

## The Genomic RNA in Ty1 Virus-Like Particles Is Dimeric

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**The yeast retrotransposon Ty1 resembles retroviruses in a number of important respects but also shows several fundamental differences from them. We now report that, as in retroviruses, the genomic RNA in Ty1 virus-like particles is dimeric. The Ty1 dimers also resemble retroviral dimers in that they are stabilized during the proteolytic maturation of the particle. The stabilization of the dimer suggests that one of the cleavage products of TyA1 possesses nucleic acid chaperone activity.**

The yeast retrotransposon Ty1 has many fundamental similarities to retroviruses. For example, the Ty1 replication cycle includes the transfer of information from RNA to DNA and back to RNA; the genomic RNA, along with the associated enzymes, is encased in a virus-like-particle (VLP) formed from a protein encoded by the element; the RNA contains short terminal repeats, while the DNA contains longer terminal repeats; and the primer for reverse transcription is a cellular tRNA molecule (2). On the other hand, Ty1 elements also differ from retroviruses in several important respects. Although Ty1 elements encode protease (PR), reverse transcriptase, and integrase, the order of these proteins within the *pol* gene is different from that in retroviruses. Further, Ty1 VLPs lack an Env protein and are not infectious; the Ty1 protein which forms the VLP, TyA1, shows no obvious homology with the corresponding retroviral protein, Gag; and during VLP maturation, TyA1 undergoes only a single cleavage, close to its C terminus (16), while retroviral Gag proteins are always cleaved into at least three characteristic fragments, termed matrix, capsid, and nucleocapsid (NC).

One hallmark of retroviruses is the fact that the genomic RNA in the virion is a dimer, consisting of two identical plus-strand RNAs joined by noncovalent bonds. While in vitro experiments with transcripts have suggested that RNAs of another yeast retrotransposon, Ty3, can form dimers (12), it is not known whether the RNAs in retrotransposon VLPs are dimeric. We now report that Ty1 RNA is present in VLPs in the form of a dimer.

Ty1 VLPs were prepared from transposition-induced cells as described by Eichinger and Boeke (6). RNA was isolated from the VLP pellet using proteinase K digestion in sodium dodecyl sulfate, followed by phenol-chloroform extraction, exactly as described for retroviral RNA (11). The preparations were digested with DNase (Promega), reextracted with phenol and chloroform, and analyzed by Northern analysis on nondenaturing gels as described elsewhere (11) except that electrophoresis was performed in TAE buffer (40 mM Tris acetate, 10 mM EDTA, 20 mM glacial acetic acid [pH 8.4]). As shown in Fig. 1, when RNA from Ty1 VLPs was analyzed in this manner, a single band was observed in the blots. This band was composed of RNA, since it was eliminated by digesting the sample with RNase A (data not shown). Heating the RNA to 55°C had

no effect on its mobility, but incubation at 65°C led to the appearance of a new species which migrated much faster than the band in the unheated sample. Treatment with temperatures over 75°C eliminated the original, slow-migrating species, apparently by converting all of it to the smaller species. This pattern is virtually identical to that observed with retroviral RNAs (10, 11). The slow-moving band seen in the unheated samples was intermediate in mobility between the dimeric and monomeric RNAs of murine leukemia virus (11), while the band that appeared upon heating the Ty1 RNA migrated more rapidly than murine leukemia virus monomeric RNA, which is 8.3 kb in length (data not shown). Ty1 genomic RNA is 5.7 kb (2). Thus, it seems very likely that the upper RNA band present in the unheated samples is a dimer of the Ty1 genome which is dissociated into monomers by treatment at 65 to 75°C.

The dimeric RNA in a retrovirus particle normally undergoes a change in conformation, rendering it more stable, after the particle is released from the cell (10, 11, 20). This change, called maturation of the dimer, was shown to depend on the activity of the viral PR (10, 11). The dimer maturation event is due to the nucleic acid chaperone activity of NC (9, 17), which is released from the Gag polyprotein by PR; this activity enables NC to catalyze rearrangements of nucleic acids into the most stable possible structures (reviewed in reference 18). We therefore examined the RNA from PR<sup>-</sup> Ty1 VLPs (4, 23) in order to determine whether, as in retroviruses, these immature particles contain an RNA dimer less stable than that observed in the wild-type VLPs. The level of Ty1 RNA obtained from the PR<sup>-</sup> VLPs was significantly lower than in comparable amounts of wild-type VLPs, as expected (23). As shown in Fig. 2, electrophoresis under nondenaturing conditions revealed that the PR<sup>-</sup> VLP RNA was also dimeric. When these samples were heated to 45 to 55°C before electrophoresis, a new band with the same electrophoretic mobility as the monomers obtained from the wild-type RNA sample appeared. Thus, the dimeric structure of the RNA in PR<sup>-</sup> VLPs is significantly less stable than in that from wild-type VLPs.

The results presented above show that Ty1 retrotransposons resemble retroviruses in two additional fundamental ways. First, the genomic RNA in a Ty1 VLP is a dimer; second, this dimer undergoes a conformational change, rendering it more stable, during maturation of the VLP.

The presence of dimeric RNA in Ty1 elements, which are only distantly related to retroviruses, suggests that this property is probably common to all long terminal repeat-containing retrotransposons. The evolutionary advantage which the dimeric genome confers on retroviruses and Ty1 elements is not fully understood. The dimer presumably affords the possibility of

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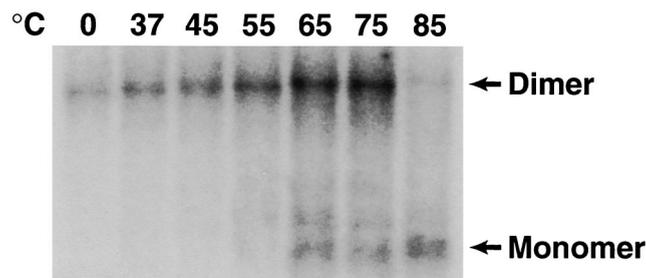


FIG. 1. Thermal dissociation of Ty1 dimeric RNA. VLPs were isolated after induction of Ty1 expression in strain GRY458 (13), using an established protocol (6). After isolation from the VLP pellets (11) and treatment with DNase, RNA was incubated for 10 min at the indicated temperatures. It was then analyzed by Northern blotting under non-denaturing conditions (11), using a  $^{32}\text{P}$ -labeled riboprobe complementary to nucleotides 241 to 493 of Ty1H3 (GenBank accession no. M18706).

recombination between retroviruses and is thus a significant source of genetic variation for the virus (15, 21). The same is undoubtedly true of Ty1 elements, which are known to recombine at high levels during retrotransposition (3). In addition, the fact that each particle contains two copies of its genetic information presumably offers the virus some protection against inactivation by breakage or other physical damage to the RNA. The similarities between retroviruses and Ty1 elements, including the nature of the terminal repeats in the DNA and RNA genomes and the use of a cellular tRNA as primer for DNA synthesis, suggest that mechanisms of reverse transcription are conserved in the two systems despite the genetic distance between them; perhaps we should not be surprised at the dimeric nature of Ty1 RNA.

We and others (10, 11, 20) have previously shown that in retroviruses, the dimeric RNA undergoes a change in conformation, rendering it more thermostable, during the maturation of the virion. This change was originally attributed to the release of NC from Gag, since NC was known to have nucleic acid chaperone activity (9–11, 17). However, studies on recombinant human immunodeficiency type 1 Gag in solution subsequently showed that this molecule actually exhibits nucleic acid chaperone activity equivalent to that of its cleavage product NC (8). Thus, the maturation of dimeric RNA is apparently not due to the appearance in the mature particle of a new molecular species possessing chaperone activity. Rather, it probably reflects the structural reorganization of the particle resulting from cleavage of Gag. The contact between Gag and

genomic RNA in the immature particle is somehow less intimate or less extensive than that between NC and genomic RNA in the mature particle, since in avian retroviruses, UV cross-linking of Gag to RNA in the immature particle is at least 1,000-fold less efficient than that between NC and RNA in the mature particle (19).

It is thus of considerable interest that the dimeric RNA undergoes maturation in Ty1 VLPs. As noted above, TyA1 protein is not cleaved into a series of fragments during Ty1 maturation. Only a single, small fragment is removed from the protein (16), and this fragment has no evident similarity to retroviral NC proteins. Therefore, the change in conformation of Ty1 dimeric RNA is probably due to the reorganization of the VLP during maturation, rather than to the release of a molecule with new nucleic acid chaperone activity. The data imply that one of the two proteins formed during maturational cleavage of TyA1 protein exhibits nucleic acid chaperone activity, and in analogy with retroviruses (5, 8), the uncleaved protein may also possess this activity.

In retroviral RNAs, the initial contact between the two monomers is probably at the kissing loop (for a review, see reference 1). This is a stem-loop with a palindromic sequence in the loop, affording the possibility of intermolecular base pairing between the loops on two monomers. In fact, this may be the only contact between the monomers in immature dimers of retroviral RNAs. In the mature dimer, on the other hand, sequences in the stem, as well as those in the loop, may also engage in intermolecular base pairing (7, 14). However, a search of Ty1 sequences did not reveal any potential kissing loops, particularly within a 285-base *cis*-acting region of the genome which is evidently sufficient for some transposition in the presence of a helper Ty1 element (22). Thus, the site of linkage between the monomers in Ty1 RNA dimers remains to be determined; it is remarkable that the linkages are so similar in thermostability to those in retroviral RNA dimers. It is conceivable that the monomers are joined to each other through the primer tRNA $_{i}^{\text{Met}}$  molecules, as recently suggested for the yeast retrotransposon Ty3 (12).

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#### ADDENDUM

After this report was submitted for publication, a paper appeared describing the ability of transcripts (~600 nt) of Ty1 RNA to dimerize *in vitro* in the presence of tRNA $_{i}^{\text{Met}}$  and the nucleic acid chaperone activity of a synthetic protein repre-

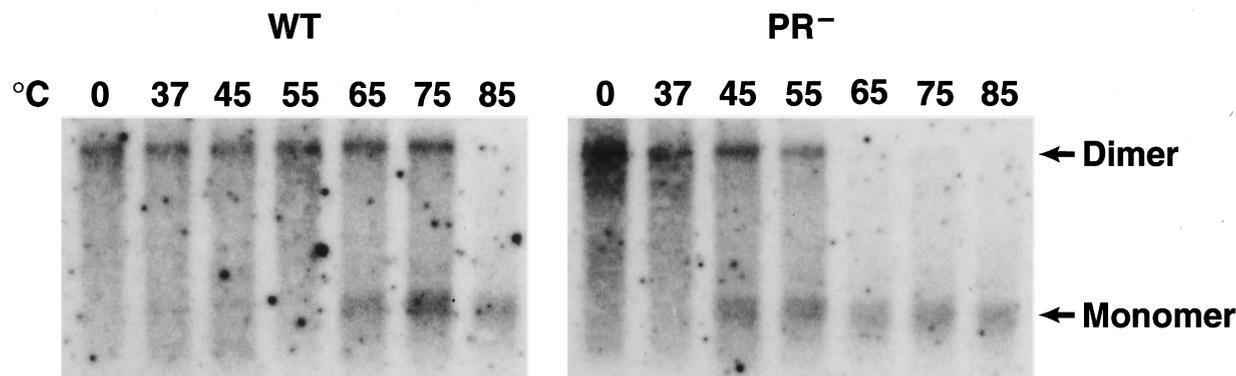


FIG. 2. Thermal dissociation of dimeric RNAs from wild-type and PR $^{-}$  VLPs. RNAs were isolated from VLPs after induction of Ty1 expression in strains GRY458 (WT [wild type]) and GM035 (4, 23). RNAs from the two preparations were incubated in parallel at the indicated temperatures and analyzed as described for Fig. 1.

senting residues 299 to 401 of TyA1 (5a). The findings of this paper are completely consistent with those in the present report.

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