1989). Only combinations of the more distantly related teosintes found in *Zea* Section Luxuriantes with W23 exhibit the tcm phenotype (ALLEN, EMENHISER and KERMICLE 1989).

Although a number of nuclear genes that regulate mitochondrial gene expression have been identified in yeast (TZAGOLOFF and MYERS 1986), with the exception of nuclear fertility restorers (HANSON and CONDE 1985), few such genes have been described in plants. We have begun to identify molecular alterations that result from incompatible combinations of teosinte cytoplasmic genomes and maize nuclear genomes as a means of studying how nuclear genes regulate mitochondrial gene expression in higher plants. We have used lines derived from accessions of three species of teosintes in Section Luxuriantes: the perennials *Z. perennis* and *Z. diploperennis*, and the annual *Zea luxurians*. The nuclear genomes of these teosintes were replaced with those of maize inbreds W23 or A619 by serial backcrossing. Recently we reported an effect of maize nuclear background on *Z. luxurians* mitochondrial protein synthesis (COOPER and NEWTON 1989). *Z. luxurians* mitochondria from the A619 line synthesize a distinctive 22-kD polypeptide, whereas those from the W23 line do not. Genetic analyses indicated that synthesis of this mitochondrial polypeptide is controlled by a single nuclear gene.

Here we report that maize nuclear background influences the number and size of transcripts from the gene encoding subunit 2 of cytochrome *c* oxidase (*cox2*) in *Z. perennis* and *Z. diploperennis* mitochondria. Three major transcripts are present in mitochondria from plants possessing an A619 nuclear background, whereas two major transcripts are found in mitochondria from plants possessing a W23 background. Nuclear background has no influence on *cox2* transcript pattern in lines carrying *Z. luxurians* cytoplasm. The major transcripts all contain both exons of the gene and lack the intron, indicating that they are processed, mature mRNAs. Two of the major transcripts were missing 3′ untranslated regions of the gene; thus some of the size heterogeneity is the result of differential termination or downstream processing. Segregation analysis showed that a single nuclear gene is responsible for determining transcript pattern. The A619 line carries the dominant allele responsible for the production of three transcripts, whereas W23 carries the recessive allele responsible for the production of two transcripts. This nuclear gene is specific for *cox2*, as no qualitative transcript differences were identified for the other two mitochondrial *cox* genes.

MATERIALS AND METHODS

Teosinte and maize stocks: The nuclear genomes of three teosinte species, *Z. perennis* (Collins and Kempton), *Z. diploperennis* (Guzman, collection 777), and *Z. luxurians* (Guatemala, Wilkes, collection 51186) were replaced with that of maize by successively backcrossing with inbred lines A619 or W23. The initial five backcrosses were made at the University of Wisconsin by JERRY KERMICLE, and backcrossing was continued at the University of Missouri. Analysis of plant materials was begun after at least six generations of backcrossing; data presented here are from materials backcrossed at least nine generations to the respective maize inbred lines. Young ear shoots were used for the preparation of mitochondria. The ear shoots were harvested from field- or greenhouse-grown material when the silks had just emerged from the surrounding husk leaves.

Preparation of mitochondrial RNA: A crude mitochondrial pellet was prepared from homogenate of 20–50 g of surface-sterilized ear shoots as described by STERN and NEWTON (1986). The pellet was resuspended in a wash buffer consisting of 0.35 M sorbitol, 50 mM Tris-HCl (pH 8) and 20 mM EDTA. The sample was layered onto a 20/35/47/60% (w/v) sucrose step gradient and centrifuged in a Beckman SW 41 rotor at 154,000 × *g* for 1 hr. The purified mitochondria were removed from the 35/47% interface and slowly diluted threefold with wash buffer. The mitochondria were collected by centrifugation at 10,000 × *g* and washed once with wash buffer. For scoring individuals, single ear shoots were homogenized and the mitochondria collected by differential centrifugation. They were washed by a second round of centrifugation at 2000 and 10,000 × *g*, but were not further purified. All manipulations of mitochondria were carried out at 4°. RNA was extracted from the mitochondria in the presence of 1 mM aurintricarboxylic acid (ATA) following the procedure of STERN and NEWTON (1986). RNA was precipitated twice in 2 M LiCl, and solubilized in 25 mM Tris-HCl (pH 8), 50 μM ATA, aliquotted, and frozen on dry ice. RNA samples were stored at −80°.

Electrophoresis and hybridization analysis of RNA: Total mitochondrial RNA (0.5 μg per lane) was electrophoresed through 1.2% agarose-formaldehyde gels (MANIATIS, FRITSCH and SAMBROOK 1982). After staining with ethidium bromide the RNAs were blotted onto uncharged nylon filters (MSI, 0.45 μm) by wicking the gels with 10 × SSC. RNA was UV crosslinked to the filters, and the filters baked for 2 hr at 80°. Filters were prehybridized overnight at 42° in 50% deionized formamide, 25 mM Pipes-NaOH (pH 6.8), 5 mM EDTA, 10 × Denhardt's solution, 0.75 M NaCl, 0.1% sodium dodecyl sulfate (SDS) and 100 μg/ml sonicated, denatured salmon sperm DNA (STERN and NEWTON 1986). Hybridization with DNA probes was carried out under the same conditions for 16 h. Following hybridization, filters were washed in 2 × SSC, 0.5% SDS followed by two changes of 1 × SSC, 0.5% SDS at 65° prior to autoradiography using Kodak XRP-5 film and Dupont Cronex intensifying screens. Estimation of RNA size was based on molecular size standards run alongside the samples (BRL RNA Ladder). Cloned restriction fragments containing the genes for subunits I (*cox1*, ISAAC, JONES and LEAVER 1985), II (*cox2*, FOX and LEAVER 1981), or III (*cox3*, HIESEL *et al.* 1987) of mitochondrial cytochrome *c* oxidase were labeled by random priming (Pharmacia Oligolabelling Kit) according to the manufacturer's instructions. Restriction fragments

which contained the intron and exons of the *cox2* gene were identified from a restriction map based on the published nucleotide sequence (FOX and LEAVER 1981). The appropriate fragments were eluted from gels and purified by standard protocols (MANIATIS, FRITSCH and SAMBROOK 1982).

Immunoblot analysis of mitochondrial proteins: Mitochondria were purified from sucrose gradients as above, then resuspended in a small volume of RNA wash buffer, and the concentration of mitochondrial protein was determined using the method of PETERSON (1977). The mitochondria were solubilized in sample buffer (LAEMMLI 1970) and the proteins separated in SDS gels with a linear polyacrylamide gradient of 12–18%. Equivalent amounts of total mitochondrial protein (20 μg) were loaded onto each gel lane alongside molecular weight markers (Bio-Rad Low). Duplicate samples were loaded on each half of the gel. Following electrophoresis, one-half of the gel was stained with Coomassie blue R-250, and the other half was used for immunoblot analysis.

Proteins were electrophoretically transferred from the gel to 0.2 μm nitrocellullose (Schleicher and Schuell, Keene NH) using a Bio-Rad Transblot cell operated at 50 V for 5 hr. Prior to transfer, gels were equilibrated in transfer buffer consisting of 12.5 mM Tris, 96 mM glycine, 0.1% SDS and 20% MeOH. Following transfer, the filter was shaken overnight in 20 mM Tris-HCl (pH 7.5), 150 mM NaCl and 3% fatty acid-free BSA to block nonspecific protein binding sites. Reaction of the filter with antibodies and probe detection using alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (IgG), 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium was essentially as previously described (DARR, SOMERVILLE and ARNTZEN 1986). The filter was incubated 16 hr with a 1:24 dilution of an affinity-purified rabbit anti-COXII IgG (NIVISON and HANSON 1989). Because the antigen used to produce the antibody was a synthetic peptide conjugated to keyhole limpet hemocyanin (KLH), 0.17 mg/ml KLH was included in the probe solution. Alkaline phosphatase-conjugated goat anti-rabbit IgG (Boehringer-Mannheim, Indianapolis Indiana) was used as a 1:1000 dilution of the stock solution. Following development, the filter was photographed using Polaroid Type 55 film, and the negative was scanned using an LKB UltroScan XL Laser Densitometer to quantitate the antibody signal. The filter was then stained for proteins with 0.1% India ink.

RESULTS

Influence of nuclear background on transcript patterns for subunits I, II and III of cytochrome *c* oxidase: Northern blot analysis of the teosinte mitochondrial RNAs was carried out using cloned restriction fragments containing the genes for subunits I, II and III of cytochrome *c* oxidase as hybridization probes (Figure 1). When the cloned *cox2* gene (FOX and LEAVER 1981) was used as a probe, both the number and size of hybridizing transcripts varied as a function of nuclear background for the *Z. perennis* and *Z. diploperennis* cytoplasms. When these teosinte mitochondria were in a maize inbred W23 nuclear background (*Z. perennis*-W23, *Z. diploperennis*-W23), two major 1.9- and 1.7-kb transcripts hybridized with the probe (Figure 1A, lanes 1 and 3). When the nuclear background was maize inbred A619 (*Z. perennis*-A619, *Z. diploperennis*-A619), three major transcripts of 1.9, 1.5 and 1.3 kb hybridized with the *cox2* probe (Figure 1A, lanes 2 and 4). Southern blot analysis indicated that the changes in transcript pattern were not the result of mitochondrial genome rearrangements or of transcription from a duplicate *cox2* gene. Teosinte mitochondrial DNAs from *Z. perennis*-W23, *Z. perennis*-A619, *Z. diploperennis*-W23, and *Z. diploperennis*-A619 were digested with 13 different restriction enzymes, and the Southern blots of the digests probed with the cloned fragment containing the *cox2* gene. For every restriction enzyme used, the number of hybridizing restriction fragments depended upon the mitochondrial source, but did not depend on the nuclear background; that is, for a given teosinte cytoplasm, the same hybridization pattern was observed irrespective of the maize nuclear background. In all but one case, only a single hybridizing fragment was observed. The presence of a restriction site within the region delineated by the cloned probe accounts for the instance in which more than one hybridizing band was observed (data not shown). Therefore all the *cox2* mRNAs were transcribed from

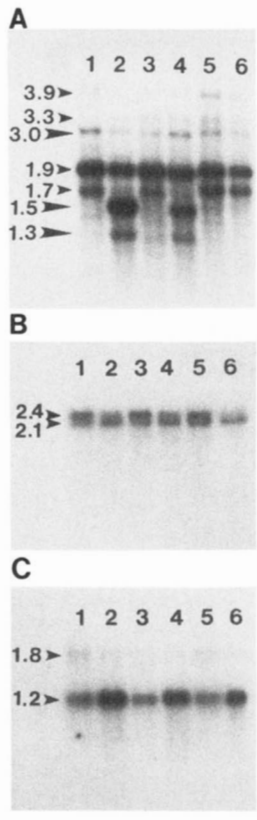

FIGURE 1.—Northern blot analysis of mitochondrial RNA extracted from plants possessing *Z. perennis* or *Z. diploperennis* teosinte cytoplasm in either W23 or A619 maize nuclear backgrounds. RNA was electrophoresed, blotted to nylon, and hybridized with radiolabeled cloned restriction fragments containing A, *cox2*, B, *cox1*; or C, *cox3* genes. Lanes: 1, *Z. perennis*-W23; 2, *Z. perennis*-A619; 3, *Z. diploperennis*-W23; 4, *Z. diploperennis*-A619; 5, *Z. luxurians*-W23; 6, *Z. luxurians*-A619. Transcript sizes in kilobases are indicated on the left.

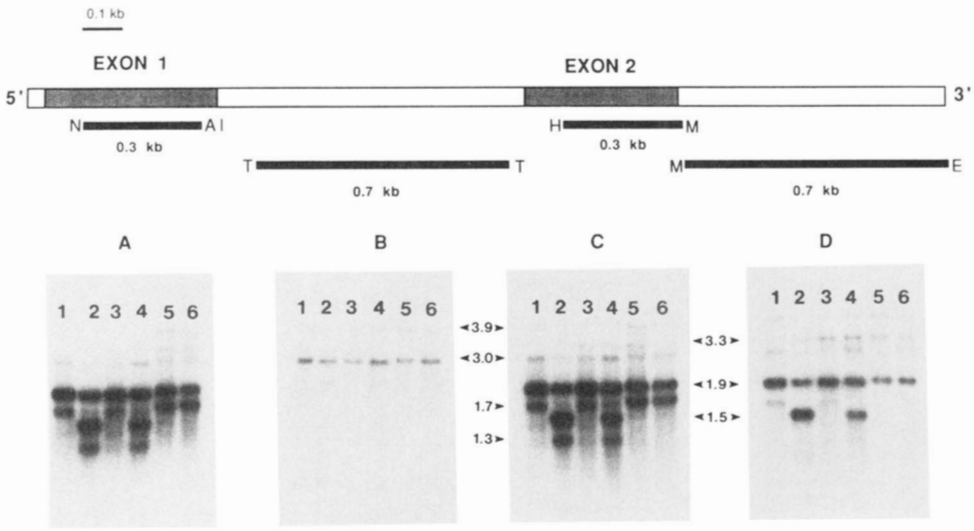

FIGURE 2.—Northern blot analysis of mitochondrial RNA isolated from plants possessing teosinte cytoplasm in either maize W23 or A619 nuclear backgrounds. Blots were probed with different regions of the maize *cox2*-containing pZmE1 clone (FOX and LEAVER 1981). At top is pictured pZmE1, a 2.4-kb *Eco*RI fragment. It contains the two *cox2* exons (shaded regions) and a 794-bp intron on the leftmost two-thirds of the fragment. Blots probed with the different regions of the clone are shown immediately under the region used as a probe. A, Blot hybridized with a 300-bp *Nco*I-*Alu*I exon 1-specific fragment; B, blot hybridized with a 700-bp *Taq*I intron fragment; C, blot hybridized with a 300-bp *Hinc*II-*Msp*I exon 2 fragment; D, blot hybridized with a 700-bp *Msp*I-*Eco*RI fragment containing 3′ noncoding sequences. Lanes: 1, *Z. perennis*-W23; 2, *Z. perennis*-A619; 3, *Z. diploperennis*-W23; 4, *Z. diploperennis*-A619; 5, *Z. luxurians*-W23; 6, *Z. luxurians*-A619. Sizes of the *cox2* transcripts are in kilobases.

a single copy of the gene, and the transcript differences did not arise due to some major rearrangement in the mitochondrial genome. Maize nuclear background apparently had no effect on the *cox2* transcript patterns for *Z. luxurians*, because two transcripts of 1.9 and 1.7 kb were detectable with the probe in both nuclear backgrounds. Minor *cox2* transcripts of 3.9, 3.3 and 3.0 kb were present in all the teosinte-maize combinations.

Transcript patterns for *cox1* and *cox3* in the teosinte-maize combinations were less complex than the *cox2* transcript patterns, and maize nuclear background had no qualitative effect on those patterns. Two major transcripts of 2.4 and 2.1 kb hybridized with the *cox1* probe in every case (Figure 1B). Teosinte mitochondria in the W23 background possess slightly higher levels of the 2.4-kb transcript than teosinte mitochondria in the A619 background. A major 1.2-kb and a less abundant 1.8-kb transcript were detected using *cox3* as the hybridization probe in all the teosinte-maize combinations (Figure 1C). Nuclear background seems to have some effect on the relative abundance of the major *cox3* transcript. Teosinte mitochondria in the W23 background contain slightly reduced amounts of the 1.2-kb transcript compared to mitochondria in the A619 background (Figure 1C).

The major teosinte *cox2* transcripts are processed and contain both exons: To characterize the *cox2* transcript patterns obtained for the two different nuclear backgrounds, restriction fragments containing different portions of the *cox2*-containing pZmE1 clone (FOX and LEAVER 1981) were used as hybridization probes on Northern blots (Figure 2). When fragments that contained either the first or second exon of the *cox2* gene were used as probes (Figure 2, A and C), the transcript patterns were found to be identical to those generated when the full length clone was used as a probe (Figure 1A). If the blots were probed with a fragment from the intron of the gene, none of the major transcripts in any of the teosinte-maize combinations hybridized to the probe, although the larger 3.9- and 3.0-kb minor transcripts did hybridize to the intron probe (Figure 2B).

When a 700-bp region flanking the 3′ end of the *cox2* gene was used as a probe, the minor transcripts hybridized in all the teosinte-maize combinations (Figure 2D). The 1.9- and 1.5-kb major transcripts also hybridized with the probe. However, the 1.3-kb transcript detectable with the full length probe failed to hybridize with the 3′ probe (Figure 2D, lanes 2 and 4). Also, the 1.7-kb transcript detectable with the full length probe hybridized only weakly with the 3′ flanking region in *Z. perennis*-W23, *Z. diploperennis*-W23 and *Z. luxurians* in both maize nuclear backgrounds (Figure 2D, lanes 1, 3, 5 and 6, respectively).

Effect of nuclear background on COXII protein levels: To determine what effect nuclear background had on the teosinte COXII protein itself, total mitochondrial proteins were extracted from *Z. perennis* and *Z. luxurians* in both the W23 and A619 nuclear backgrounds, and subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting. Equivalent amounts of total mitchondrial protein were loaded onto each lane of the gel. The proteins were blotted

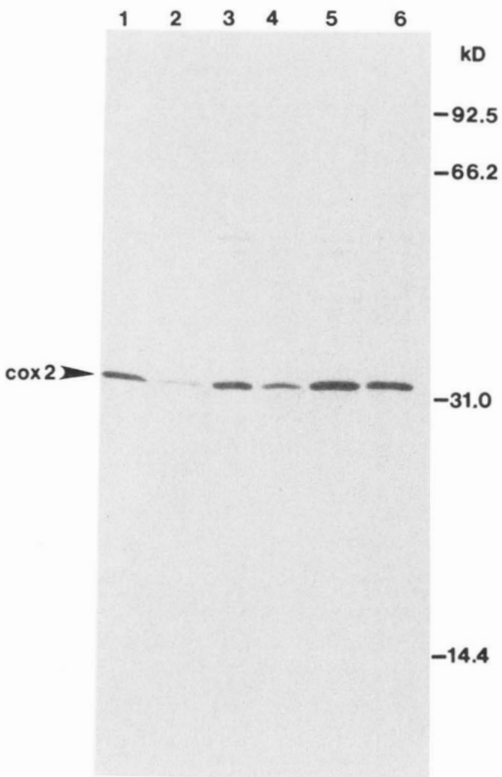

FIGURE 3.—Immunoblot analysis of the COXII polypeptide in mitochondria from plants possessing teosinte cytoplasms in maize W23 or A619 nuclear backgrounds. Mitochondrial proteins were separated on 12–18% linear polyacrylamide gels containing SDS. Proteins were blotted to nitocellulose and the blots were incubated with anti-COXII rabbit IgG. Bound antibody was detected using an alkaline phosphatase-conjugated secondary antibody. Lanes: 1, *Z. perennis* cytoplasm, W23 background (*Z. perennis*-W23); 2, *Z. perennis*-A619; 3, *Z. perennis*-W23, sib of plant in lane 1; 4, *Z. perennis*-A619, sib of plant in lane 2; 5, *Z. luxurians*-W23; 6, *Z. luxurians*-A619. Positions of molecular mass markers shown in kilodaltons on the right.

to nitrocellulose and reacted with the COXII antibody (NIVISON and HANSON 1989). Staining the blot with India ink following antibody treatment confirmed that the transfer of proteins from the gel to the nitrocellulose was uniform both within and between lanes (not shown). The COXII antibody bound to a protein of molecular mass = 34 kD (Figure 3). Three-times more COXII polypeptide was present in *Z. perennis* mitochondria in a W23 background (Figure 3, lanes 1 and 3) than in an A619 background (Figure 3, lanes 2 and 4). More COXII protein was present in *Z. luxurians* mitochondria as compared to *Z. perennis*, but there was no significant difference in the level of the protein as a function of nuclear background for *Z. luxurians* mitochondria (Figure 3, lanes 5 and 6).

A single nuclear gene determines *cox2* transcript pattern: The number of nuclear genes responsible for the effect on the *cox2* transcript pattern in *Z. perennis* was determined by genetic analysis. Plants possessing *Z. perennis* cytoplasm were used for these studies. *Z. perennis*-W23 plants, which exhibit the "two-transcript" pattern (*i.e.*, 1.9 and 1.7 kb *cox2* transcripts as in Figure 1A, lane 1), were crossed by A619. *Z. perennis*-A619 plants, which exhibit the "three-transcript" pattern (*i.e.*, 1.9-, 1.5- and 1.3-kb transcripts as in Figure 1A, lane 2) were crossed by W23. The F_1 hybrids *Z. perennis*-W23 × A619 (Figure 4, lane 2) and *Z. perennis*-A619 × W23 (not shown) both exhibited the three-transcript pattern upon Northern analysis using the *cox2* clone as probe. Thus, the two-transcript pattern is recessive to the three-transcript pattern.

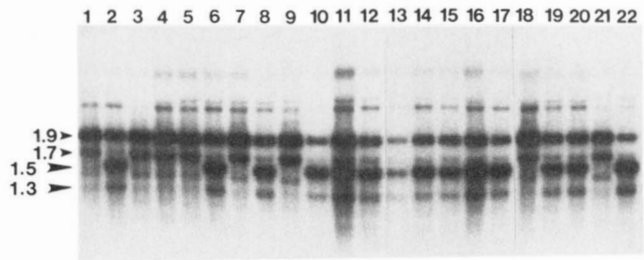

FIGURE 4.—Segregation analysis of *cox2* transcript patterns by Northern blot hybridization. Mitochondrial RNA was extracted from individual ear shoots, electrophoresed, blotted, and probed with the full length *cox2* gene. Lanes: 1, *Z. perennis* cytoplasm, W23 background (*Z. perennis*-W23); 2, F_1 hybrid *Z. perennis*-W23 × A619; 3–10, backcross (*Z. perennis*-W23 × A619) × W23; 11–22 F_2, *Z. perennis*-W23 × A619 self-pollinated. Sizes of major transcripts in kilobases are indicated at left.

An F_2 generation was produced by self-pollinating *Z. perennis*-W23 × A619 plants. *Z. perennis*-W23 × A619 plants were also backcrossed by maize inbred W23 plants. Seeds from these crosses were planted and mitochondrial RNA was extracted from individuals. Transcript patterns were assessed by probing Northern blots of the mitochondrial RNA with the *cox2* clone. A representative blot is shown in Figure 4A, lanes 3–22. The segregation of transcript patterns for F_2 and backcross generations are summarized in Table 1. If a single nuclear gene is responsible for the transcript differences, then a 3:l segregation in favor of the three-transcript pattern is expected in the F_2 generation, and a 1:1 segregation is expected in the backcross generation. Out of 35 F_2 individuals scored, 25 possessed the three-transcript pattern, and 10 possessed the two-transcript pattern. Chi-squared analysis of the 25:10 ratio showed that it did not deviate significantly from 3:1 (Table 1, analysis 1). For the backcross, 3 of 8 individuals scored possessed the three-transcript pattern. The probability of observing a 3:5 ratio by chance when a 1:1 ratio was expected was found to be 0.73 (Table 1, analysis 2). Thus at the conventional 5% level of significance, these data support the hypothesis that a single nuclear gene is responsible for the differences in *cox2* transcript size and number.

DISCUSSION

Differential efffects on mitochondrial *cox2* gene expression are observed when *Z. perennis* or *Z. diplo-*

TABLE 1

Segregation of *Z. perennis cox 2* transcript patterns in F_2 and backcross generations

Analysis	Cross	Expected ratio	n	Individuals with three transcripts	Individuals with two transcripts	χ^2	$P(x)$
1	*Z. perennis*-W23 × A619 ×	3:1	35	25	10	0.08	$0.80 > P > 0.70$
2	*Z. perennis*-(W23 × A619) × W23	1:1	8	3	5		0.73

Analysis 1: F_2 sample from two sibling ears grown during two field seasons. d.f. = 1, two-sided χ^2 calculated using Yate's continuity correction.

Analysis 2: backcross from a single ear. Two-sided binomial exact test.

perennis cytoplasms are in association with certain maize nuclear backgrounds. When the cytoplasm is in a W23 background, two major *cox2* transcripts of 1.9 and 1.7 kb are present, whereas in an A619 background the 1.7-kb transcript is replaced by two smaller transcripts of 1.5 and 1.3 kb. Although plant mitochondria possess genes encoding subunits I, II and III of the cytochrome *c* oxidase complex, only *cox2* transcript number is influenced by nuclear background in the teosinte-maize combinations. Altering nuclear background had no major qualitative effects on *cox1* or *cox3* transcripts. The *cox2* transcript patterns for *Z. luxurians* mitochondria were not affected by maize nuclear background; the 1.9- and 1.7-kb transcripts were present in mitochondria from plants possessing either the W23 or the A619 nuclear background.

Z. perennis, a tetraploid perennial teosinte, is thought to have derived from the diploid perennis *Z. diploperennis* (ILTIS *et al.* 1979). Thus the similar effects of nuclear background on the *cox2* transcripts patterns for the two species is not surprising. Teosinte mitochondria in their own nuclear background possess a two-transcript pattern (P. COOPER, unpublished results); therefore the three-transcript pattern, seen when perennial teosinte mitochondria are present with A619 nuclear genes, represents an abnormal situation.

Although miniature kernels and CMS are two other phenotypic consequences of placing, *Z. perennis*, *Z. diploperennis* and *Z. luxurians* cytoplasms in association with maize nuclear genes, there appears to be no direct causal relationship between *cox2* transcript pattern and either of these two developmental abnormalities. Miniature kernels result when these teosinte cytoplasms are in association with a W23 background, and normal kernel size can be recovered by outcrossing to A619 (ALLEN, EMENHISER and KERMICLE 1989). We have found no linkage between altered *cox2* transcript patterns and kernel/plant size. In an F_2 population segregating 3:1 for large and small kernels, *cox2* transcript patterns segregated independently from kernel size (P. COOPER, unpublished results). Plants possessing *Z. perennis* or *Z. diploperennis* cytoplasm in an A619 nuclear background exhibit CMS but fertility is restored by outcrossing to W23 (NEWTON and COE 1989). However, the three-transcript pattern is present in the fertile F_1 hybrids as well as in the sterile *Z. perennis*- or *Z. diploperennis*-A619 plants. Thus there is no direct correlation between the expression of CMS and the altered *cox2* transcripts.

Segregation analyses of the *cox2* transcript patterns in *Z. perennis*-maize populations support the hypothesis that a single nuclear gene is responsible for the observed differences in the major *cox2* transcripts. The inbred A619 line carries the dominant allele of this gene, which we designate as *Mct* (for modifier of *cox2* transcripts). Plants possessing *Z. perennis* cytoplasm in an A619 nuclear background also exhibit a threefold reduction in accumulated levels of the COXII polypeptide relative to the plants carrying the same cytoplasms in a W23 background. These data imply a reduced stability or translational efficiency of the smaller transcripts. Alternatively, the resulting polypeptides may be less stable.

How might the *Mct* gene alter *cox2* transcript size? Transcript size heterogeneity can be the result of multiple transcription initiation sites, multiple transcription termination sites, and/or post-transcriptional processing. In maize mitochondria, the transcript size heterogeneity of the protein coding genes *atp9*, *cox3* and *cob* results from multiple sites of transcription initiation (MULLIGAN, LAU and WALBOT 1988; MULLIGAN, MALONEY and WALBOT 1988). Because all of the teosinte major *cox2* transcripts lack the intron, they represent processed forms of the mRNA. Given that the coding region for the *Z. diploperennis cox2* gene is virtually identical to that of maize (GWYNN *et al.* 1987), and that the minimum length for a spliced mRNA would be 825 bp (FOX and LEAVER 1981), then a substantial portion of each of the teosinte *cox2* transcripts consists of 5′ and 3′ untranslated regions. The presence of multiple transcripts in all of the teosinte-maize combinations is in part due to heterogeneity in the 3′ untranslated region, as judged by the lack of hybridization of the 3′ noncoding region of the pZmE1 clone to the 1.7- and 1.3-kb transcripts. This is in contrast to maize *atp9*, *cob* and *cox3* transcripts in maize (MULLIGAN, LAU and WALBOT 1988; MULLIGAN, MALONEY and WALBOT 1988) and *atpA*

and *cox2* transcripts in Oenethera (SCHUSTER *et al.* 1986), in which no 3′ heterogenity has been identified. Although we have not yet analyzed the full extent of 5′ heterogenity in the teosinte *cox2* transcripts, we can deduce from our data that the 1.7-kb transcript found in plants possessing a W23 nuclear background arises from an initiation or processing event approximately 400 bp further upstream from a similar event that gives rise to the 1.3-kb transcript found in plants possessing an A619 nuclear background. The transcripts are similar in their sequence content in that they possess both exons (825 bases) but have no intron and little or no 3′ untranslated regions (Figure 2). Precise mapping of the 5′ and 3′ ends of the teosinte *cox2* transcripts by S1 nuclease and primer extension analyses will determine whether these conclusions are true.

We wish to acknowledge JERRY KERMICLE for generously providing us with the teosinte-maize stocks. We are grateful to CHRIS LEAVER for providing us with the maize *cox1* and *cox2* clones, and to AXEL BRENNICKE for the Oenethera *cox3* clone. We also thank HELEN NIVISON and MAUREEN HANSON for the gift of the COXII antibody, and KAREN CONE for critically reading the manuscript. Support was provided by the National Science Foundation (DCB 885827), the Missouri Center for Advanced Technology, and a McKnight Foundation Individual Investigator Award.

LITERATURE CITED

ALLEN J. O., G. K. EMENHISER and J. L. KERMICLE, 1989 Miniature kernel and plant: interaction between teosinte cytoplasmic genomes and maize nuclear genomes. Maydica **34:** 277–290.

COOPER, P., and K. J. NEWTON, 1989 Maize nuclear background regulates the synthesis of a 22-kDa polypeptide in *Zea luxurians* mitochondria. Proc. Natl. Acad. Sci. USA **86:** 7423–7426.

DARR, S. C., S. C. SOMERVILLE and C. J. ARNTZEN, 1986 Monoclonal antibodies to the light-harvesting chlorophyll *a/b* protein complex of photosystem II. J. Cell Biol. **103:** 733–740.

DOEBLEY, J., and H. H. ILTIS, 1980 Taxonomy of *Zea*. I. Subgeneric classification with key to taxa. Am J. Bot. **67:** 982–993.

DUVICK, D. N., 1965 Cytoplasmic pollen sterility in corn. Adv. Genet. **13:** 1–56.

FOX, T. D., and C. J. LEAVER, 1981 The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. Cell **26:** 315–323.

GRACEN, V. E., and C. O. GROGAN, 1974 Diversity and suitability for hybrid production of different sources of cytoplasmic male sterility in maize. Agron J. **66:** 654–657.

GRUN, P., 1976 *Cytoplasmic Genetics and Evolution.* Columbia University Press, New York.

GWYNN, B., R. E. DEWEY, R. R. SEDEROFF, D. H. TIMOTHY and C. S. LEVINGS III, 1987 Sequence of the 18S-5S ribosomal gene region and the cytochrome oxidase II gene from mtDNA of *Zea diploperennis*. Theor. Appl. Genet. **74:** 781–788.

HANSON, M. R., and F. CONDE, 1985 Function and variation of cytoplasmic genomes: lessons from cytoplasmic-nuclear interactions affection male fertility in plants. Int. Rev. Cytol. **94:** 213–267.

HIESEL, R., W. SCHOBEL, W. SCHUSTER and A. BRENNICKE, 1987 The cytochrome oxidase subunit I and subunit III genes of *Oenethera* mitochondria are transcribed from identical promoter sequences. EMBO J. **6:** 29–34.

ILTIS, H. H., J. F. DOEBLEY, R. GUZMAN and B. PAZY, 1979 *Zea diploperennis* (Graminae): a new teosinte from Mexico. Science **203:** 186–188.

ISAAC, P. G., V. P. JONES and C. J. LEAVER, 1985 The maize cytochrome *c* oxidase subunit I gene: sequence, expression, and rearrangement in cytoplasmic male sterile plants. EMBO J. **4:** 1617–1623.

KERMICLE, J. L., and J. H. LONNQUIST, 1973 Nucleo-cytoplasmic interaction in the determination of a defective seed trait. Maize Genet. Coop. Newslet. **47:** 209–211.

LAEMMLI, U. K., 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685.

LAUGHNAN, J. R., and S. GABAY-LAUGHNAN, 1983 Cytoplasmic male sterility in maize. Annu. Rev. Genet. **17:** 27–48.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MULLIGAN, R. M., G. T. LAU and V. WALBOT, 1988 Numerous transcription intitiation sites exist for the maize mitochondrial genes for subunit 9 of the ATP synthase and subunit 3 of cytochrome oxidase. Proc. Natl. Acad. Sci. USA **85:** 7998–8002.

MULLIGAN, R. M., A. P. MALONEY and V. WALBOT, 1988 RNA processing and multiple transcription initiation sites result in transcript size heterogeneity in maize mitochondria. Mol. Gen. Genet. **211:** 373–380.

NEWTON, K. J., and E. H. COE, JR., 1989 Multiple ferility restorer genes for EP (Z.p.) cytoplasm. Maize Genet. Coop. Newslet. **63:** 68–69.

NIVISON, H. T., and M. R. HANSON, 1989 Identification of a mitochondrial protein associated with cytoplasmic male sterility in petunia. Plant Cell **1:** 1121–1130.

PETERSON, G. L., 1977 Simplification of the protein assay of Lowry *et al.* which is generally more applicable. Anal. Biochem. **83:** 346–356.

SCHUSTER, W., R. HIESEL, P. G. ISAAC, C. J. LEAVER and A. BRENNICKE, 1986 Transcript termini of messenger RNAs in higher plant mitochondria. Nucleic Acids Res. **14:** 5943–5954.

STERN, D. B., and K. J. NEWTON, 1986 Isolation of plant mitochondrial RNA. Methods Enzymol. **118:** 488–496.

TZAGOLOFF, A., and A. M. MYERS, 1986 Genetics of mitochondrial biogenesis. Annu. Rev. Biochem. **55:** 249–285.

Communicating editor: M. R. HANSON