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Interaction of nuclear and mitochondrial genomes in the alteration of maize mitochondrial *orf221* transcripts

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Abstract The interaction of nuclear and mitochondrial genomes in the alteration of maize (*Zea mays* L.) mitochondrial *orf221* transcript patterns was examined. Northern analyses involving specific maize nuclear genotypes associated with N, C or S cytoplasms revealed considerable *orf221* transcript heterogeneity. F_1 progenies were developed from maize inbred-cytoplasm combinations that differed for *orf221* transcript patterns. Northern analyses revealed that the presence or level of abundance of certain *orf221* transcripts was dependent on nuclear genotype. The maize inbred B37(C) exhibits *orf221* transcripts of 3500, 3200, 2800, and 1300 nt whereas the F_1 of B37(C)×Ky21(N) does not exhibit a 2800-nt transcript but does give transcripts of 2100 and 1250 nt in addition to 3500-, 3200- and 1300-nt transcripts. Northern analyses also suggested that the size or the presence of certain *orf221* transcripts was related to the mitochondrial genome configuration. Maize inbred A619 exhibits a 2300-nt *orf221* transcript when associated with N cytoplasm and a 2100-nt *orf221* transcript when associated with C and S cytoplasms. As a result of deletion of the gene T-*urf13*, the A188(T7) mitochondrial mutant exhibits only a 3100-nt *orf221* transcript and not the very complex T-*urf13*/*orf221* transcript pattern associated with A188(T). The genetic stock A188(T7)×W64A(N)^2 gives a highly abundant 2100-nt *orf221* transcript not detected in A188(T7). Deletion of T-*urf13* has enabled the nuclear genotype of W64A(N) to alter *orf221* transcript patterns in a manner not detected in T cytoplasm. This observation suggests that alteration of the mitochondrial genomic configuration adjacent to *orf221* results in a different response to nuclear gene products from that observed when *orf221* is present in the T mitochondrial genome configuration.

Key words Maize (*Zea mays* L.) · Mitochondrial mRNA transcription · *orf221* · Nuclear-mitochondrial interactions

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Introduction

Mitochondrial function requires the coordinated expression of nuclear and mitochondrial genes. When a cytoplasm is transferred to a different nuclear background, the absence of specific nuclear alleles may result in the expression of mitochondrial mutations. Altered maize (*Zea mays* L.) phenotypes due to nuclear-mitochondrial incompatibility include cytoplasmic male sterility (CMS) (Laughnan and Gabay-Laughnan 1983), and the "teosinte cytoplasm associated miniature" (tcm) trait (Kermicle and Lonnquist 1973; Allen et al. 1989). Cytoplasmic male sterility causes pollen abortion and the tcm condition results in small kernels that germinate and give rise to short-statured, pale-green plants. The CMS and tcm phenotypes are converted to normal phenotypes when specific nuclear restorer or rectifier genes, respectively, are crossed into the nuclear background.

Investigations on maize nuclear-mitochondrial interactions have revealed effects of the nuclear genome on the presence or abundance of specific mitochondrial (mt) mRNA transcripts. Certain maize mitochondrial mRNA processing events have been associated with specific nuclear genes or genotypes. The maize nuclear gene *Rf1*, which is one of two restorer genes required for restoration of T cytoplasmic male sterility to normal fertility, has been shown to alter mitochondrial T-*urf13*/*orf221* transcripts (Dewey et al. 1986, 1987; Kennell and Pring 1989). T-*urf13* is associated with male sterility and sensitivity to T toxin in maize (For a review see Pring and Lonsdale 1989). The function of *orf221* (formerly ORF25), which is co-transcribed with T-*urf13* in T cytoplasm maize, remains to be

determined (Stamper et al. 1987). Recently, Prioli et al. (1993) identified a 24.7-kDa polypeptide product of *orf221* that is mitochondrial membrane-bound. The presence of *Rf1* is associated with a processing event which reduces the abundance of 2013 and 1830-nt T-*urf13/orf221* transcripts and generates a novel 1571-nt T-*urf13/orf221* transcript (Dewey et al. 1987). A maize T cytoplasm 1100-nt *orf221*-specific transcript that is dependent on nuclear genotype has also been reported (Kennell et al. 1987; Rocheford et al. 1992). Two other nuclear-genotype-controlled events alter T-*urf13/orf221* transcript patterns, producing transcripts of 1475 and 1400 nt. Genetic analyses demonstrated that the control of the presence of the 1400-nt transcript is independent of *Rf1* effects on T-*urf13/orf221* transcription (Rocheford et al. 1992).

When maize nuclear genotypes are associated with cytoplasms from teosinte, a wild relative of maize, nuclear effects on mitochondrial gene transcription have been revealed. Alterations in mitochondrial gene *cox2* transcripts were observed when teosinte cytoplasms became associated with specific maize nuclear genotypes (Cooper et al. 1990). Genetic analyses determined that a single dominant nuclear gene, *Mct*, is responsible for alteration of the *cox2* transcript pattern. In other plant species, the presence or absence of large *atpA* mRNA transcripts has been associated with specific nuclear genotypes for radish and sunflower (Makaroff and Palmer 1988; Siculella and Palmer 1988). In the above studies, mRNA processing was suggested as the event responsible for the transcriptional changes.

We present results demonstrating considerable nuclear effects on the transcription pattern of maize mitochondrial *orf221*. *orf221* appears to be unique to plant mitochondria (Prioli et al. 1993). DNA sequencing analyses have identified sequences homologous to maize *orf221* in the mitochondrial genomes of tobacco (Stamper et al. 1987), wheat (Bonen et al. 1990), *Arabidopsis* (Brandt et al. 1992) and the liverwort *Marchantia polymorpha* (Oda et al. 1992). In contrast, computer searches have not identified sequences homologous to *orf221* in animal and fungal mitochondria (Prioli et al. 1993). Furthermore, the ORF221 membrane protein has been detected in maize, tobacco and wheat, but not in yeast (Prioli et al. 1993). Although the function of the ORF221 protein is still unknown, a recent report suggests that ORF221 may be important in plant mitochondria (Prioli et al. 1993).

Heterogeneity in *orf221* transcript pattern has been detected among the four major maize cytoplasm types N, C, S, and T (Stamper et al. 1987). Variation in *orf221* transcript patterns has also been observed among mtRNAs from the N cytoplasm grouping but with different nuclear genotypes (Kennell et al. 1987). A difference in the presence of a 2300-nt *orf221* transcript between two different maize N cytoplasm inbreds was observed by Wang et al. (1991) while Gupta and Abbott (1991) detected the 2300-nt *orf221* transcript in both reciprocal F_1s of these two inbreds. This indicated that nuclear effects influence the presence of this 2300-nt *orf221* transcript.

We performed experiments designed to examine the interaction of mitochondrial genome configuration and nuclear genotype in the alteration of *orf221* transcript patterns. F_1 progeny from crosses of maize inbred-cytoplasm combinations that differed for *orf221* transcript pattern were evaluated. We examined *orf221* transcript patterns of the T cytoplasm tissue-culture mutant T7 in association with two different nuclear backgrounds. The mutant T7 has deleted the gene T-*urf13* (Umbeck and Gengenbach 1983). Northern analyses on the above genetic stocks revealed that *orf221* transcript pattern variation could be attributed to nuclear effects as well as to mitochondrial genome configuration effects.

Materials and methods

Open reading frame terminology and abbreviations

The maize mitochondrial open reading frame ORF25 was originally characterized in T cytoplasm by Dewey et al. (1986). ORF25 has been sequenced in four maize cytoplasms and, based on putative initiation and termination sites, ORF25 has a predicted amino-acid size of 221 residues in T, C, and S cytoplasms and 219 residues in N cytoplasm (Stamper et al. 1987). Consequently, the designations *orf221* in T, C, and S cytoplasms and *orf219* in N cytoplasm have been assigned (see Fig. 1). For simplicity and clarity in the text, we shall use the term *orf221* for N, T, C, and S cytoplasms.

Genetic stocks

The C and S cytoplasm maize inbreds and the N cytoplasm version of the same inbreds were supplied by M. Smith, except for Vg(S) which was provided by J. Laughnan. The N and T cytoplasm maize inbreds came from W. F. Pederson, except for Wf9(N) and Wf9(T) which were provided by M. Albertsen and A188(N) which was provided by B. Gengenbach. The genetic stocks of A188(T7)×W64A^2 and A188(T2)×W64A^2 were developed and provided by B. Gengenbach. The *Zea diploperennis* stock was supplied by D. Timothy. In the figures and the text the designation 187-2 is used as an abbreviation for maize inbred C.I. 187-2.

Isolation and hybridization analysis of mtRNA

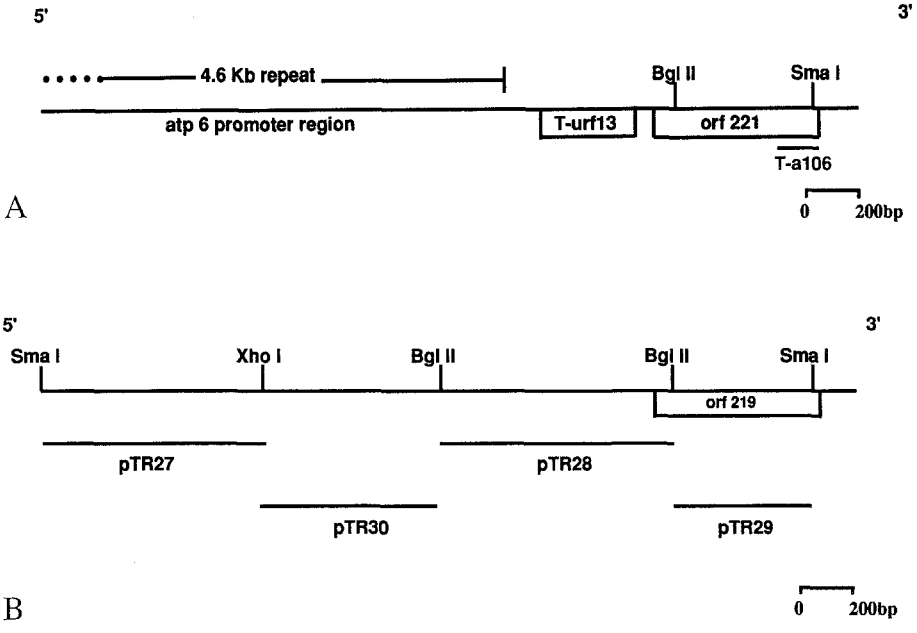
Mitochondria were isolated from etiolated coleoptiles and all procedures were essentially as described previously (Kennell et al. 1987; Rocheford et al. 1992). Whenever unique *orf221* patterns were observed for inbreds or F_1 progeny an additional Northern analysis was performed with the same mtRNA preparation and/or a new mtRNA preparation.

Subcloning of mtDNA

The cosmid N8A1, developed from B37(N) mtDNA, was provided by C. M.-R. Fauron (Fauron and Havlik 1988). A 2.8-kb *Sma*I fragment containing the single copy of *orf219* in the maize mitochondrial genome was isolated from N8A1 and a contiguous set of subclones (see Fig. 1B) were developed in pUC119.

Results and discussion

Northern analysis with the *orf221* probe T-a106 (Fig. 1A) on mtRNA from a series of maize inbred-cytoplasm com-



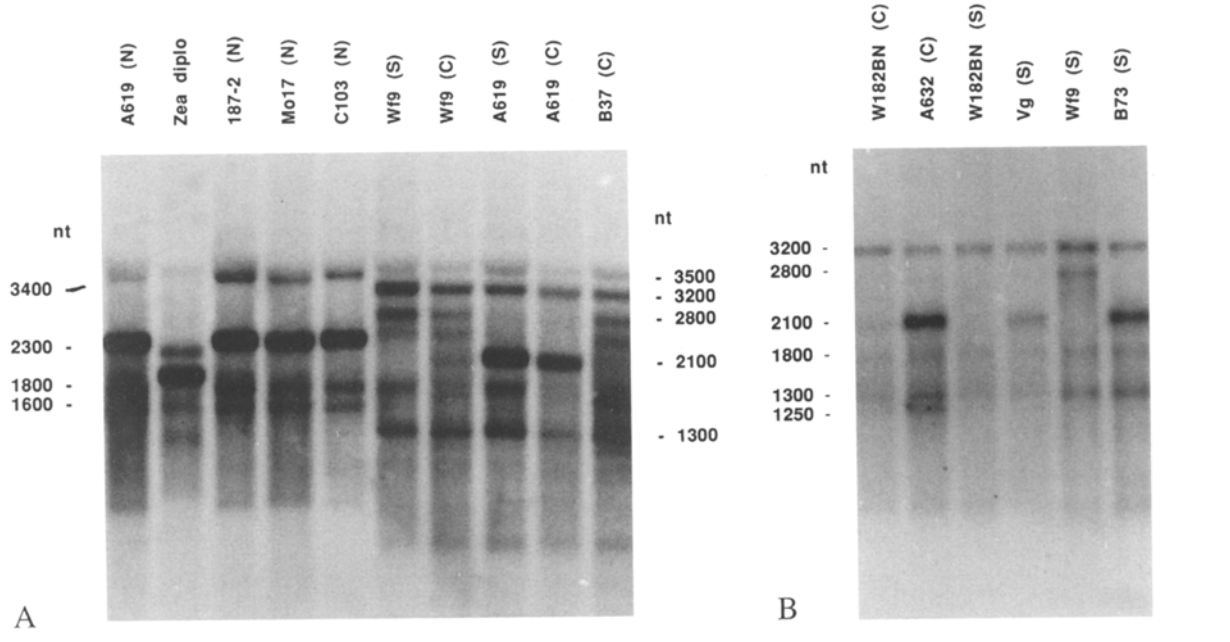


Fig. 1A Schematic representation of the T-*urf13/orf221* region in T cytoplasm. **B** Schematic representation of the *orf219* region in N cytoplasm maize. Clones were subcloned from the cosmid N8A1 (Fauron and Havlik 1988)

Fig. 2A, B Northern analysis with *orf221* clone T-a106 of mitochondrial mRNA from maize inbreds associated with N, C and S cytoplasms and a *Z. diploperennis* stock

binations revealed multiple transcripts and considerable transcript pattern variability among different stocks (Fig. 2). Some *orf221* transcripts observed were consistent with previous reports. For example the 3400-, 2300-, 1800- and 1600-nt transcripts detected for A619(N), 187-2(N), Mo17(N) and C103(N) and the 3200-, 2100- and 1300-nt transcripts detected for A619 (S) and (C) have all been previously observed (Kennell et al. 1987; Stamper et al. 1987). In Fig. 2A, the 1800- and 1600-nt transcripts are overexposed for A619(N), 187-2(N), and Mo17(N) but are distinguishable for C103(N). We also detected additional *orf221* transcripts: a 3500-nt transcript for Wf9(S), Wf9(C), A619(S), A619(C) and B37(C); a 2800-nt transcript for Wf9(S), Wf9(C) and B37(C); and a 1800-nt transcript for Wf9(S) and A619(S) (Fig. 2A). The detection of additional transcripts suggests *orf221* transcript patterns are more complex than previously reported. A unique *orf221* transcript pattern of 2200 and 1900 nt for an open pollinated stock of *Z. diploperennis* cytoplasm was also observed. This relative of maize transcribes *orf221* but has a different transcript pattern from those observed for *Z. mays* L.

The Stamper et al. (1987) report involved stocks that differed for both nuclear genotype and cytoplasm type, preventing specific determination of whether nuclear or mitochondrial effects were responsible for some of the *orf221* transcript pattern differences they observed. Although the

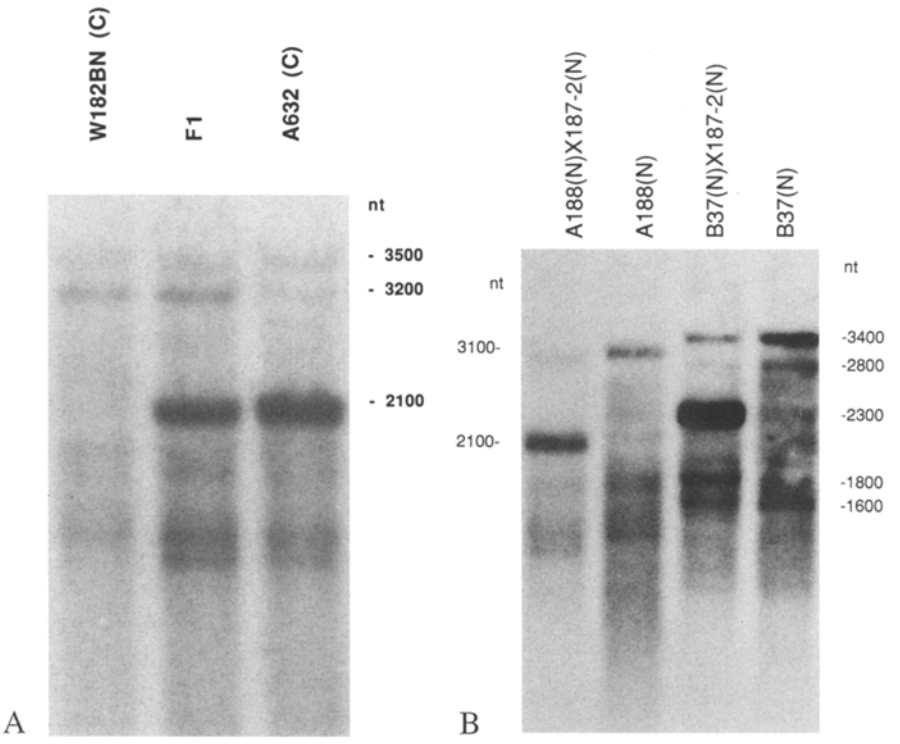


Fig. 3A Northern analysis with T-a106 of two C cytoplasm maize inbreds and their F_1 progeny. **B** Northern analysis with pTR29 of two maize inbreds and two F_1 progenies of maize inbreds, all associated with N cytoplasm

Kennell et al. (1987) study involved only N cytoplasm stocks with different nuclear genotypes, it is not known if the mitochondrial configuration was identical among the different stocks since the N cytoplasm grouping is very broad and diverse. In the present study, the transcript size variation detected among the A619 isonuclear cytoplasm stocks (N, C, S) in Fig. 2A is probably due to mitochondrial genome configuration differences since the nuclear genotype was constant. At least nine generations of backcrossing in the A619 nuclear genotype was performed on each cytoplasm stock (M. Smith, personal communication).

Some *orf221* transcripts were associated with specific nuclear genotypes within a cytoplasm group. A 2800-nt *orf221* transcript was detected for Wf9(C) and B37(C) but not for A619(C), W182BN(C), and A632(C) (Fig. 2A, B). A 2800-nt *orf221* transcript was detected for Wf9(S) but not for A619(S), W182BN(S), Vg(S) and B73(S) (Fig. 2A, B). A 2100-nt *orf221* transcript was detected for A619(C) and A632(C) but not for Wf9(C), B37(C) and W182BN(C). (Note that a very low abundance 2100-nt transcript appears to be present for W182BN(C).) The 2100-nt *orf221* transcript of A619(S), Vg(S) and B73(S) was not present in Wf9(S) and W182BN(S) (Fig. 2A, B). These observations suggested that the presence of the 2800- and 2100-nt *orf221* transcripts in C and S cytoplasm is controlled by the nuclear genotype.

Collectively, the above results indicate that both the mitochondrial genome configuration and the nuclear genotype can influence *orf221* transcript pattern. A series of experiments were performed to more precisely confirm whether presence or absence of specific *orf221* transcripts could be attributed to mitochondrial genome configuration differences or nuclear genotype differences. The maize inbred W182BN(C), which does not exhibit the 2100-nt *orf221* transcript, was used as the female parent and the maize inbred A632(C), which shows the highly abundant 2100-nt *orf221* transcript, was used as a pollen source in the development of F_1 progeny. The highly abundant 2100-nt *orf221* transcript was detected in the F_1 progeny of W182BN(C)×A632(C) (Fig. 3A). Genetic analyses showed that the presence of either the abundant 2300- or 2100-nt *orf221* transcript can be conferred on F_1 progeny of N cytoplasm maize inbreds that do not exhibit the abundant 2300- or 2100-nt *orf221* transcript (Fig. 3B). Northern analysis with probe pTR29 (Fig. 1B) on inbred B37(N) detects transcripts of 3400, 2800, 1800 and 1600 nt but does not detect a highly abundant 2300-nt transcript. The F_1 of B37(N)×187-2(N) exhibits an abundant 2300-nt transcript in addition to 3400-, 1800-, and 1600-nt transcripts whereas the 2800-nt transcript is greatly reduced in abundance (Fig. 3B). The presence of the 2300-nt transcript in the F_1 of B37(N)×187-2(N) is similar to the results of Wang et al. (1991) who demonstrated that the 2l300-nt transcript was present in the F_1 of B37(N)×B73(N). The inbred A188(N) exhibits a 3100-nt transcript but does not show either the 2300- or the 2100-nt transcript (Fig. 3B). The F_1 of A188(N)×187-2(N) exhibits a highly abundant 2100-nt transcript. The observation of a 2100-nt, but not a 2300-nt *orf221* transcript in an N cytoplasm stock suggests that the mitochondrial DNA configuration surrounding *orf221* in C and S cytoplasm which may be associated with the 2100-nt transcript size may also be present in some N cytoplasm maize stocks such as A188(N). Further evidence

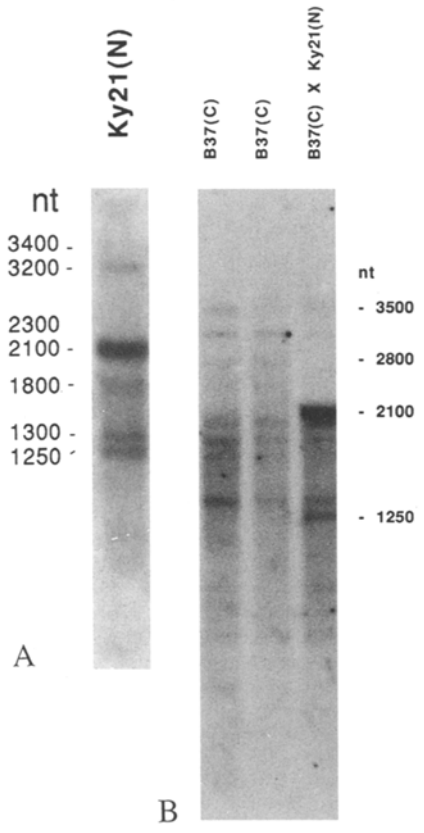


Fig. 4A Northern analysis of maize inbred Ky21(N). **B** Northern analysis of C cytoplasm stocks associated with different nuclear genotypes. The probe for both Northerns was T-a106

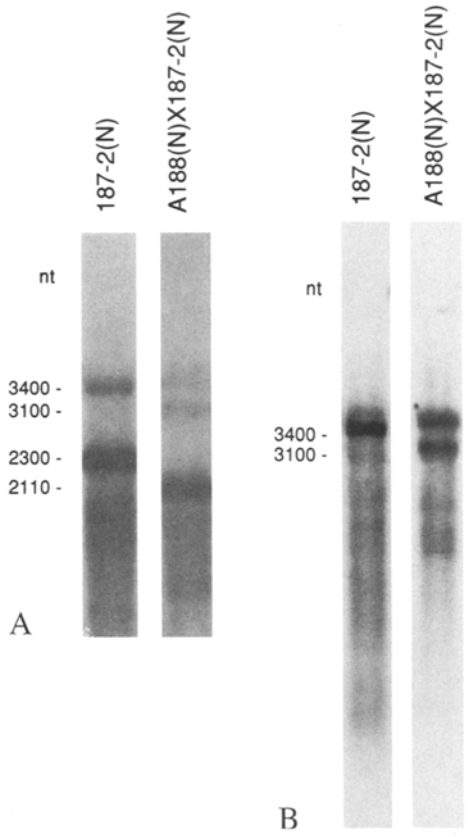


Fig. 5A Northern analysis with probe T-a106 of a maize inbred and an F_1 progeny. **B** Northern analysis of the same stocks with probe pTR27 which is 5′ to *orf221*

comes from the observation that the maize inbred B73(N) exhibits a 2300-nt *orf221* transcript (Gupta and Abbott 1991) whereas B73(S) has the 2100-nt *orf221* transcript (Fig. 2B).

B37(C) gives *orf221* transcripts of 3500, 3200, 2800, and 1300 nt (Figs. 2A and 4B) and inbred Ky21(N) gives *orf221* transcripts of 3200, 2100, 1800, 1300 and 1250 nt (Fig. 4A). Ky21 is unique among N cytoplasm stocks in that it exhibits a 3200-nt transcript but not a 3400- or 3100-nt *orf221* transcript. When Ky21(N) is crossed as the pollen source to B37(C) the F_1 progeny do not show the 2800-nt transcript detected in B37(C) but does exhibit transcripts of 2100 and 1250 nt in addition to reduced abundance transcripts of 3500 and 3200 nt, and the 1300-nt transcript present in B37(C). This reveals that the presence or absence of three different *orf221* transcripts in C cytoplasm (2800, 2100, 1250 nt) were affected by the nuclear genotype in this single cross. [Note the 1250-nt transcript was also detected for A632(C) in Fig. 2B but is difficult to distinguish in this exposure.]

The mtRNA isolated from inbred A188(N) also exhibits a somewhat unique *orf221* transcription pattern in that the largest transcript is 3100 nt (Fig. 3B; Kennell et al. 1987) rather than the 3400-nt transcript observed for most N cytoplasm stocks (Figs. 2A, 3B; Kennell et al. 1987; Wang et al. 1991). In a separate cross and mtRNA preparation from that used in Fig. 3B, the inbred 187-2(N), which displays the 3400-nt *orf221* transcript (Fig. 2A), was crossed as pollen source to A188(N). The resultant F_1 hybrid exhibited both the 3400- and 3100-nt *orf221* transcripts in Northern analysis (Fig. 5A) using probe T-a106. The 3400- and 3100-nt transcripts were more visible when the pTR27 probe, which is 5′ to *orf221* and is a larger DNA fragment than T-a106 (Fig. 1), was used for Northern analysis (Fig. 5B). The results establish that association of A188(N) cytoplasm with the heterozygous A188/187-2 nuclear genotype resulted in the presence of both the 3400- and 3100-nt transcripts but not the single 3400- or 3100-nt transcript found in association with 187-2(N) or A188(N), respectively. This demonstrates that the nuclear genotype can influence the presence of the largest detectable *orf221* transcripts in N cytoplasm.

The tissue-culture mutant A188(T7) has deleted the gene T-*urf13* (Wise et al. 1987) which is normally 5′ to, and co-transcribed with, *orf221* in T cytoplasm maize (Dewey et al. 1986). The *orf221* transcript pattern of A188(T7) is identical to that of A188(N), exhibiting just the major 3100-nt transcript (Fig. 6; Wise et al. 1987). The genetic stock W64A(N) has the abundant 2300-nt *orf221* transcript (Kennell et al. 1987). Northern analysis of

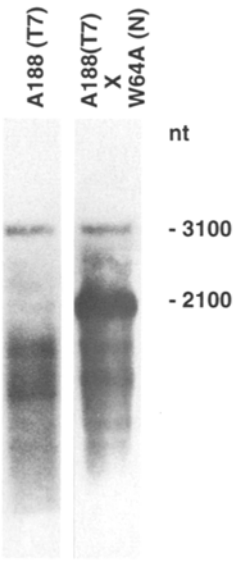


Fig. 6 Northern analysis of T-*urf13* deletion mutant T7 associated with two different nuclear genotypes. The probe was pTR29

A188(T7)×W64A(N)^2 with the *orf221* probe pTR29 detected a highly abundant 2100-nt transcript in addition to the 3100-nt transcript (Fig. 6). The deletion mutant A188(T2) also has the T-*urf13* region deleted and the genetic stock A188(T2)×W64A^2 also exhibited the 3100- and 2100-nt *orf221* transcript pattern (data not shown).

The major difference in *orf221* transcript patterns between A188(T7) and A188(T7)×W64A(N)^2 is in contrast to apparent identical T-*urf13*/*orf221* transcript patterns for A188(T), W64A(T), and other T cytoplasm genotypes. Selected nuclear backgrounds, however, can differentially confer the abundant 2300-nt *orf221* transcripts in N cytoplasm (Kennell et al. 1987; Rocheford et al. 1992). For example, A188(T) and A632(T) give identical T-*urf13*/*orf221* transcript patterns, in contrast to *orf221* transcript patterns in N cytoplasm versions, which may exhibit the abundant 2300-nt transcript [A632(N)] or show absence of the 2300- or 2100-nt transcript [A188(N] (Kennell et al. 1987; Rocheford et al. 1992). These observations indicate that the deletion of T-*urf13* from T cytoplasm to produce the T7 mutant resulted in a change to the mitochondrial genome which enabled it to respond to the nuclear gene product(s) associated with W64A(N) that determine the presence of the highly abundant 2100-nt *orf221* transcript. This concept is consistent with the report of Fauron et al. (1990) which indicated that the V3 deletion mutant of T-*urf13* from a T cytoplasm progenitor has an N-like mitochondrial configuration. The possibility therefore exists that the T7 deletion mutant may also have an N-like configuration.

Clones 5′ to *orf221* (Fig. 1B) were used as probes in the Northern analysis of different cytoplasm stocks to characterize transcription in the region 5′ to *orf221*. Northern analysis with clone pTR28 identified transcripts of 3400, 2300, and 1800 nt in N cytoplasm stocks 187-2, Mo17, and C103 (Fig. 7A) but did not identify the 1600-nt transcript shown by T-a106 in the same stocks (Fig. 2A). Similarly, analysis of Wf9(C), A619(C), B37(C), Wf9(S) and A619(S) with pTR28 did not identify the 1300-nt transcript (Fig. 7A) identified with T-a106 (Fig. 2A). This finding suggests the 1600-nt transcript in N cytoplasm and the 1300-nt transcript in C and S cytoplasms may have 5′ termini that are internal to *orf221* since the pTR28 probe extends 86 bp into *orf221* and does not detect these transcripts in Northern analysis. In T cytoplasm, Kennell et al. (1987) identified a 1100-nt T-*urf13*/*orf221* transcript with a 5′ terminus internal to *orf221*. Northern analysis with probe pTR30, which is 5′ to pTR28 (Fig. 1B), detected the same transcripts as pTR28 for the above N, C and S cytoplasm stocks (data not shown). Northern analysis with probe pTR27 gave only a 3400-nt transcript for 187-2(N) and Wf9(N) (Fig. 7B). This suggests that the 5′ termini of the 2300- and 1800-nt *orf221* transcripts in N cytoplasm are in the DNA region 5′ to *orf221* covered by clone pTR30 (Fig. 2B). This result is consistent with the finding of Wang et al. (1991) who probed 5′ to *orf221* and performed S1 nuclease mapping experiments which indicated that there were two different 5′ termini for the 3400- and 2300-nt transcripts and placed the 5′ terminus of the 2300 *orf221* transcript in the region encompassed by pTR30.

Since T-*urf13*/*orf221* transcriptional patterns are complex and are influenced by the *atp6* promoter (Fig. 1A), it is possible that nuclear effects on *orf221* in the T-*urf13*/*orf221* configuration may be masked by the complex transcriptional pattern. Therefore a Northern analysis was performed on T cytoplasm inbreds with pTR30 since it is 5′ to *orf221* in the N cytoplasm configuration and the 5′ terminus of the 2300 *orf221* transcript in N cytoplasm most likely occurs in the DNA region included in pTR30. However, pTR30 did not detect any mRNA transcription for the T cytoplasm inbreds (Fig. 7C), including 33-16 (T), whose N cytoplasm version exhibits the abundant 2300-nt *orf221* transcript (data not shown), and Wf9(T), whose N cytoplasm version does not exhibit the 2300-nt transcript. This observation indicates that in the T configuration there is little or no transcription in the homologous region that is 5′ to *orf221* in the N cytoplasm configuration. On the same Northern blot N cytoplasm inbreds exhibited differences for *orf221* transcript pattern (Fig. 7C), including the presence of the 2300-nt *orf221* transcript for CO220(N) but not for Wf9(N) (Fig. 7C). Therefore, certain nuclear effects on *orf221* transcription which we have identified in N, C and S cytoplasm may not be able to occur in the T cytoplasm configuration. However, the detection of an abundant 2100-nt *orf221* transcript in the A188(T7)×W64A^2 stock (Fig. 6) suggests that deletion of T-*urf13* might have altered the mitochondrial genome into a configuration more like that of an N configuration and thus enabled a response to the nuclear genome effects that confer the presence of the abundant 2100-nt *orf221* transcript.

Variation in maize mitochondrial transcript size has been attributed to mRNA processing, multiple transcription initiation sites, or multiple transcription termination sites (Mulligan et al. 1988a, b). The results in the present paper add to the growing body of evidence consistent with the occurrence of nuclear genotype specific effects that may influence one or more of these mechanisms. We have

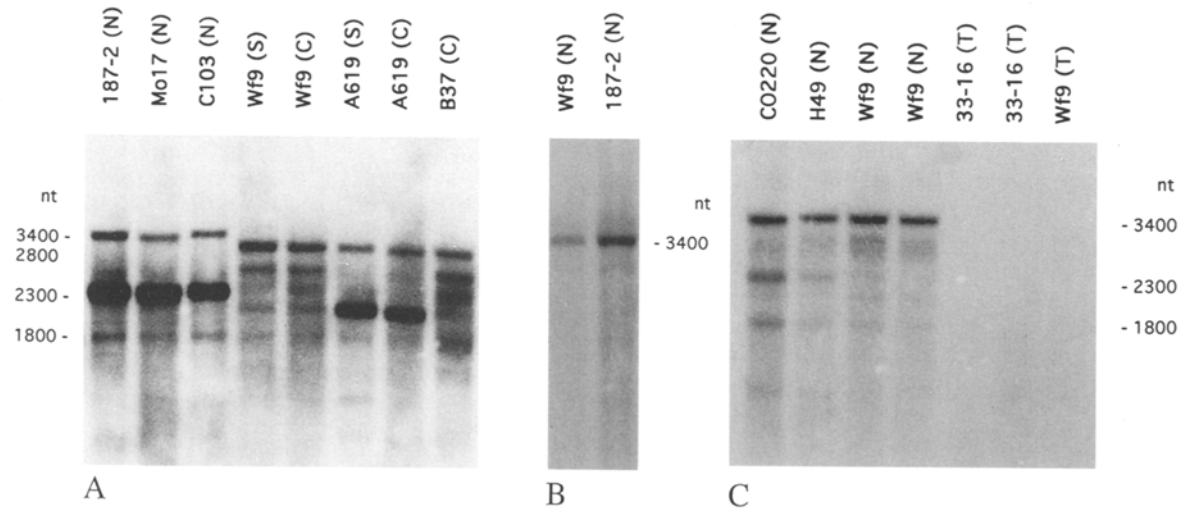


Fig. 7A Northern analysis of maize inbreds associated with N, C and S cytoplasms with probe pTR28. **B** Northern analysis of two N cytoplasm inbreds with probe pTR27. **C** Northern analysis of maize inbreds associated with N cytoplasm and T cytoplasm with probe pTR30

detected alterations in *orf221* transcripts where the responsible mechanism may be the nuclear control of mRNA processing. For example, the 1250-nt transcript detected in the F_1 progeny of B37(C)×Ky21(N), but which was not present in B37(C) (Fig. 5B), may be a result of mRNA processing of a transcript larger than 1250 nt. Similar explanations could be given for the observation of a 2800-nt transcript in B37(N) that is absent or reduced in abundance in the F_1 progeny of B37(N)×187-2 (N) which also displays a 2300-nt *orf221* transcript not present in B37(N). Although these observations are consistent with mRNA processing, possible nuclear control of initiation of these transcripts can not be ruled out.

We repeatedly detected the presence of multiple *orf221* transcripts in a variety of nuclear-cytoplasm stocks which suggests that there may be more than one *orf221* transcription initiation site. Multiple transcription initiation sites have been identified for the maize mitochondrial genes T-*urf13* (Kennell and Pring 1989), *coxIII* and *atp9* (Mulligan et al. 1988b). Our Northern analyses in the region 5′ to *orf221* revealed that the 5′ terminus region for the 3400-nt transcript is different from that of the 2300-nt *orf221* transcript. One or both of these higher-molecular-weight transcripts may be initiated. For most maize N cytoplasm inbreds the largest *orf221* transcript is 3400 nt. For A188(N) the highest-molecular-weight transcript is 3100 nt and the 3400-nt transcript is not present. However, the F_1 progeny of A188(N)×187-2(N) can exhibit both the 3400-nt and 3100-nt transcripts. The presence of the largest transcript (3400 nt) in the F_1 of A188(N)×187-2(N) may be due to nuclear control of the initiation of this transcript. Alternatively, nuclear control of mRNA processing, which results in the presence of both the 3400- and 3100-nt transcripts, is an alternative explanation.

The genetic analyses in this study suggest that different nuclear loci can alter the *orf221* transcript pattern. In some cases, the effect of these nuclear genes appears very specific to different mitochondrial configurations. We have identified different nuclear genotypes associated with the presence, or relative abundance, of the following *orf221* transcripts: 3400 nt in N cytoplasm, 2800 nt in N, C and S cytoplasms, 2300 or 2100 nt in N, C, or S cytoplasms and 1250 nt in N and C cytoplasms. Additionally, the presence of a 1100-nt *orf221* transcript in T cytoplasm has been associated with different nuclear genotypes (Kennell, et al. 1987). Collectively, our results suggest there is extensive nuclear-controlled alteration of *orf221* transcripts. The *orf221* transcription pattern presently appears to be more extensively affected by nuclear genotype than other plant mitochondrial genes including T-*urf13* (Dewey et al. 1986; Kennell et al. 1987; Rocheford et al. 1992), *atp6* (Makaroff and Palmer 1988; Siculella and Palmer 1988) and *cox2* (Cooper et al. 1990). There is no clear explanation for the extensive variation in *orf221* transcription.*orf221* is in a mitochondrial region that has undergone novel recombinations to generate the *atp6* promoter/T-*urf13*/*orf221* configuration (Dewey et al. 1986) and also novel recombination events during tissue culture to delete the T-*urf13* region (Wise et al. 1987; Fauron et al. 1990). If *orf221* is in a region of the mitochondrial genome that frequently undergoes recombination, different mitochondrial genome configurations surrounding *orf221* may have coevolved with different nuclear alleles that influence *orf221* transcription in different mitochondrial genome configurations.

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