The Gag-like Protein of the Yeast Ty1 Retrotransposon Contains a Nucleic Acid Chaperone Domain Analogous to Retroviral Nucleocapsid Proteins*

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The reverse transcription process for retroviruses and retrotransposons takes place in a nucleocore structure in the virus or virus-like particle. In retroviruses the major protein of the nucleocore is the nucleocapsid protein (NC protein), which derives from the C-terminal region of GAG. Retroviral NC proteins are formed of either one or two CCHC zinc finger(s) flanked by basic residues and have nucleic acid chaperone and matchmaker properties essential for virus replication. Interestingly, the GAG protein of a number of retroelements including Spumaviruses does not possess the hallmarks of retroviral GAGs and in particular lacks a canonical NC protein. In an attempt to search for a nucleic acid chaperone activity in this class of retroelements we used the yeast Ty1 retrotransposon as a model system. Results shows that the C-terminal region of Ty1 GAG contains a nucleic acid chaperone domain capable of promoting the annealing of primer tRNA\(^{\text{Met}}\) to the multipartite primer binding site, Ty1 RNA dimerization and initiation of reverse transcription. Moreover Ty1 RNA dimerization, in a manner similar to Ty3 but unlike retroviral RNAs, appears to be mediated by tRNA\(^{\text{Met}}\). These findings suggest that nucleic acid chaperone proteins probably are general co-factors for reverse transcriptases.

Retroelements form a large family of mobile genetic elements, including retroviruses, retrotransposons (or LTR\(^1\) retrotransposon for long terminal repeats containing retrotransposon) and retroposons (or non-LTR retrotransposon). Despite their tremendous diversity and dispersity among living organisms, their replicative cycle shares several similarities. They all encode a reverse transcriptase (RT) that converts the genomic RNA into a double-stranded DNA subsequently integrated into the cellular genome by integrase or endonuclease (1). Retroelements efficiently spread within eukaryotic organisms and have a major evolutive impact on the genomes that they inhabit. As repeated sequences they represent a high potential for homologous recombination of the host genome and as elements with transcriptional activity, their integration triggers both insertional mutagenesis and deregulation of gene expression (2, 3). Furthermore by means of cellular RNAs recruitment and reverse transcription, retroelements have been involved in pseudogene formation and exon shuffling in plants and mammals (4–6), long term immunity against nonretroviral RNA viruses in mammals (7), and intron loss in yeast (8, 9).

The reverse transcription process for retroviruses and probably most LTR retrotransposons occurs in a nucleocore also called nucleocapsid. This nucleoprotein structure is composed of the RNA genome and the primer tRNA coated with nucleocapsid (NC) protein molecules and of the RT and integrase enzymes. The NC protein has one or two highly conserved CXXC\(_{4}\)C\(_{4}\)X\(_{4}\)C zinc finger(s) flanked by basic residues with key functions in particle formation and reverse transcription (10). The NC protein exerts major roles in the replication of retroviruses that can be attributed to its nucleic acid chaperone and match-maker activities (10, 11). The NC protein annealing and strand transfer activities are involved in genomic RNA dimerization (12, 13), initiation of reverse transcription (12, 14, 15), and both in minus DNA (16–20) and plus DNA (21, 22) strand transfers required for the generation of the LTRs.

The yeast retrotransposon Ty1 is a LTR-retrotransposon with two overlapping open reading frames TYA1 and TYB1. TYA1 codes for a protein that can assemble into virus-like particles (VLPs) and where reverse transcription occurs while TYB1 codes for the protease, integrase, and RT enzymes similar to those of retroviruses (23–27). Interestingly, the TYA1 protein appears to be functionality homologous to the retroviral GAG polyproteins, although it does not have the canonical hallmarks. In fact TYA1 protein has no zinc finger motif, no major homology region, and is not processed into matrix, capsid, and NC by the protease (28). Several LTR retrotransposons such as Gypsy, ZAM, 17.6, 297, tom, 412 (Drosophila melanogaster), TED (Autographa californica), or Tt1 (Arabidopsis thaliana), and the members of spumavirus family share these properties in common with Ty1 (29–32).

In view of the ubiquitous nature of the nucleic acid chaperone and match-maker activities of the NC protein of retroviruses and of a number of LTR retrotransposon, such as Ty3 (33), or retroposon, such as I-Factor (34), we have studied Ty1 as a model system to look for a nucleic acid chaperone in this class of noncanonical GAG proteins. In the present report we show that a domain derived from the TYA1 protein exhibits a nucleic acid chaperone activity promoting primer tRNA\(^{\text{Met}}\) annealing to the multipartite primer binding site (PBS) and dimerization of Ty1 RNA \textit{in vitro}. 

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§ The abbreviations used are: LTR, long terminal repeat; RT, reverse transcriptase; NC, nucleocapsid; VLP, virus-like particle; PBS, primer binding site; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; HIV-1, human immunodeficiency virus, type 1; Fmoc, N-(9-fluorenyl)methoxycarbonyl; MLV, murine leukemia virus; nt, nucleotide(s); ss-cDNA, strong-stop cDNA.
DNA encoding Ty1 5'-RNA under the T7 promoter was generated by PCR using Ty1-H3 clone (GenBank™ accession number M18706), a 5'-oligonucleotide containing an EcoRI site, T7 promoter, and 15 nucleotides of the 5'-end of Ty1 RNA (5'Ty1) and a 3'-oligonucleotide complementary to positions 563–578 of Ty1 RNA and containing a RI site, T7 promoter, and 15 nucleotides of the 3'-end of Ty1 RNA (5'Ty1). The amplified DNA was cloned into pSP64 (Promega) to obtain pTy1-CG73. Template DNA was linearized at position 587 (3' Ty1) and dissolved at 1 mg/ml in sterile water. For biotinylated RNA synthesis, 5'Ty1 was cut by HincII and the 5'-oligonucleotide was subcloned into pSP64 (Promega) to obtain pCG90. Template DNA was linearized at position 73 (3'BstNI), and RNA<sup>trans</sup> was generated in vitro using T7 RNA polymerase (RiboMax, Promega). Mutations in the rRNA<sup>trans</sup> (see Fig. 6) were introduced by PCR using plasmid pCG90 as template, a 5'-oligonucleotide containing an EcoRI site, the T7 promoter, the mutations in the 5'-acceptor stem, and the 11 first nucleotides of the rRNA<sup>trans</sup> (5'TRNA<sup>met</sup>), under the T7 promoter, and 15 nucleotides of the 3'-acceptor stem, and the last 11 nucleotides of the rRNA<sup>trans</sup> (3'TRNA<sup>met</sup>10'10'). The amplified DNA was cloned into pSP64 to obtain pTy1-Van5, and RNA synthesis was performed as for Ty1 5'-RNA.

DNA encoding tRNA<sup>met</sup> derived from plasmid pHG300 (which contains the tRNA<sup>met</sup> sequence under the T7 promoter, pHG300 (36)) was cut by BamHI and the 5'-oligonucleotide was subcloned into pSP64 (Promega) to obtain pCG90. Template DNA was linearized at position 73 (BstNI), and RNA<sup>trans</sup> was generated in vitro using T7 RNA polymerase (RiboMax, Promega). Mutations in the rRNA<sup>trans</sup> (see Fig. 6) were introduced by PCR using plasmid pCG90 as template, a 5'-oligonucleotide containing an EcoRI site, the T7 promoter, the mutations in the 5'-acceptor stem, and the 11 first nucleotides of the rRNA<sup>trans</sup> (5'TRNA<sup>met</sup>), under the T7 promoter, and 15 nucleotides of the 3'-acceptor stem, and the last 11 nucleotides of the rRNA<sup>trans</sup> (3'TRNA<sup>met</sup>10'10'). The amplified DNA was cloned into pSP64 to obtain pVan6 and RNA synthesis was performed as for tRNA<sup>met</sup> wild-type.

All RNAs were purified by spin-column chromatography (Pharmacia S-300 HR) and dissolved at 1 mg/ml in sterile water. For biotinylated RNA synthesis the final concentration of rNTPs was 1 mM except for rUTP (0.8 mM), and 0.2 mM bio-16-UTP (Sigma) was added. For <sup>32</sup>P-UMP-labeled RNA synthesis, the final concentration of rNTPs was 1 mM except for rUTP (0.1 mM) and 10 μM (α-<sup>32</sup>P)-UTP (Amersham Pharmacia Biotech) was added. tRNA<sup>met</sup> was synthesized in vitro using T7 RNA polymerase, purified by 8% PAGE in 15 M urea, and recovered and dissolved at 0.1 mg/ml in sterile water after ethanol precipitation. It was then dialyzed against water, heat-denatured at 95 °C for 10 min in the presence of 1 mM MgCl<sub>2</sub> for proper folding. All mutations were verified by DNA sequencing.

DNA encoding Gag protein and its derivatives were obtained by PCR amplification using Ty1-H3 as template, a 5'-oligonucleotide containing a BamHI site (5'Ty1A-E2 or 5'Ty1A-D284) and a 3'-oligonucleotide containing a BgII site (5'Ty1A-A283 or 3'Ty1-H401). The amplified DNA was cloned into pQE-16 (Qiagen) and then subcloned into the BamHI and HindIII sites of pET-21a (Novagen), which allows expression by coupled in vitro transcription-translation as described by the manufacturer (TNT system, Promega).

Highly pure HIV-1 NCp7, MLV NCp10, Ty3 NCp9 (wild-type and mutant), and Ty1 TYA1-D peptide were synthesized by the Fmoc/o linker method and purified by high performance liquid chromatography as described previously (33, 37, 38). The sequence of the TYA1-D peptide derives from the Ty1-H3 clone (GenBank™ accession number M18706). All peptides were at 1 mg/ml in 20 mM Tris acetate, pH 6.5, 30 mM NaCl, and 1.5 equivalents of ZnCl<sub>2</sub>.

RESULTS

The C-terminal Domain of p54<sup>TYA1</sup> Directs RNA Binding—Reverse transcription of Ty1 RNA occurs in VLPs in which Ty1 RNA is specifically packaged (23, 41). The major protein of Ty1 VLPs is the product of TYA1, which is expressed as a 58-kDa precursor and processed by cleavage at position 401 to give rise to a small acidic C-terminal peptide to give rise to the p54<sup>TYA1</sup> protein (28). As already shown, p58<sup>TYA1</sup> and p54<sup>TYA1</sup> can bind RNA and DNA (24). Computer analysis indicates that the C-terminal domain of the p54<sup>TYA1</sup> protein possesses three stretches of basic amino acids, which could mediate nucleic acid binding (cf. Fig. 1A). To examine this possibility, we set up an assay with biotinylated Ty1 5'-RNA coupled to streptavidin-magnetic beads. p54<sup>TYA1</sup> and different deletion mutants (see Fig. 1B) were expressed by coupled in vitro transcription/translation in the rabbit reticulocyte lysate system in the presence of <sup>35</sup>S-methionine (cf. Fig. 1C). These <sup>35</sup>S-labeled products were incubated with the biotinylated RNA bound to the streptavidin beads. Proteins bound to RNA were recovered by magnetic separation and analyzed by SDS-PAGE. As shown in Fig. 1D,
p54TYA1 (lane a) and its C-terminal domain (lane c) were able to bind RNA (lanes 4 and 6) and not to the beads alone (lanes 1 and 3), while the C-truncated protein (lane b) did not interact with RNA-coupled beads (lane 5) or with beads alone (lane 2). These results confirm that the p54TYA1 protein can interact with RNA as described previously (24) and suggest that the C-terminal part of this protein is sufficient for RNA binding.

**Binding of the C-Terminal Domain of p54 TYA1 to RNA Generates High Molecular Weight Nucleoprotein Complexes**—To further investigate the properties of the C-terminal domain of the p54TYA1 protein, we synthesized by Fmoc-pentafluorophenyl ester chemistry a 103-amino acids peptide encompassing amino acids Asn299 to His401 and named TYA1-D. The RNA binding ability of this TYA1-D peptide was examined by band-shift assays. 32P-Labeled Ty1 5'-RNA was incubated with the TYA1-D peptide at increasing protein to nt molar ratios, and complexes were analyzed by PAGE in non-denaturing conditions. As a negative control we used a GST-cEngrailed2 fusion protein that belongs to the homeoprotein family known to bind to nucleic acids (42). At a protein to nt ratio of 1:20 to 1:10 virtually all the RNA molecules were present in high molecular weight nucleoprotein complexes (see Fig. 2A, top of gel). Since no intermediary complexes could be detected, the binding of the TYA1-D peptide to Ty1 RNA probably takes place in a cooperative manner. The TYA1-D peptide was found to bind to other RNAs and DNAs in a similar manner (cf. Fig. 3 and data not shown), suggesting that it has a strong affinity for nucleic acids. We have also investigated the ability of the TYA1-D-RNA complexes to be pelleted by centrifugation. As shown in Fig. 2B all the radiolabeled RNA was found in the pellet at a 1:12 protein:nt molar ratio. Taken together, these results indicate that the C-terminal domain of the Ty1 p54 protein can interact with nucleic acids generating high molecular weight complexes.

**The TYA1-D Peptide Exhibits Nucleic Acid Annealing Activity**—Because the previous properties of TYA1-D peptide were reminiscent of nucleic acid chaperone proteins, we have tested its effect on the annealing of two complementary single-strand DNA oligonucleotides. For this purpose we used a 32P-labeled Tar(−) and a Tar(+) oligonucleotides derived from the HIV-1 5'-sequence as described previously (39). The annealing of Tar(−) to Tar(+) is most probably prevented at 30 °C due to extensive secondary structures (see Fig. 3C, lane 1, and Fig. 3A, lanes 1–7, with Tar(−) structures migrating as two different forms in the gel). Addition of the TYA1-D peptide promoted a rapid annealing of the two complementary oligonucleotides with most of the duplexes formed at 10 min and at a peptide to nt molar ratio of 1:5 (cf. Fig. 3A, lane 15, Fig. 3B and C, lane 14). To further characterize the annealing activity of the TYA1-D peptide, we compared it to nucleocapsid protein NCp9 of the yeast retrotransposon Ty3. Interestingly the Ty3 NCp9 and Ty1 TYA1-D were found to behave similarly regarding TAR oligonucleotide annealing (see Fig. 3C, lanes 4–8 for NCp9 and lanes 10–14 for TYA1-D, and Fig. 3D). In conclusion the TYA1-D peptide has strong nucleic acid annealing activity. The TYA1-D Peptide Promotes the Annealing of Primer tRNA\textsubscript{Met} onto Ty1 RNA—The next step was to study the TYA1-D annealing activity in a context relevant to Ty1 replication. For this purpose, we have designed an *in vitro* model system comprising the 5'-part of Ty1 genomic RNA (5'Tyr RNA) with its multipartite primer binding site (43, 44), 32P-labeled primer tRNA\textsubscript{Met} (45) and TYA1-D. As shown in Fig. 4, TYA1-D allowed the annealing of primer tRNA\textsubscript{Met} to Ty1 RNA at a peptide:nt ratio of 1:10 to 1:5 (cf. Fig. 4, A and B). Furthermore the TYA1-D peptide promoted the dimerization of Ty1 RNA and tRNA at a similar protein to nt ratio (Fig. 4A, lanes 9–10, and Fig. 4B, lanes 4 and 5). Interestingly RNA dimerization did not occur in the absence of primer tRNA (Fig. 4A, lanes 1–5). These results indicate that the TYA1-D peptide promotes the annealing of primer tRNA onto genomic RNA and Ty1 RNA dimerization.

To compare the chaperone activity of the TYA1-D peptide with NC proteins from different retroelements, we tested the ability of NCp10 of Moloney murine leukemia virus, NCp7 of the HIV-1, and NCp9 from the yeast Ty3 retrotransposon or of its deletion mutant without the zinc finger (NCp9 dd) to direct
Clearly NCp9 was as efficient as TYA1-D in promoting tRNA annealing and Ty1 RNA:tRNA dimerization (cf. Fig. 5, compare lanes 1–4 with lanes 13–16). The NCp9 dd mutant was less efficient, since higher protein:nt ratios were required to obtain a high level of tRNA annealing and dimerization (cf. Fig. 5, compare lanes 1–4 with lanes 17–20). On the other hand, NCp7 and NCp10 were almost completely inactive in these processes.
tRNAi or MLV NCp10, can efficiently promote annealing of primer C, acceptor stem (from G2 to C13), which is palindromic (see Fig. 9). tRNA-tRNA dimerization is probably mediated by the 5'As previously suggested for Ty3, cf. Fig. 6 for a description of mutations) have been performed. It should be noted that all NC proteins are not equivalent in the Ty1 model system, since only Ncp9 from the Ty3 retrotransposon was as active as the TYA1-D peptide in vitro despite the absence of sequence homologies. Mutations in Primer tRNA\textsuperscript{Met} or in the Multiparite PBS Impaired Ty1 RNA:tRNA Dimerization in Vitro Induced by the TYA1-D Peptide—As previously suggested for Ty3, in vitro RNA:tRNA dimerization is probably mediated by the 5'-tRNA acceptor stem (from G2 to C13), which is palindromic (see Fig. 6C, wt panel). To examine if this hypothesis also holds true for Ty1, mutations in primer tRNA\textsuperscript{Met} and compensatory mutations in the PBS (cf. Fig. 6A for Ty1 RNA structure and Fig. 6B for a description of mutations) have been performed. It should be noted that the tRNA\textsuperscript{Met} mutations were introduced in the 5'- and 3'-strands of the acceptor stem to maintain the stem structure. We selected these mutations made in vivo, since the PBS or tRNA\textsuperscript{Met} mutations alone abolished Ty1 transposition (from G2 to C13), which is palindromic (pKc10 and pKc66 constructed by Chapman et al. (45)). The only differences between the in vitro and in vivo mutations are at positions A1G/U72C of tRNA\textsuperscript{Met} instead of A1U/U72A to allow efficient in vitro transcription by T7 RNA polymerase. C, effect of the mutations introduced in the 3'-acceptor stem of tRNA\textsuperscript{Met} on the putative palindromic tRNA:tRNA interaction. D, mutations in the 5'-end of tRNA\textsuperscript{Met} acceptor stem alters Ty1 RNA:tRNA dimerization but not RNA annealing to the PBS. Nucleoprotein complexes were formed using either wt (lanes 1–6) or mutant (lanes 7–12) Ty1 5'-RNA in the presence of wild-type (lanes 1–3 and 7–9) or mutant (lanes 4–6 and 10–12) 32P-labeled primer tRNA\textsuperscript{Met} with increasing doses of the TYA1-D peptide as described under “Experimental Procedures.” Nucleoprotein complexes were processed as described previously. The gel has been autoradiographed to visualize 32P-labeled tRNA. M and D are monomeric and dimeric Ty1 5'-RNA or tRNA\textsuperscript{Met}, respectively. The protein to nt molar ratios are indicated at the top of the gel.

As shown in Fig. 6D, mutations in tRNA\textsuperscript{Met} or the multipartite PBS drastically decreased tRNA annealing to Ty1 RNA (compare lanes 4–6 or lanes 7–9 with lanes 1–3) and completely inhibited dimerization induced by the TYA1-D peptide. When compensatory mutations in tRNA and Ty1 RNA were used (lanes 10–12), tRNA annealing was restored but not RNA dimerization. Interestingly the mutant tRNA\textsuperscript{Met} could not form homodimers unlike wild-type tRNA\textsuperscript{Met} (see Fig. 6D, lanes 11 and 12). These results indicate that Ty1 RNA dimerization probably occurs via a tRNA:tRNA hybridization process.

Thus each strand of the tRNA\textsuperscript{Met} acceptor stem appears to achieve distinct functions, since the 3'-strand allows priming of reverse transcription once hybridized to the PBS while the 5'-strand promotes RNA:tRNA dimerization (cf. Fig. 6, B and C).

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verse transcription by tRNA annealing to the PBS of Ty1 RNA while inhibiting self-primed cDNA synthesis.

**DISCUSSION**

Ty1, a yeast LTR retrotransposon distantly related to oncoretroviruses and lentiviruses, encodes a major protein TYA1, considered to be equivalent to retroviral GAG, although it lacks most of the hallmarks of GAG. We have investigated the nucleic acid binding properties of the p54<sup>TYAI</sup> protein and mapped the nucleic acid-binding domain within the C-terminal region of p54<sup>TYAI</sup> (Fig. 1). In an attempt to extensively characterize the properties of this domain, it was synthesized in vitro as a highly pure 103-amino acid peptide, designated TYA1-D. This TYA1-D peptide binds both RNA and DNA in vitro and forms high molecular weight nucleoprotein complexes (Fig. 2).

Also the TYA1-D peptide was found to promote the annealing of complementary DNA oligonucleotides in a manner very similar to NCP9 of the yeast retrotransposon Ty3 and of retroviral NC proteins (Fig. 3), raising the possibility that the TYA1-D peptide has nucleic acid chaperone and match-maker activities similar to previously characterized NC zinc finger proteins (10, 33, 34). Using an in vitro reconstituted Ty1 replication system, the TYA1-D peptide was able to direct the hybridization of primer tRNA<sub>Met</sub><sup>Met</sup> to the multipartite PBS of Ty1 RNA, allowing elongation of tRNA<sub>Met</sub><sup>Met</sup> by RT (Figs. 4 and 7). Furthermore due to its nucleic acid chaperone and match-maker activities, the TYA1-D domain is expected to direct the DNA strand transfers during Ty1 reverse transcription as shown for retroviral NC proteins (16–22), and this is presently under investigation. Both the TYA1-D peptide and Ty3 NCP9 appeared to be efficient in primer annealing to the PBS and in the formation of Ty1 RNA:tRNA<sub>Met</sub><sup>Met</sup> dimers, while HIV-1 NCp7 was poorly active and MLV NCp10 inactive. The common use of replication primer tRNA<sub>Met</sub><sup>Met</sup> in Ty1 and Ty3 (45, 49), the nature of their multipartite PBS, and the extended tRNA<sub>Met</sub><sup>Met</sup>-PBS interactions (33, 44) might explain the differences seen between Ty1 TYA1-D peptide and Ty3 Ncp9, and the retroviral NC proteins. In agreement with this, HIV-1 primer tRNA<sub>Met</sub><sup>Met</sup> probably also interacts with genomic sequences flanking the PBS (50), but to a lesser extent than in Ty1 and Ty3. The nucleic acid binding and chaperoning activities of the TYA1-D peptide suggest that the C-terminal domain of p54<sup>TYAI</sup> probably is a major factor driving genomic RNA and tRNA<sub>Met</sub><sup>Met</sup> packaging required for the formation of functional Ty1 VLPs. In agreement with this notion, binding of tRNA<sub>Met</sub><sup>Met</sup> to the multipartite PBS appears to mediate its recruitment into VLPs (51). Biochemical data presented in Fig. 4 are the first direct evidences indicating that Ty1 RNA can exist in a dimer form. According to genetic data (52) intermolecular DNA strand transfers take place in the course of Ty1 replication in agreement with Ty1 RNA dimers. The observation that Ty1 RNA dimerization only occurs in presence of tRNA<sub>Met</sub><sup>Met</sup> leads us to propose a model where two genomic RNAs are linked via two tRNA<sub>Met</sub><sup>Met</sup> molecules (see Fig. 8). Dimerization of tRNA<sub>Met</sub><sup>Met</sup> would be mediated by 5′-5′ inter-actions involving a 12-nucleotide palindrom (position 2–13, GCGCGUGGCGC, see Fig. 6D). In support of this dimerization model, mutations in the 5′-tRNA palindrome impaired dimerization (Fig. 6D). Interestingly, Chapman et al. (45) found that these tRNA mutations (cf. Fig. 6C) inhibited retrotransposition, probably by interfering with tRNA<sub>Met</sub><sup>Met</sup> annealing to the PBS and reverse transcription. However, mutating the PBS to insert compensatory mutations did not restore a wild-type level of retrotransposition, but only 15% of it. In the light of our in vitro data, it is tempting to speculate that this might be due to the low level of Ty1 RNA:tRNA dimerization.

In many aspects Ty1 is much different from other retrotransposons like Ty3 or retroviruses, for example in respect to the
structure and maturation of its GAG precursor, called TYA1. In fact, Ty1 does not contain a retroviral (CX₃CX₄HₓC) zinc finger motif, the major homology region, and is not processed into mature matrix, capsid, and NC proteins. The sole maturation event occurs by cleavage of p58 resulting in the release of p54. The cleavage product, a very acidic peptide of 40 amino acids, cannot be detected and is probably degraded (28). It is likely that the TYA1-D peptide will exhibit nucleic acid chaperone and match-maker activities in the context of the full-length p54TYA1 protein. As already shown for other nucleic acid chaperone proteins like hnRNP C1/C2, the chaperone activity was found in both the full-length protein and in the RNA-binding domain alone (53). Furthermore in HIV-1, the nucleic acid chaperone activity was found in the mature NCp7, in the precursor NCp15 (13) and in the Gag polyprotein (54). Thus p54₃⁄₄TYA1 might be a multidomain protein with several associated functions rather than a polyprotein-like in retroviruses. The mapping of a minimal nucleic acid chaperone domain in Ty1-A1 is under way. A number of retroelements such as Gypsy, ZAM, 17.6.297, tom, 412, TED, or Ta1 and the Spumaviruses share these properties in common with Ty1, since they all possess a domain with stretches of basic amino acids with possible nucleic acid chaperone properties (29–32).

Due to the general property of single-stranded nucleic acids to fold into and to be kinetically trapped in several conformations (55), reverse transcriptases might be especially sensible to the existence of nonfunctional initiation complexes. Based on our datas and previous reports on retroviruses (10), Ty3, another yeast very distant LTR retrotransposon (33, 38) and I, a long interspersed nuclear element-like element in Drosophila (34), we propose that RT activity, whatever the reverse transcription mechanism, should be associated with a nucleic acid chaperone protein that acts in setting up functional and specific initiation complexes. This would be achieved through two complementary mechanisms: first by annealing the primer to the template and second by inhibiting nonspecific priming events. In agreement with this notion, it has recently been reported (56) that hnRNP A1, a nucleic acid-binding protein known to exhibit a strong chaperoning activity (53), probably interacts with telomeric repeats so as to stimulate telomere elongation by telomerase, distantly related to RT’s (57). Thus functional interactions between RTs and a nucleic acid chaperone protein might be a general feature of genome maintenance in retroelements and their cellular hosts.

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