The yeast ROAM mutation – identification of the sequences mediating host gene activation and cell-type control in the yeast retrotransposon, Ty

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ABSTRACT

When the yeast retrotransposon, Ty, integrates into the 5' flanking region of a gene it can activate the expression of that gene. At the same time the activated gene is brought under cell-type specific control such that expression is high in haploid a or α cells but low in a/α diploids. These Ty mediated mutations are known as ROAM mutations. In this study we have used a ROAM mutation created in vitro to identify the sequences within Ty that mediate this phenomenon. We show that a single activator located within the coding region of the Ty element is responsible for ROAM activation. This sequence, which is regulated by the mating type of the cell, differs from classical enhancer elements in that its activity is strictly orientation dependent. An independent activator located downstream of the ROAM sequence activated transcription only in the non-ROAM orientation. This sequence may be part of an internal promoter that controls expression of the sub-genomic 5.0kb Ty transcript.

INTRODUCTION

Ty elements are a family of dispersed transposable elements found in the yeast Sacharomyces cerevisiae which are closely related to the retroviral proviruses of higher eukaryotes in both structure and expression strategies (1,2,3). A typical element is about 5.9kb long and consists of long terminal repeats (called delta sequences) of 340 base pairs that flank an internal 5.2kb 'epsilon' region (4). The major transcript is a 5.7kb RNA that starts in the 5' LTR and finishes in the 3' LTR such that it has a terminal redundancy of about 50 nucleotides (5,6). This RNA encodes two overlapping genes, TYA which is functionally analogous to the retroviral gag protein (7), and TYB. TYB is expressed by a retroviral-like frameshift mechanism (8) and encodes proteins with homology to retroviral reverse transcriptase, integrase and acid protease (9,10). In addition to these organisational similarities it has recently been shown that

Ty transposes through an RNA intermediate (12) and produces virus-like particles (Ty-VLPs) which contain Ty-encoded proteins, reverse transcriptase and RNA (13,14). A sub-genomic RNA of 5.0kb that initiates some 1150bp into the element has been described (11, Figure 5B) but its function and control remain unclear.

Ty also shares with retroviruses an ability to activate cellular genes (1,15). This may take the form of overexpression (15) or constitutive expression of otherwise regulated genes (16). In all cases the affected gene is simultaneously brought under control of yeast cell type. Expression is high in haploid a or α cells but low in a/α diploids (17). These Ty-mediated mutations are known as ROAM (Regulated Overproducing Alleles responding to Mating type) mutations. Although this activation is in many ways similar to retroviral activation of cellular oncogenes (18) the spatial relationship between Ty and the activated gene is rather more restricted. Whereas retroviral enhancers are generally position and orientation independent (19) a ROAM mutation always results from integration of Ty upstream of a gene and in such a manner that Ty transcription is away from the activated gene (1). Ty-mediated activation of cellular genes is therefore strictly position and orientation dependent. It is also found that the size and initiation site of the overexpressed RNA are unchanged (20). This means that activation is not simply a consequence of transcription from a Ty promoter and corresponds only to the category III class of retrovirus activation as defined by Payne *et. al.* (18).

We have undertaken an analysis of a ROAM mutation constructed *in vitro* to identify the Ty sequences responsible for this phenomenon. We show that Ty activation is mediated by a single activating sequence that is located within the TYA open reading frame, just downstream from the 5' delta. This activator is strictly orientation dependent and functions only in the ROAM orientation. Furthermore it is closely associated with a sequence mediating cell-type regulation. We have also identified a second less powerful activator located within the TYA gene. This sequence cannot be involved in ROAM-type activation because it functions only in the non-ROAM orientation. It may be part of an internal 'promoter' that controls the expression of the 5.0kb transcript.

MATERIALS AND METHODS

Strains and Media

Strains used were *Escherichia coli* AKEC28 (C600, thrC, leuB6, thyA, trpC1117, hsdR_k, hsdM_k) and *Saccharomyces cerevisiae* MD40/4c (MATx, ura2, trp1, leu2.3, leu2.112, his3.11, his3.15), AH22 (MATa, leu2.3, leu2.112, his4), SF-1 (MATa/ α , leu2.3, leu2.112) and MD40/4c (Diploid) (MAT α / α , ura2, trp1, leu2.3, leu2.112, his3.11, his3.15). Strain MD40/4c (Diploid) was a generous gift from Dr. M.F. Tuite, University of Kent. *E. coli* cultures were grown in Luria broth (21) or selectively in Luria broth plus Ampicillin (Sigma, 30 μ g/ml). Yeast cultures were grown at 30°C in YEPD or, for RNA analysis, in defined minimal medium (22) plus appropriate amino acids. Diploid yeast transformants were sporulated on Vogel's medium (23) and haploid segregants were selected as Leu⁺, His⁻ colonies.

Plasmid Constructions

The molecules used in this analysis were derivatives of the plasmid pMA27 (Figure 1B (i), 24). This is a pBR322 derivative containing a LEU2/2 μ origin selection and replication module from pJDB219 (25) and a 2.95kb HindIII fragment which carries the yeast PGK gene. The PGK UAS in pMA27 was removed by bidirectional BAL31 deletion and replaced with a BamHI restriction site to generate pMA766 (Figure 1B, (ii)) in which expression of PGK is substantially reduced (26). This plasmid, which can be used to screen for potential yeast activator sequences, is referred to here as pMA27AT (Activator Trap). Both pMA27 and pMA27AT transform yeast at a high frequency and are stably maintained at about 100 copies/cell (24). Fragments of Ty were inserted into the BamHI site of pMA27AT in both orientations. The 'r' orientation corresponds to the orientation of Ty in a ROAM mutation while the 'non-ROAM' orientation is designated 'n'.

Figures 1A and 3A show the Ty1-15 fragments cloned into pMA27AT as part of this analysis. Fragments 11 and 35 were obtained from molecules in which the Ty XhoI site had been converted to BamHI. Blunt ended fragments (20-24) and fragment 35 were initially inserted into HincII or HincII/BamHI cut pSp4-6 (26) a derivative of pSp6-4 (27) in which the HindIII restriction site has been converted to BglII. Fragments were subsequently excised from this vector by BamHI/BglII digestion and cloned into

the BamHI site of pMA27AT. Fragment 31 was cloned into pMA27AT as a BglIII fragment following conversion of the flanking XhoI and PvuII restriction sites to BglIII by digestion, end-filling and ligation in the presence of excess BglIII linkers (Pharmacia). Other fragments were inserted directly into pMA27AT. Reagents were from BRL and were used in accordance with the manufacturer's specifications. All cloning procedures used standard techniques described elsewhere (28).

Transformation of yeast and *E. coli*.

E. coli was transformed using standard methods (28). Haploid and diploid yeast were transformed as described by Hinnen *et al.*, (22). Yeast strains containing a plasmid are termed T (transformant) followed by the number of the relevant plasmid.

DNA and RNA Isolation

Plasmid DNA was isolated from *E. coli* by the method of Chinault and Carbon (29). Total yeast RNA for Northern blot analysis was isolated from cells grown to $4-6 \times 10^6$ cells/ml as described previously (30). Total yeast DNA for copy number determination was prepared according to Cryer *et. al.* (31) from the same culture as was used for RNA preparation.

Hybridisation Conditions and Probes

Northern and Southern filters were hybridised using standard conditions (32). Probes were nick translated (33) in the presence of α - ^{32}P -dTTP (Amersham International) to a specific activity of $4-6 \times 10^7$ cpm/ug. PGK and rRNA specific probes were purified from EcoRI digested pMA27 (24) and pYIRG12 (34) respectively. To quantitate RNA levels, regions of the filter corresponding to specific bands were excised and counted by liquid scintillation. Background was determined by counting 2 other areas of the filter. Figures were standardised with respect to the levels of PGK RNA in untransformed cells and the rRNA loading controls.

RNA Analysis and Copy Number Determination

Total RNA isolated from yeast transformants was electrophoresed on 1% agarose/6% formaldehyde gels, transferred bidirectionally to nitrocellulose filters and probed with PGK or rRNA specific probes. The level of rRNA was used as an internal loading control.

For determination of plasmid copy number, total yeast DNA was digested with EcoRI, electrophoresed on 1% agarose gels and transferred to nitrocellulose (35). Filters were probed with rDNA and PGK specific probes nick translated to the same specific

activity. The relative intensities of PGK and rDNA (100-140 copies/cell) bands reflect the copy number of the plasmid.

The initiation site of PGK transcripts was determined by the riboprobe method as described by Ogden *et. al.* (26).

RESULTS

Construction of an in vitro ROAM Mutation

All previous analyses of the ROAM phenomenon have been carried out on ROAM mutations isolated in vivo. It has proved difficult to define exactly the nature of the Ty signals controlling activation because these mutations often occur in the promoters of genes that are not fully characterised. The Ty elements may also vary between different ROAM mutations and in some cases are deleted and/or rearranged (36,37). To overcome these difficulties we constructed an in vitro ROAM mutation in the well characterised (26) promoter of the yeast PGK gene. Ty normally displaces the functional UAS of the target gene in a ROAM mutation. To simulate this apparent positional requirement we used the 'Activator Trap' plasmid pMA27AT in which the PGK UAS has been deleted and replaced by a BamHI restriction site (Figure 1B). Fragments of Ty were inserted into this site to create an in vitro ROAM mutation. The levels of PGK RNA directed by the deleted promoter alone are very low. A Ty fragment that normally produces a ROAM mutation could therefore be recognised by its activation of the PGK promoter resulting in increased mRNA levels.

Previous investigations have shown that the Ty signals required for gene activation are located within the first 2.0kb of the element (38). To test the validity of our in vitro assay for ROAM mutations we inserted the 1.35kb 'll' fragment from the 5' end of Tyl-15 into the BamHI site of pMA27AT in both orientations to produce the plasmids pMA27AT-llr and pMA27AT-lln (Figure 1B,(iii)). In this and in all following constructions the fragment orientation that corresponds to the orientation of Ty in a ROAM mutation is designated 'r' and the 'non-ROAM' orientation is designated 'n'. As a negative control a Ty fragment that is not thought to contain any activator sequences (fragment 40) was also cloned into pMA27AT in both orientations to give plasmids pMA27AT-40r and pMA27AT-40n (Figure 1B,(iii)). These plasmids and the controls pMA27 and pMA27AT were transformed into the haploid (α mating type) yeast MD40/4c and the level of steady state PGK

Fig 1. A, Organisation of the Ty element used in this study, Ty1-15. Open boxes are the delta sequences. The major 5.7kb transcript and open reading frames (TYA and TYB) are indicated. Fragments 11 and 40 are the internal regions of Ty used in the initial localization of activator sequences (see text). **B,** Structure of the PGK genes in plasmids pMA27 (i), (24) and pMA27AT (ii), (26) and construction of an *in vitro* ROAM mutation (iii). The important features of pMA27 and the construction of pMA27AT are described in the text. Fragments of Ty were inserted into the BamHI site of pMA27AT in both orientations (iii). The 'r' orientation corresponds to the orientation of Ty in a ROAM mutation while the 'non-ROAM' orientation is designated 'n'. X, XhoI; Pv, PvuII; H, HpaI; Bg, BglII; S, SalI; H3, HindIII.

mRNA produced from the transformants was measured by Northern blot hybridisation. Transformants were designated T27AT-11r, T27AT-11n, T27AT-40r, T27AT-40n, T27 and T27AT respectively.

Figure 2A confirms previous data (26) that removal of the PGK UAS in pMA27AT results in substantially reduced levels of PGK RNA compared to pMA27 (Lanes 5,6). Insertion of the Ty '11' fragment into pMA27AT can elevate PGK RNA levels but only when it is in the 'r' orientation (Lanes 1,2). By contrast, fragment 40 has no activating effect in either orientation (Lanes 3,4). An identical result was obtained when the same constructions were transformed into a second haploid strain (AH22) of opposite mating type (a) to MD40/4c (Figure 2B). Filters were probed for rRNA to ensure that none of the observed differences in RNA levels were caused by unequal loading of samples.

As anticipated for a ROAM mutation, activation of PGK

Fig. 2. Analysis of an *in vitro* ROAM mutation. The molecules described in Fig. 1, pMA27AT-11r, pMA27AT-11n, pMA27AT-40r, pMA27AT-40n, pMA27 and pMA27AT, were transformed into yeast strains of different mating type and the steady state levels of PGK RNA produced from transformants containing these molecules was measured by Northern blot hybridisation. **A**, strain MD40/4c (α/α haploid), **B**, strain AH22 (a haploid), **C**, strain SF-1 (a/ α diploid). Filters were probed for PGK RNA or for rRNA which served as an internal loading control. **D**, Northern blot analysis of PGK transcripts from pMA27AT-11r in 9 randomly selected haploid segregants (S1-S9) obtained by sporulation of a diploid (SF-1) transformant containing pMA27AT-11r. Lane 1, pMA27AT-11r in the diploid strain SF-1. Lanes 2 and 3, pMA27AT-11r in the haploid strains AH22 and MD40/4c respectively. Lanes 4-12, S1-S9. **E**, 5' mapping of PGK transcripts from MD40/4c transformants T27AT-11r and T27 (which has the wild type RNA start site, 26) by ribonuclease digestion. Markers were Sau3AI digested pBR322. **F**, Copy number determination of plasmids pMA27AT-11r, pMA27AT-11n, pMA27AT-40r, pMA27AT-40n, pMA27 and pMA27AT in MD40/4c. PI denotes plasmid specific bands.

transcription was abolished in an a/ α diploid (SF-1) containing pMA27AT-11r (Figure 2C). Sporulation of this diploid yielded numerous haploid segregants in which activation was restored (Figure 2D). Furthermore, transformation of these molecules into a homozygous (α/α) diploid strain (MD40/4c (Diploid)) resulted in orientation dependent activation identical to that seen in haploids (result not shown). This shows that fragment 11 can impose cell-type specific regulation on PGK expression and that repression of activation in the diploid is not just a non-specific effect of the diploid state.

Fig. 3. A, Diagrammatic representation of the fragments inserted into pMA27AT and a summary of their effects on PGK transcription. The number of each fragment is given in the left hand column. 'r' and 'n' are 'ROAM' and 'non-ROAM' orientations as described in Fig. 1. **B**, Northern blot analysis of PGK transcripts from pMA27AT containing fragments shown in A in haploid (MD40/4c) yeast. **C**, Northern blot analysis of PGK transcripts from pMA27AT containing fragments shown in A in diploid (SF-1) yeast. Track 18 in B and C are the non-transformed parental yeast strains MD40/4c and SF-1 respectively.

To confirm that the start site of the overexpressed RNA was unchanged in our in vitro system we mapped the 5' ends of PGK RNA from transformants T27 and T27AT-11r and found them to be identical (Figure 2E). Finally, the copy number of each plasmid used in this analysis was measured and shown to be constant relative to the number of rDNA repeats (Figure 2F). None of the RNA level differences in Figure 2 can therefore be due to differences in template copy number.

It is clear that the properties of T27AT-11r duplicate exactly the known properties of an in vivo ROAM mutation. The gene activation is orientation specific and subject to cell-type control while the size and 5' end of the overexpressed transcript are unchanged. These data indicate that the pMA27AT system is a useful assay for the identification of yeast activator sequences and provides a valid model for ROAM mutations in vitro. They also demonstrate that there is sufficient information within the Ty1-15 '11' fragment to direct ROAM activation.

Identification of Activators by Subdivision of Fragment 11

To increase the resolution of this analysis, subfragments of fragment 11 were inserted into the BamHI site of pMA27AT in both the 'r' and 'n' orientations and PGK RNA levels were assayed in haploid and diploid transformants containing these constructions. A diagram of the fragments used and a summary of the results of the Northern blot analysis is shown in Figure 3A. Of the various fragments used only 2 non-overlapping fragments, 31 and 21, activated PGK transcription in pMA27AT (Figure 3B, Lanes 6,12). These data identify two distinct regions of Ty that can act as activators in pMA27AT.

Fragment 31 activated PGK transcription to high levels in pMA27AT and must therefore contain an activator sequence. This sequence, which we have called the Ty Activator Sequence 1 (TAS1), can be assigned to a position between the arrowed Sau3AI and PvuII sites in Figure 3A because fragment 30, which substantially overlaps fragment 31 (Figure 3A), did not activate PGK expression in pMA27AT (Figure 3B, Lanes 14,15). TAS1 is therefore located within the transcribed region of the Ty element but outside the delta sequence. The activator is strictly orientation dependent as it stimulates PGK transcription in the 'r' but not the 'n' orientation in pMA27AT (Figure 3B, Lanes 12,13). This duplicates the directionality of the ROAM phenomenon. Alone, TAS1 can activate PGK transcription to the

same levels as the entire '11' fragment (Figure 3B, Lanes 5,12) indicating that no other sequences need be postulated to account for ROAM activation. Fragment 31 (TAS1) also mediates cell-type specific regulation as shown by the loss of TAS1 activation in diploid yeast (Figure 3C, Lane 12).

The position of a second independent activator, designated TAS2, can be deduced from the activation of PGK transcription in pMA27AT by fragment 21 but not fragment 22 (Figure 3B, Lanes 6,7,16,17). This activator is also located within the transcribed region of the Ty element, between the arrowed HpaI and RsaI restriction sites shown in Figure 3A. TAS2 can activate PGK transcription to only 25% of the levels seen in T27AT-31r (TAS1) as determined by scintillation counting (Figure 3B, lanes 6,12), and differs from TAS1 in two important respects. Firstly, although TAS2 is also orientation dependent, it activates in the opposite orientation to TAS1 i.e. in the direction of Ty transcription. This automatically precludes this activator from any participation in the ROAM phenomenon. Secondly, TAS2 cannot be associated with cell-type regulatory sequences within the 264bp of fragment 21 because TAS2 mediated activation of PGK transcription is retained in diploid yeast (Figure 3C, Lane 6).

Previous investigations have implicated an additional activator, located between the arrowed PvuII and HpaI sites in Figure 3A, in the ROAM phenomenon (39,40). This activator would be on fragment 24 which failed to stimulate PGK transcription in either orientation in pMA27AT. Although this lack of activation may be a consequence of interfering sequence arrangements it is unlikely that an activator in this position could contribute to the levels of PGK RNA observed in the ROAM mutation T27AT-11r since these can be accounted for by TAS1 alone.

To confirm that the control signals that comprise TAS1 reside within the deduced Sau3AI-PvuII region and do not, for example, span the Sau3AI site, we subcloned fragment 35 (Figure 3A) into pMA27AT in both orientations, transformed the resulting molecules (pMA27AT-35r and pMA27AT-35n) and appropriate controls into yeast and measured the steady state levels of PGK RNA by Northern blot analysis. As can be seen in Figure 4A, fragment 35 could mediate orientation dependent, high level activation of PGK transcription in pMA27AT in haploid (MD40/4c) yeast cells. This activation was identical to that mediated by fragment 31 (TAS1). Furthermore, activation was lost in diploid (SF-1) yeast (Figure

Fig. 4., Localisation of TAS1-related sequences. A, Northern blot analysis of PGK transcripts from pMA27AT containing fragments 31 and 35 (Fig. 3A) in haploid (MD40/4c) yeast. **B,** Northern blot analysis of PGK transcripts from pMA27AT containing fragments 31 and 35 in diploid (SF-1) yeast.

4B, lane 4). This result confirms that the activation, orientation dependence and mating type control sequences that comprise TAS 1 and control the ROAM phenomenon are located within a 90bp region of the TYA open reading frame.

DISCUSSION

We have created an in vitro ROAM mutation using the 1.35kb '11' fragment from the 5' end of Tyl-15 and the 'activator trap' pMA27AT. Characterisation of this mutation showed it to duplicate the known properties of an in vivo ROAM mutation, namely

orientation dependent activation, cell-type specific regulation and an unchanged start site of the overexpressed RNA.

More detailed analysis led to the identification of two activator sequences within the TYA open reading frame. Both of these possessed the rather unusual property of activating in an orientation dependent manner but only one of them, TAS1, could function in the direction of the ROAM activation. This activator was also regulated by yeast mating type even on a small (90bp) fragment. These features duplicate the known properties of both in vitro and in vivo ROAM mutations and implicate TAS1, which has not been identified previously in any other type of analysis, as the activator responsible for the ROAM effect. The position of TAS1 coincides with the position of a possible positive element for Ty gene expression (41). TAS1 may therefore play a role in Ty transcription in addition to mediating cellular gene activation. Interestingly, transcription of the closely related copia element of Drosophila appears to be controlled by sequences downstream from the RNA start site (42). It has, however, recently been suggested that the signals controlling Ty transcription and gene activation are distinct (43).

Two important conclusions arise from this work. First, TAS1, the ROAM activator, is functionally equivalent to a yeast UAS as it can replace the PGK UAS in vivo. Second, TAS1 functions only in the 'r' orientation even when it is out of the Ty 'context' and on a small 90bp fragment. This orientation dependence is not a property of the PGK promoter environment since both the GAL1-10 UAS (Cousins, Wilson, Kingsman and Kingsman, unpublished data) and PGK UAS (26) function well in both orientations in pMA27AT. Although the orientation dependence of ROAM mutations has long been observed, this is the first result to identify the cause of this directionality. Clearly it is an integral function of the activator sequence and is not simply a consequence of the activator being located at one end of the element and unable to act over the large distance required when Ty integrates in the non-ROAM orientation.

A second independent activator, TAS2, was found some 350-530 nucleotides downstream from TAS 1. This activator is less powerful than TAS1 but is also orientation dependent as it activates only in the 'n' (non-ROAM) orientation. Previous reports have indirectly implicated a sequence in the same region as TAS2 in Ty-mediated gene activation by deletion analysis

Fig. 5. A, Homology between TAS2 in Ty1-15 and the SV40 enhancer core consensus sequence. The Ty sequence runs from nucleotide 829 to nucleotide 846. **B,** Diagrammatic representation of a ROAM mutation. Filled in boxes are the unidirectional activators TAS1 and TAS2. 'MAT' represents the cell-type regulation sequence defined by its effect on TAS1. The positions of the Ty transcripts and open reading frames are indicated. Arrows represent the assumed activation of TAS1 and TAS2.

(37,39). This previously described activator differs from TAS2 in that it mediates mating type regulation, is orientation independent and activates only in association with distant Ty sequences (37). Our results show that TAS2 cannot be important in ROAM activation because it activates in the wrong orientation. Rather we would suggest an alternative role for this sequence as a UAS for the 5.0kb Ty transcript. The 5' initiation site of this transcript has been mapped to a position some 1150bp into the Ty element (11). TAS2 is located 120-250bp upstream of this site, within the normal functional range of a yeast UAS (44). We would predict that TAS2 has a very localised effect because it is only a weak activator and it does not function as an activator in the 11 fragment i.e., no activation is seen with 11n (Figure 2). This could mean either that TAS2 is sensitive to position or that its effect is suppressed in some way by other sequences in the Ty element. Such suppression could be required to restrict the influence of TAS2. The potential role of TAS2 as a local activator for the 5.0kb mRNA is currently being investigated.

The region defining TAS2 shares some homology with the SV40 enhancer core consensus sequence (Figure 5A, 39) but the TAS1 region has no such homology. Analysis of the nucleotide sequence of the Sau3AI-PvuII fragment that contains the cell-type specific activator TAS1 also failed to reveal any substantial homology to the consensus MATx1/ α 2 repression sequence (45). This raises the possibility that cell-type control of ROAM mutations is mediated

by a different method from that directly controlling cellular mating type.

The observation that TAS1 can replace the PGK UAS provides at least a superficial mechanism for Ty activation of cellular genes (Figure 5B). When Ty integrates between the UAS and RNA initiation site (RIS) of a gene it displaces the UAS by 5.9kb and replaces it with the Ty activator sequence TAS1. The gene will be removed from its cognate UAS-associated control and come under Ty-mediated cell-type control. If activation confers an easily scorable phenotype a ROAM mutant will be isolated (e.g. CYC7 (15); ADH1 (16)).

A distinctive feature of both TAS's is their orientation dependence. This distinguishes them from yeast UAS's (46, 47, 26) and most higher eukaryotic enhancers, in particular the enhancers that mediate retroviral activation of cellular oncogenes (19) which function well in both orientations. It is interesting that an enhancer of the myc oncogene found downstream of the RNA start site within an exon that is transcribed but not translated is also orientation dependent (48). This may prove to be a feature of activator sequences located within transcribed regions.

The location of TAS1 within the Ty element also distinguishes this activator from its retroviral counterparts. In most cases sequences that mediate retroviral activation of cellular genes reside within the non-transcribed region of the LTR (19). By contrast, TAS1 is not within the Ty LTR (delta sequence) but is located approximately 150 nucleotides into the TYA open reading frame. This explains why solo delta sequences, which often result from Ty recombination (1) have not been shown to mediate the ROAM effect. The TYA gene is central to Ty function as it encodes the major structural proteins of Ty-VLPs (7). It will therefore be of interest to see how Ty combines the need to transcribe TYA with the binding of protein factors that may mediate TAS1 and TAS2 activation. This problem could be overcome by the use of a 'finger binding' protein such as TFIIIA (49) which has already been shown to bind and function on transcribed DNA.

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