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Genetic analysis of nuclear control of T-*urf13*/*orf221* transcription in T cytoplasm maize

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Summary. The mitochondrial gene T-*urf13* in T cytoplasm maize is associated with sensitivity to disease toxins and with cytoplasmic male sterility. T-*urf13* is co-transcribed with an open reading frame designated *orf221*. We have detected alterations in the transcription of the T-*urf13*/*orf221* region that are affected by nuclear genotype. There are multiple mRNA transcripts generated from the T-*urf13*/*orf221* region, one of which is a processed 1538-nucleotide (nt) transcript. This 1538-nt transcript is present in Wf9 (T), but was not found in mitochondrial RNAs (mtRNAs) from maize inbreds B14A (T) and 33-16 (T). For B14A (T) a 1500-nt transcript was detected and for 33-16 (T) a 1400-nt transcript was detected. In F1 progeny of the cross of Wf9 (T) × 33-16 (N), only the 1400-nt transcript was present. Genetic analyses revealed this processing event is nuclear controlled with dominant gene action and is independent of nuclear restorer gene *Rf1*-associated processing events. T-*urf13*/*orf221* transcriptional patterns were shown to vary in both sterile and fertile states. Segregation analysis of a 1100-nt *orf221*-specific transcript indicated that the genetic basis of nuclear control for the presence of this transcript was relatively simple. Analysis of the A188 (T4) tissue culture mutant, which has reverted to male fertility but displays the same T-*urf13*/*orf221* transcript pattern as A188 (T), indicated no DNA sequence differences between T4-*orf221* and T-*orf221*. Presence of the nuclear gene *Rf2* was not necessary for expression of the T4 cytoplasm-associated male-fertile phenotype.

Key words: Nuclear – mitochondrial interactions – Cytoplasmic male sterility – Maize – Mitochondrial gene transcription

Introduction

Cytoplasmic male sterility (cms) of T cytoplasm maize (*Zea mays* L.) is associated with a T cytoplasm-specific mitochondrial gene, T-*urf13*, which is co-transcribed with an open reading frame designated *orf221* (Dewey et al. 1986). The T cytoplasm also confers unusual disease susceptibility to two fungal pathogens and their host-specific toxins (HmT and Pm), as well as sensitivity to the insecticide methomyl (see review by Pring and Lonsdale 1989). A relationship of T-*urf13* to cms and HmT toxin sensitivity was shown by the deletion or truncation of this gene and retention of *orf221* in tissue culture-derived mutants that were male-fertile and toxin-resistant (Rottman et al. 1987; Wise et al. 1987a). T-*urf13* encodes a 13 kDa polypeptide (Wise et al. 1987b), designated URF13, that is membrane-bound (Dewey et al. 1987). When T-*urf13* is expressed in *E. coli*, exposure to either HmT-toxin or methomyl causes spheroplast swelling and inhibits bacterial respiration (Dewey et al. 1988, Braun et al. 1989). Similarly, expression of T-*urf13* in *Saccharomyces cerevisiae* results in sensitivity to HmT-toxin and methomyl (Huang et al. 1990, Glab et al. 1990). Collectively, these results strongly suggest that T-URF13 is responsible for HmT-toxin sensitivity in *E. coli*, *S. cerevisiae* and cms-T maize.

T-*urf13* presumably arose through a series of unique recombination events within the mitochondrial genome (Dewey et al. 1986). T-*urf13* contains 345 bp with significant sequence similarity to mitochondrial DNA (mtD-

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NA) located within and 3′ to *rrn26*; 263 bp have 87% similarity to the 3′ flanking region of the *rrn26* gene and 58 bp of nearly perfect similarity to an internal *rrn26* sequence. Another mitochondrial recombinational event resulted in duplication of a 5-kb region flanking the *atp6* gene. The 3′ edge of this 5-kb repeated region is 5′ to T-*urf13* (Dewey et al. 1986) and sequences within this 5-kb region promote transcription of T-*urf13* and *orf221* (Kennell and Pring 1989).

The male-sterile phenotype associated with T cytoplasm is converted to normal microsporogenesis and pollen development in the presence of the nuclear fertility-restoration genes *Rf1* and *Rf2* (see review by Laughnan and Gabay-Laughnan 1983). Dewey et al. (1986, 1987) established that fertility restoration of T cytoplasm maize, or the presence of just *Rf1*, results in the appearance of a unique 1600-nt T-*urf13* transcript. This 1600-nt transcript was shown to be processed, with the 5′ terminus at position +10, in T-*urf13* (Kennell and Pring 1989). Although this processing event is associated with only a slight reduction in the abundance of three mature transcripts (Dewey et al. 1987; Kennell and Pring 1989), a considerable reduction of the 13-kDa T-*urf13* gene product occurs when it is associated with *Rf1* (Dewey et al. 1987). No molecular effects of *Rf2* on fertility restoration have been documented to-date.

Quantitative and qualitative variation in the T-*urf13*/*orf221* transcriptional pattern was observed among five maize inbreds (Kennell et al. 1987). The most notable observation was the presence or absence of a novel 1100-nt *orf221*-specific transcript. Evaluation of mitochondrial RNA (mtRNA) from F1 progeny of a cross involving two of these five maize inbreds revealed that the presence of the 1100-nt transcript was under nuclear control (Kennell et al. 1987). These observations, and the report of differences among T cytoplasm maize inbreds for sensitivity to T-toxin (Danko, Gengenbach and Daly, personal communication), led us to further investigate nuclear effects on the transcription of T-*urf13*/*orf221*.

We included the A188 (T4) tissue-culture mutant of T cytoplasm maize which has reverted to fertility (Umbeck and Gengenbach 1983). The A188 (T4) mutant has a 5-bp insertion that causes a frameshift in the progenitor T-*urf13* sequence (Wise et al. 1987a). This results in truncation of the open reading frame to 222 bp and loss of the 13-kDa gene product (Wise et al. 1987b). The tissue culture-induced alteration of the A188 T-*urf13* DNA sequence prompted us to investigate whether tissue culture had caused a similar alteration in the adjacent and co-transcribed *orf221* DNA sequence. We also performed genetic experiments to determine whether *Rf2*, present in A188 (T4) plants, is necessary for the fertile phenotype associated with the T4-mutant cytoplasm.

Table 1. Maize genetic stocks evaluated

Inbred	Phenotype	*Rf* genotype	Source
A188 (T)	Sterile	*rf1rf1Rf2Rf2*	B. Gengenbach
A188 (T4)	Fertile	*rf1rf1Rf2Rf2*	B. Gengenbach
A188 (T7)	Fertile	*rf1rf1Rf2Rf2*	B. Gengenbach
A619 (T)	Restored	*Rf1Rf1Rf2Rf2*	W. Pederson
A632 (T)	Restored	*Rf1Rf1Rf2Rf2*	P. Chourey
B14A (T)	Sterile	*rf1rf1* –	W. Pederson
B73 (T)	Sterile	*rf1rf1* –	M. Albertsen
C.I.64A (T)	Sterile	*rf1rf1* –	W. Pederson
Ky21 (T)	Restored	*Rf1Rf1Rf2Rf2*	E. Patterson
Mo17 (T)	Sterile	*rf1rf1* –	W. Pederson
N6D (T)	Restored	*Rf1Rf1Rf2Rf2*	W. Pederson
R177 (T)	Restored	*Rf1Rf1Rf2Rf2*	W. Pederson
R213 (T)	Sterile	*Rf1Rf1rf2rf2*	E. Patterson
Tx29 (T)	Restored	*Rf1Rf1Rf2Rf2*	W. Pederson
W182BN (T)	Sterile	*rf1rf1* –	M. Smith
Wf9 (T)	Sterile	*rf1rf1rf2rf2*	M. Albertsen
Wf9 (T)	Restored	*Rf1Rf1Rf2Rf2*	B. Gengenbach
33-16 (T)	Sterile	*rf1rf1Rf2Rf2*	W. Pederson
R213 (N)	Fertile	*Rf1Rf1rf2rf2*	E. Patterson
Wf9 (N)	Fertile	*rf1rf1rf2rf2*	M. Albertsen
33-16 (N)	Fertile	*rf1rf1Rf2Rf2*	W. Pederson

Cross	F_1 phenotype
A188 (T) × R213 (N)	Restored
A188 (T) × Wf9 (N)	Sterile
33-16 (T) × R213 (N)	Restored
33-16 (T) × Wf9 (N)	Sterile
Wf9 (T) × R213 (N)	Sterile
Wf9 (T) × 33-16 (N)	Sterile

Materials and methods

Genetic materials

Table 1 lists the T cytoplasm maize inbreds evaluated. The restorer gene complements were either previously determined, previously determined and confirmed by our evaluations, or solely determined by our evaluations. Table 1 also lists F1 progenies developed for both the determination of restorer gene complements and for experimental analyses.

The genetic stocks used in the analysis for segregation of the 1100-nt *orf221* transcript were: P1 = A188 (T), does not exhibit the 1100-nt transcript; P2 = Wf9 (T) restored to fertility, exhibits the 1100-nt transcript; F1 = P1 × P2; $BC^1P1 = P1 \times F1$; $BC^2P1 = P1 \times BC^1P1$. Tissue for mtRNA isolation was sampled from individual young plants grown from kernels of one BC^1P1 ear and one BC^2P1 ear. Kernels from the same BC^1P1 ear sampled for mtRNA isolation were planted in the nursery and the resulting plants were used as the pollen source for the BC^2P1 ears produced.

The genetic stocks used for evaluating whether the presence of *Rf2* in A188 (T4) is required for expression of the fertile phenotype were: P1 = A188 (T4) (*rf1rf1Rf2Rf2*); P2 = Wf9 (N) (*rf1rf1rf2rf2*); F1 = P1 × P2; F2 = self of F1. The F1 progeny should have a *rf1rf1Rf2rf2* complement, and the corresponding F2 progeny should segregate 1:2:1 for *rf1rf1Rf2Rf2*: *rf1rf1Rf2rf2*: *rf1rf1rf2rf2*. Several ears of F1 seed of the cross of A188 (T4) × Wf9 (N) were produced. F1 seed from several different ears of the A188 (T4) × Wf9 (N) cross was planted and the

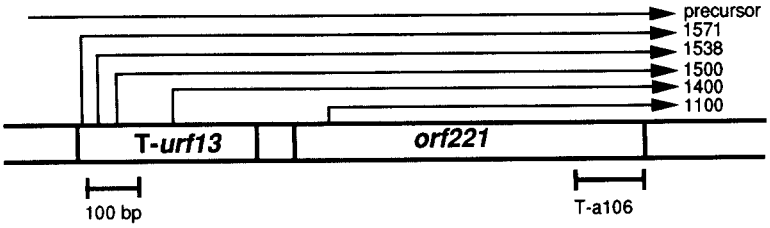

Fig. 1. Schematic representation of the maize T cytoplasm T-*urf13*/*orf221* region and transcripts detected with probe T-a106

resulting plants were self-pollinated. Two F2 generations, developed on F1 plants that descended from F1 seed on different A188 (T4) plants, were evaluated. F1 progeny of A188 (T7) × Wf9 (N) were developed, the F1 seed planted, and the resulting plants self-pollinated to develop F2 seed. A188 (T7) (*rf1rf1Rf2Rf2*) is a mutant that has lost T-*urf13* (Umbeck and Gengenbach 1983). The F2 generation involving the A188 (T7) mutant was used as a control with the expectation that all F2 progeny would express the fertile phenotype.

Field and greenhouse evaluations

Plants were visually evaluated for exertion of anthers, and tassels were shaken at the appropriate time of day to evaluate the pollen shed. Most evaluations were performed in field plots though some were performed in the greenhouse.

Two separate F2 generations developed from the cross A188 (T4) × Wf9 (N) were planted in the field in a randomized complete block design with two replicates of 120 plants for each F2 generation. One F2 generation of A188 (T7) × Wf9 (N) was planted in the field in a randomized complete block design with two replicates of 120 plants. Pollen shed was evaluated and tassels of fertile plants were cut off once pollen shed was detected.

Preparation and analysis of RNA

Mitochondrial RNA from etiolated coleoptiles or greenhouse-grown young plants was isolated as described (Wise et al. 1987a). For each sample, 10 μg of mtRNA was electrophoresed in 1.2% agarose gels using 10 mM sodium phosphate buffer. Blotting and hybridizations were performed essentially as described in Kennell et al. (1987). The probe used for Northern analyses was T-a106, a 140-bp clone at the 3′-terminus of T cytoplasm *orf221* (Fig. 1).

DNA sequencing

A series of contiguous clones from A188 (T4) mtDNA encompassing *orf221* (Wise et al. 1987a) were sequenced by double-stranded procedures (Boehringer Mannheim), or else were subcloned into mp18 and mp19 and sequenced by single-stranded procedures (Bethesda Research Laboratories).

Results

Northern analysis with *orf221* clone T-a106 (Fig. 1), detects up to seven transcripts from the T-*urf13*/*orf221* region (Figs. 2–5), which is consistend with previous reports (Dewey et al. 1986; Kennell et al. 1987; Kennell and Pring 1989). The largest transcript (3900-nt) is apparently initiated; smaller transcripts have also been mapped (Kennell and Pring 1989) and can be assigned maximum size based on sequence data (Dewey et al. 1986). The

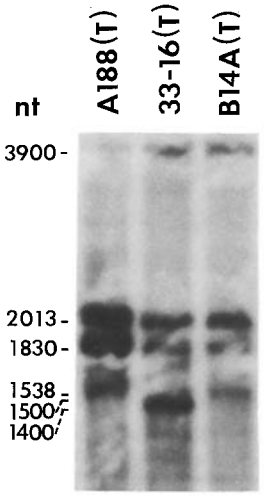

Fig. 2. Transcriptional patterns of T-*urf13*/*orf221* detected with the clone T-a106. Transcripts of inbreds A188 (T), 33-16 (T), and B14A (T) are compared. *Numbers* are transcript sizes in nucleotides

detectable transcripts include: a processed transcript of 2013-nt; an initiated transcript of 1830-nt; a processed transcript of 1785-nt (which is unresolved in most gels); processed transcripts of 1571 (*Rf1*-specific) and 1538-nt; and a 1100-nt *orf221* transcript (nuclear-genotype specific). The 2013-, 1830-, and 1785-nt species represent major, mature transcripts with 5′ termini that are 5′ to T-*urf13* (Kennell and Pring 1989). The stoichiometry of these three transcripts is influenced by nuclear background (Kennell et al. 1987) as well as by the *Rf1* gene (Dewey et al. 1986, Kennell and Pring 1989). Some of these transcriptional variations are apparent in our current analyses (Figs. 2–5).

Nuclear genotype effects on T-urf13/orf221 transcripts

A survey of 15 T cytoplasm maize inbreds (Table 1) with various pedigrees indicated that all inbreds except 33-16 and B14A were characterized by the presence of the 1538-nt T-*urf13*/*orf221* transcript (data not shown). The inbreds 33-16 and B14A did not exhibit the 1538-nt transcript characteristic of A188 (T), for example, but exhibited smaller transcripts of approximately 1400- and 1500-nt, respectively (Fig. 2).

A genetic experiment was performed to determine whether presence of the 1400-nt transcript for 33-16 (T)

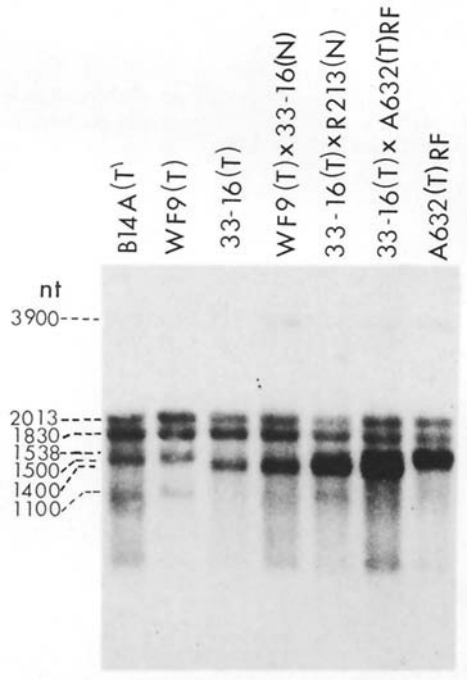

Fig. 3. T-*urf13/orf221* transcriptional patterns detected with the clone T-a106. Transcripts of B14A (T), Wf9 (T), 33-16 (T) are compared with WF9 (T) × 33-16 (N), 33-16 (T) × R213 (N), 33-16 (T) × A632 (T) Rf and A632 (T) Rf. Rf refers to an inbred restored to fertility. *Numbers* are transcript sizes in nucleotides

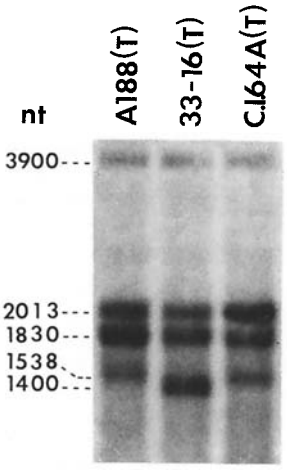

Fig. 4. Transcriptional patterns of T-*urf13/orf221* detected with clone T-a106. A188 (T), 33-16 (T) and C.I.64A (T) are compared. *Numbers* are transcript sizes in nucleotides

and absence of the 1538-nt transcript was under nuclear control or was due to mitochondrial genomic differences. Inbred 33-16 (N), the maintainer line of 33-16 (T), was crossed as male to Wf9 (T), which exhibits only the 1538-nt transcript. Northern analysis of mtRNA from the F1 progeny revealed that the 1538-nt transcript was not evident but that the novel 1400-nt transcript was present (Fig. 3). This indicates that the presence of the 1400-nt transcript is under nuclear control and is associated with disappearance of the 1538-nt transcript.

T-urf13/orf221 transcript interactions

We also investigated the relationship of the T-*urf13/orf221* processing event, associated with *Rf1*, which reduces the abundance of the 2013-nt and 1830-nt transcripts and generates the 1571-nt transcript, as well as the event associated with presence of the novel 1400-nt transcript. We crossed inbred R213(N) (*Rf1Rf1rf2rf2*) as male to 33-16 (T) (*rf1rf1Rf2Rf2*). The T-*urf13/orf221* transcriptional pattern of the F1 progeny displays the *Rf1*-associated reduction in abundance of the 2013-nt and 1830-nt transcripts (Fig. 3). The T-*urf13/orf221* transcriptional pattern of the F1 progeny also exhibited mRNAs (or an mRNA) that encompass the 1400–1571-nt size range (Fig. 3). The two transcripts (or a single transcript) are presumably the 1400-nt transcript associated with 33-16 and the 1571-nt transcript associated with *Rf1*. These two transcripts cannot be resolved by our Northern analyses. The processing event associated with *Rf1*, which reduces the abundance of the 2013-nt and 1830-nt transcripts (and also generates the 1571-nt transcript), appears to be independent of the putative processing event generating the novel 1400-nt transcript, since both events are detected in the F1 progeny.

The novel 1400-nt transcript was present in the F1 progeny of the cross Wf9 (T) × 33-16 (N), as well as the F1 progeny of the cross 33-16 (T) × R213 (N) (Fig. 3). As indicated, Wf9 (T) displays the 1538-nt transcript. R213 (N) is the maintainer line of R213 (T) (which exhibits the 1538-nt transcript but not the 1400-nt transcript). The detection of the 1400-nt transcript in both of these F1 progenies suggests that gene action is dominant for the presence of this transcript.

The generation of the 1400-nt T-*urf13/orf221* transcript may be associated with a reduction in the abundance of the 2013-nt T-*urf13/orf221* transcript. Visual comparison of the abundance of the 1400-nt transcript in 33-16 (T) with the abundance of the 1538-nt transcript in Wf9 (T) revealed a higher level of the 1400-nt transcript (Fig. 3). For these same samples, a visual comparison of the abundance of the 2013-nt transcript in 33-16 (T) and the abundance of the 2013-nt transcript in Wf9 (T) revealed a lower level for the former. This observation was repeated with a different 33-16 (T) mitochondrial mRNA preparation and a comparison made with other maize inbreds (Fig. 4). In this evaluation, 33-16 (T) exhibits a 1400-nt transcript that is of higher abundance than that of the 1538-nt transcript of A188 (T) and C.I.64A (T), whereas the 2013-nt transcript of 33-16 (T) appears to be of lower abundance than that of the 2013-nt transcript of A188 (T) or C.I.64A (T) (Fig. 4). This suggests that the

2013-nt transcript may be processed to generate the 1400-nt transcript.

Relevant male fertility information was gained by evaluating the pollen shed, and the corresponding T-*urf13*/*orf221* transcript patterns, for some of the genetic stocks in this study. Inbred 33-16 (T) (male-sterile), the F1 progeny of 33-16 (T) × R213 (N) (male-fertile) and 33-16 (T) × A632 (T) restored to fertility (male-fertile), all exhibit the 1400-nt transcript that was not previously associated with T cytoplasm stocks in either a sterile [i.e., Wf9 (T)] or a fertile [i.e., A632 (T) restored to fertility] condition (Fig. 3). Therefore, T-*urf13*/*orf221* transcriptional patterns can be variable in both sterile and fertile states.

The F1 progeny of 33-16 (T) × R213 (N) exhibited a very low abundance 1100-nt transcript that is not present in 33-16 (T) (Fig. 3). The 1100-nt transcript can, therefore, be present in association with the 1400-nt transcript as well as the 1538-nt transcript. This observation supports the independent genetic control of the events generating the different transcripts. Interestingly, we did not detect the 1100-nt transcript in the F1 progeny of Wf9 (T) × 33-16(N) (Fig. 3).

Surveys of the T-*urf13*/*orf221* transcriptional patterns of several maize inbreds (data not shown) indicated that R213 (T) and Tx29 (T) also display the 1100-nt *orf221*-specific transcript detected previously in Wf9 (T) and W64A (T) (Kennell et al. 1987). Tx29 has a very different pedigree from Wf9 and the Wf9 derivatives R213 and W64A. Therefore, the gene(s) controlling the generation of the 1100-nt *orf221* transcript is present in at least one maize inbred that is not closely related to Wf9.

Segregation of the 1100-nt orf221 transcript in backcross generations

Evaluation of backcross generations that were derived from the cross of A188 (T) (*rf1rf1Rf2Rf2*) as female parent (P1) and Wf9 (T) restored to fertility (*Rf1Rf1Rf2Rf2*) as male parent (P2) revealed segregation for the presence/absence of the 1100-nt transcript. A188 (T) does not exhibit this transcript whereas Wf9 (T) restored to fertility does (Fig. 5). For progenies sampled from the BC^1P1 generation, this transcript was present in 24 plants and absent in seven plants. This segregation exhibited a poor fit to a 1:1 ratio ($\chi^2 = 8.24$, $0.005 > P > 0.001$), which suggests that more than one nuclear locus may control the presence of the 1100-nt transcript. A better fit to a 3:1 ratio for this segregation was observed ($\chi^2 = 0.096$, $0.80 > P > 0.70$), suggesting two independent dominant nuclear loci may be involved. Evaluation of samples from one BC^2P1 generation revealed the presence of the transcript in five plants and its absence in 11. This segregation demonstrated a better fit to a 1:1 ($\chi^2 = 1.52$, $0.30 > P > 0.20$) than to a 3:1 ($\chi^2 = 14.08$, $0.001 > P$) ratio. However, the sample size for the BC^2P1 is small, which limits interpretation, and the fit to a 1:1 ratio is not very strong. The observed BC^2P1 ratio is consistent with a relatively simple genetic control of this processing event. A single dominant gene responsible for mRNA processing may be segregating in this particular BC^2P1 generation, which is consistent with the possibility of two dominant genes segregating in the BC^1P1 generation.

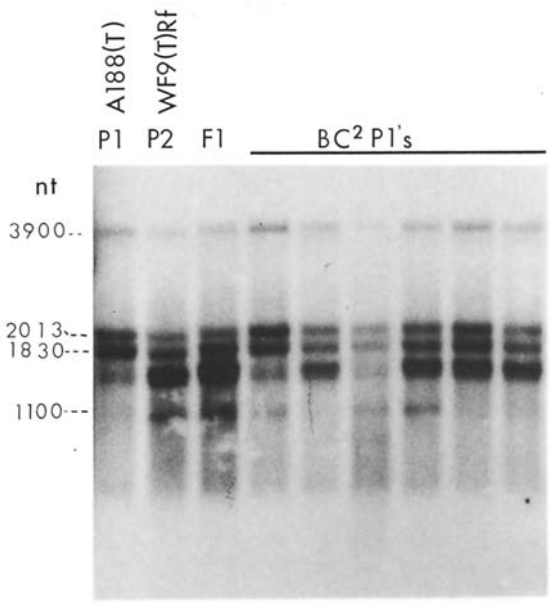

Fig. 5. Transcriptional patterns of *T-urf13/orf221* in A188 (T) – parent one (P1), Wf9 (T) RF – parent two (P2), the F1 of P1 × P2, and six BC^2 P1s detected with probe T-a106. Rf refers to an inbred restored to fertility. *Numbers* are transcript sizes in nucleotides

The independence of the 1100-nt *orf221* transcript and *Rf2*, suggested by Kennell et al. (1987), is confirmed by the segregation of the 1100-nt transcript in the BC^2P1 backcross progenies, which are all *Rf2Rf2* (Fig. 5). The independence of the *Rf1*-associated effect that generates the 1571-nt transcript and the events that generate the 1100-nt transcript (Kennell et al. 1987) was confirmed by our observation of the presence or the absence of this transcript in backcross progenies that exhibit, as well as those that do not exhibit, the *Rf1*-associated processing effects that generate the 1571-nt T-*urf13*/*orf221* transcript and the reduction in the 2013-nt and 1830-nt transcripts (data not shown).

A188 (T4) orf221 DNA sequence

The A188 (T4) mutant does not display any T-*urf13*/*orf221* transcriptional differences from A188 (T) (Wise et al. 1987a). Maize mitochondrial *orf221* has previously not been sequenced in T cytoplasm tissue-culture mu-

tants beyond 88 bp extending into the open reading frame of the V3 deletion mutant (Rottmann et al. 1987). Our sequencing analysis of the entire coding region of A188 (T4) *orf221* revealed no variation from the *orf221* T cytoplasm sequence of Stamper et al. (1987). Therefore, the DNA composition of T4-*orf221*, in contrast to that of T4-*urf13*, did not undergo nucleotide changes associated with tissue culture and reversion to male fertility.

Evaluation of T4 cytoplasm stocks segregating for Rf2 and rf2

All plants scored from the F2 generation developed from the cross of A188 (T4) (*rf1rf1Rf2Rf2*) × Wf9 (N) (*rf1rf1rf2rf2*) were fertile. A 3:1 ratio for normal pollen development/male sterility would be observed if *Rf2* was necessary for normal pollen development. The detection of normal pollen development in all F2 plants indicates that the presence of the dominant restorer gene *Rf2* is not required for expression of the fertile phenotype of maize plants with a T4 mutant cytoplasm. All plants sampled from the control F2 generation developed from the F1 progeny of the cross of A188 (T7) (*rf1rf1Rf2Rf2*) × Wf9 (N) (*rf1rf1rf2rf2*) exhibited normal pollen development, as expected.

Discussion

The combined results of this study and previous studies have identified four nuclear-controlled events that affect the abundance and size of T-*urf13*/*orf221* transcripts. These events, which we assume involve mRNA processing, are: *Rf1*-specific processing resulting in the 1571-nt transcript; processing associated with nuclear genotype B14A resulting in an approximately 1500-nt transcript; processing associated with nuclear genotype 33-16 resulting in an approximately 1400-nt transcript; and the nuclear genotype-specific processing resulting in an approximately 1100-nt *orf221* transcript. Additionally, since the 1538-nt species, which is a processed transcript (Kennell and Pring 1989), is not detected in all T cytoplasm maize inbreds (absent in B14A and 33-16), maturation of this transcript also appears to be under nuclear control. The genetic analyses involving some of these events indicated that they were under independent genetic control in relation to one another.

Our detection of novel transcripts associated with different maize inbreds suggests an inordinate amount of variation in the nuclear regulation of T-*urf13*/*orf221* transcript processing. The novel transcript patterns observed in our studies were revealed by the evaluation of only 15 maize inbreds. Although several of these were intentionally selected because of their diverse pedigrees, the inbreds evaluated are primarily limited to U.S. Corn Belt germplasm. Consequently, our survey encompasses only a relatively narrow sample of maize germplasm overal. Additionally, our detection of the 1100-nt *orf221*-specific transcript in Tx29, which is not related to Wf9, reveals that the nuclear gene(s) responsible for this event are not unique to Wf9-type material.

Dewey et al. (1986, 1987) provided the first evidence that the nuclear genome can influence transcription of plant mitochondrial genes, specifically that the presence of the nuclear restorer gene *Rf1* alters transcription of the cms-associated gene T-*urf13*. Nuclear effects on mitochondrial transcription have been shown in the male-sterile *Ogura* radish cytoplasm (Makaroff and Palmer 1988) and of cms89 sunflower (Sicullela and Palmer 1988). These investigators observed the disappearance of large *atpA* transcripts, presumably due to differential mRNA processing, when different nuclear genotypes were crossed onto the male-sterile lines. Cooper et al. (1990) reported alterations in mitochondrial gene *cox2* transcripts when teosinte cytoplasms became associated with certain maize nuclear genotypes. Genetic analyses revealed that a single dominant nuclear gene, *Mct*, is responsible for the *cox2* transcript differences.

The preponderance of the processing of mature transcripts within the T-*urf13*/*orf221* coding region raises two considerations: why events which reduce the abundance or size of mature mitochondrial transcripts occur, and why different nuclear genotypes are associated with processing events at different mitochondrial mRNA locations. These considerations suggest that the nuclear genome can encode gene products that recognize and cleave specific mitochondrial mRNA sequences. The genetic basis for the control of some of these processing events is still not clear. For example, events generating the 1538-nt or 1400-nt T-*urf13* transcripts may be controlled by allelic variants of a single locus. Alternatively, these events may be controlled by independent loci. In either case, putative gene products associated with the maize inbred 33-16, for example, appear dominant or epistatic in action by virtue of the observation that the associated mRNA-processing activity results in the smaller mRNA size class. This activity precludes the detection of processing events that generate the larger mRNA size class.

Sequences internal to T-*urf13* which are the sites of four nuclear genotype-specific processing events are highly similar to sequences 3′ to *rrn26* (Dewey et al. 1986). Recent data on the maturation of *rrn26* in B37 (N) maize mitochondria suggests a relationship to the T-*urf13* processing. Maloney et al. (1989) identified two apparent precursor RNA molecules, of 4000-nt and 3750-nt, and a presumably mature 3550-nt species, which could result from 3′ processing. The apparent longest, initiated transcript, 4000-nt, was processed 5′ to yield a 3750-nt transcript with a mature 5′-terminus. Based on

these data and on determinations of the major 3′-terminus (Kennell and Prig 1989), the size of the mature *rrn26* can be calculated as 3544-nt. A presumed processing site approximately 200-nt 3′ to the major 3′ *rrn26* terminus occurs within T-*urf13* sequences. If a 3′ *rrn26* processing step occurs in this region, it is possible that similar activity may be imposed on T-*urf13* sequences, resulting in transcripts with 5′-termini within the gene.

Our observations are pertinent to T-*urf13* expression and disease toxin resistance, since the gene product is strongly implicated in toxin susceptibility (Dewey et al. 1988; Braun et al. 1989; Glab et al. 1990; Huang et al. 1990). Processing events which generate the 1571-nt, 1538-nt, 1500-nt, and 1400-nt transcripts presumably reduce the abundance of the mature 2013-nt, 1830-nt, and 1785-nt transcripts. It is not known whether these events are related to T-toxin sensitivity. Since genotypic differences for toxin sensitivity have been detected (Danko, Gengenbach and Daly, personal communication), it may be worthwhile to investigate whether there are T-*urf13*/*orf221* transcriptional differences among a set of genotypes differing for toxin sensitivity and to evaluate the toxin sensitivity of 33-16 (T) and B14A (T).

The analyses involving the T4 mutant provide data that further support mutation in the T-*urf13* coding region as the basis for reversion to a fertile phenotype. Sequence analysis of T4-*orf221* revealed no nucleotide composition differences from the T-*orf221* sequence. This eliminates a DNA mutation in *orf221* as a factor in the reversion to fertility in A188 (T4). Presence of the *Rf2* gene does not appear to be necessary for expression of the fertile phenotype associated with the mutant T4 cytoplasm. This lends further support to the hypothesis that the TURF13 mutation in A188 (T4) is alone responsible for reversion to fertility since the T4 cytoplasm is associated with fertility even in the absence of both *Rf1* and *Rf2* restorer genes.

In contrast to T cytoplasm that has been restored to fertility, the T4 mutant which has reverted to fertility exhibits a transcriptional pattern that is essentially the same as male-sterile T-*urf13*/*orf221* transcriptional patterns. (Wise et al. 1987a). Although we present no further evidence that suggests *orf221* is involved in male sterility, a possible role has not been eliminated. Since *orf221* is only 80 bp 3′ to T-*urf13* or T4-*urf8.3*, the possibility exists that translation or expression of T-*urf13* or T4-*urf8.3* may affect the expression of *orf221*.

The results in this paper provide further evidence for the complexity of nuclear-mitochondrial interactions. Our results also illustrate that an alteration of a transcript from the cms-associated T-*urf13* region is under nuclear control but that it is independent of fertility restoration. T-*urf13* transcripts are subject to nucleus-dependent processing events that collectively appear to be more extensive than has been documented for other higher plant mitochondrial genes. The observation of various nuclear genotype-associated differences in the transcription of the mitochondrial gene *T-urf13* indicates the need for, and importance of, considering and specifying the associated nuclear genotype in mitochondrial gene expression studies.

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