Intracellular Targeting of Gag Proteins of the *Drosophila* Telomeric Retrotransposons

S. Rashkova, A. Athanasiadis, and M.-L. Pardue*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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*Drosophila* has two non-long-terminal-repeat (non-LTR) retrotransposons that are unique because they have a defined role in chromosome maintenance. These elements, *HeT-A* and *TART*, extend chromosome ends by successive transpositions, producing long arrays of head-to-tail repeat sequences. These arrays appear to be analogous to the arrays produced by telomerase on chromosomes of other organisms. While other non-LTR retrotransposons transpose to many chromosomal sites, *HeT-A* and *TART* transpose only to chromosome ends. Although *HeT-A* and *TART* belong to different subfamilies of non-LTR retrotransposons, they encode very similar Gag proteins, which suggests that Gag proteins are involved in their unique transposition targeting. We have recently shown that both Gags localize efficiently to nuclei where *HeT-A* Gag forms structures associated with telomeres. *TART* Gag does not associate with telomeres unless *HeT-A* Gag is present, suggesting a symbiotic relationship in which *HeT-A* Gag provides telomeric targeting. We now report studies to identify amino acid regions responsible for different aspects of the intracellular targeting of these proteins. Green fluorescent protein-tagged deletion derivatives were expressed in cultured *Drosophila* cells. The intracellular localization of these proteins shows the following. (i) Several regions that direct subcellular localizations or cluster formation are found in both Gags and are located in equivalent regions of the two proteins. (ii) Regions important for telomere association are present only in *HeT-A* Gag. These are present at several places in the protein, are not redundant, and cannot be complemented in *trans*. (iii) Regions containing zinc knuckle and major homology region motifs, characteristic of retroviral Gags, are involved in protein-protein interactions of the telomeric Gags, as they are in retroviral Gags.

Transposable elements are abundant in the genomes of metazoa. Two *Drosophila* non-long-terminal-repeat (non-LTR) retrotransposons, *HeT-A* and *TART*, are particularly interesting because they form the telomeres of this organism (reviewed in references 17 and 18). Thus, they offer insight into both chromosome structure and properties of retrotransposable elements.

Transposable elements and viruses usually encode only proteins needed for their own replication and movement around the genome. For retrotransposons, these include proteins forming the ribonucleoprotein complexes that transport their RNA through the cell and proteins with enzymatic activities that reverse transcribe the RNA and insert the element in a new chromosomal site. In many retrotransposons and in retroviruses, these functions are divided into open reading frames 1 and 2 (ORF1 and -2). In retrotransposons, ORF1 encodes presumed structural components and ORF2 encodes enzymatic activities, an organization similar to that of retroviruses, where ORF1 encodes Gag protein and ORF2 encodes Pol protein (reviewed in reference 3). Like retroviruses, many retrotransposons link the two ORFs by a translational frameshift or leaky stop codon so that Gag is the more abundant product of translation (12).

Although Gag proteins are the major products of both retrotransposons and retroviruses, they are much less understood than Pol proteins. To explore the possibility that *HeT-A* and *TART* Gags are involved in directing their RNA transposition intermediates to telomeres, we have analyzed the intracellular localization of these proteins. The Gag coding sequences were tagged with sequence for green fluorescent protein (GFP) and were expressed in transiently transfected cultured *Drosophila* cells. For comparison with these telomeric Gags, we also studied Gag proteins of three typical non-LTR elements that never transpose into telomere arrays, i.e., Doc, jockey, and *I* factor (21). We found that the intracellular localizations of these proteins differ dramatically. Gag proteins from both *HeT-A* and *TART* are efficiently transported into the nucleus, while the other Gag proteins enter the nucleus inefficiently or remain in the cytoplasm. Thus, *HeT-A* and *TART*, the two elements with a bona fide role in the *Drosophila* genome, have Gag proteins with effective nuclear targeting, whereas the elements that are generally considered parasitic have proteins with impeded access to transposition sites. The different intracellular localizations of the five Gags suggest that each interacts differently with cellular components.

Both *HeT-A* and *TART* Gags move into the nucleus efficiently, but once inside the nuclei, their distribution differs markedly (21). In interphase nuclei, *HeT-A* Gag forms discrete dots, which we refer to as Het dots. Het dots are preferentially associated with chromosome ends and remain intact when cells are centrifuged onto slides. In contrast, *TART* Gag forms many small clusters of less discrete material. *TART* Gag clusters do not associate with chromosome ends. *TART* Gag clusters are dissociated when cells are centrifuged onto slides, suggesting...
FIG. 1. Comparison of amino acid sequences from HeT-A, TART, and other insect Gags with sequence from HIV-1 Gag MHR. Amino acids identical to those of HIV Gag are in boldface. Amino acids identical to those of HeT-A Gag are shaded. Asterisks mark the four invariant amino acids of the retroviral MHR (8). For each protein, the region shown is just N terminal of the zinc knuckle region. Sequences shown are from Gags of human immunodeficiency virus: accession no. AAC09330, amino acids 274 to 353; HET-M, HeT-A from D. melanogaster, accession no. AAC17188, amino acids 525 to 611; HET-Y, HeT-A from Drosophila yakuba, accession no. AAC01742, amino acids 517 to 603; TART, accession no. AYO35776, amino acids 617 to 703; jock, jockey, accession no. M22874, amino acids 290 to 332; Doc, accession no. X17551, amino acids 280 to 335. Sequences were aligned with the ClustalW algorithm (31) in the MegAlign program of the Lasergene package (DNASTAR, Inc.).

that they differ from Het dots not only in morphology but also in their molecular associations (22).

The difference in localization of HeT-A and TART Gags, seen when the proteins are expressed separately, is eliminated when the proteins are coexpressed. The two proteins enter an association that is strong enough to withstand centrifugation (22). HeT-A Gag localizes to its characteristic Het dots. On the other hand, TART Gag does not show its characteristic distribution; instead, it completely colocalizes with HeT-A Gag in the nuclear Het dots. Thus, the HeT-A protein determines the intranuclear localization of TART Gag. The complete colocalization suggests that the proteins form heteromers. The interaction between HeT-A and TART Gags is element specific. HeT-A Gag does not affect the localization of Gags of the three nontelomeric retrotransposons that were studied (22).

HeT-A Gag also forms a distinctive cytoplasmic structure not formed by TART Gag. In a fraction of the cells, HeT-A Gag forms a single smooth-edged body, which we refer to as the Het body (21). Het bodies are seen only in cells with Het dots. The percentage of cells carrying Het bodies increases with time after transfection, possibly reflecting saturation of the amount of HeT-A Gag that can be targeted into the nucleus. In contrast, cells expressing TART Gag have much less cytoplasmic protein and never have cytoplasmic structures similar to Het bodies.

Our previous studies showed that these Gag proteins are capable of precise subcellular targeting. Therefore, to identify protein regions responsible for the specific localization of HeT-A and TART Gags and regions involved in possible interactions between these two proteins, we have designed deletion derivatives of HeT-A and TART Gags and expressed them in cultured Drosophila melanogaster cells. In these experiments, we have been guided in part by similarities between the coding regions of the telomeric retrotransposon Gags and retroviral Gags (14, 19, 26). HeT-A and TART both have the two conserved motifs that characterize retroviral Gag proteins.

The first motif is one or more copies of a sequence, CXXC X3HX3C, which has been called a CCHC box, a zinc finger, or a zinc knuckle (4, 29). In retroviruses, the zinc knuckle region is implicated in binding retroviral RNA and in contributing to the interactions between Gag monomers (10, 23, 30). HeT-A and TART Gags both have three well-conserved zinc knuckles (19, 26). The second motif in retroviral Gag proteins, QGXXEXR, is located N terminal to the zinc knuckles and is called the major homology region (MHR) because it is the only segment with significant homology among different groups of retroviruses. Genetic evidence implicates the MHR in viral assembly, maturation, and infectivity (4, 5, 9, 15, 28). Mutations in a similar region of the protein of yeast retrotransposon Ty3 affect multiple steps in retrotransposition (16). The family of insect non-LTR retrotransposons most closely related to HeT-A and TART has a similar, strongly conserved sequence in a position analogous to that of the viral MHR (19). Sequence alignment of this region shows highest similarity with MHR from human immunodeficiency virus type 1 Gag protein (Fig. 1).

Other specific features include a length polymorphism region in HeT-A Gag where different elements have insertions and/or deletions of 1 to 31 codons (19) and a repeat region in TART Gag where elements differ in the number of 73-amino-acid repeats (26; our unpublished data). These two regions appear in relatively similar positions near the amino-terminal ends of their respective proteins. In addition, HeT-A and TART Gags both have a coiled coil region at the very C-terminal end, which resembles a leucine zipper motif (13). Although not a characteristic motif in Gag proteins, leucine zippers have been detected in Gag proteins of non-LTR and LTR retroelements (6, 11), as well as in retroviral Gags (20). The importance of this motif in Gag proteins has not been investigated in detail.

The experiments reported here show that regions of HeT-A and TART Gags with the most sequence similarities to retroviral Gags are, as in the retroviral proteins, implicated in Gag-Gag interactions. Regions of HeT-A and TART Gags with the fewest sequence similarities to retroviral Gags, those at the amino-terminal end of each protein, seem to have targeting properties different from those of the retroviral proteins. While signals in the amino-terminal half of retroviral Gags direct the proteins to the plasma membrane, amino-terminal
regions in the telomeric Gags are important for targeting these proteins into the nucleus. The final step in targeting to telomeres, a step that only HeT-A Gag can accomplish, requires signals in several parts of the protein.

MATERIALS AND METHODS

Recombinant DNA and plasmid construction. Each gag coding sequence was amplified by PCR with primers that included an initial Met codon placed within an optimal translation initiation sequence (CCACCATGTT). The 3′ primers included the final codons fused in frame with the GFP coding sequence. Each of the amplified coding sequences was inserted into vector p.PL17. Plasmid p.PL17, which expresses enhanced GFP, was a kind gift from Ilaria Rebay. This plasmid was derived from Clontech vector pEGFP-N1 by replacing the cytomegalovirus promoter with the Armadillo promoter (32). When proteins were to be expressed, each was recloned into either pSR24 or pSR25, which respectively express enhanced cyan fluorescent protein (CFP) and the enhanced yellow fluorescent protein (YFP) under the Armadillo promoter. These were constructed by inserting the BamHI-SvuI fragments from Clontech vectors pECFP-N1 and pEYPF-N1 into p.PL17 cut with BamHI and partially cut with SvuI, thus replacing the coding sequence of the enhanced GFP in p.PL17 with the coding sequence of enhanced CFP or enhanced YFP. Cloning of full-length HeT-A Gag and TART A ORF1 has been described previously (22). All HeT-A deletion derivatives were made from DNA isolated from element 23Zn-1 (GenBank accession no. U06920); TART deletion derivatives were derived from TART A ORF1 (GenBank accession no. AY035776). Plasmid DNA was purified by using ion-exchange columns (Qiagen, Inc.). Plasmid Tag DNA Polymerase High Fidelity (Gibco BRL) was used for all PCR.

Cell culture and transfection. Drosophila Schneider line 2 (SL2) cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 0.5% laurylmaltose, and 10 mM nontoxic amino acids. For transfection, cultures at 2.5 × 10⁷ cells/ml in 5 ml of DMEM were incubated at 25ºC for 18 to 20 h. Transfections were carried out in 2.5 ml of serum-free DMEM by using a Cytofectene Transfection Reagent Kit (Bio-Rad) and 5 to 10 µg of plasmid DNA purified with our cytological assays.

The medium with the transfection cocktail was replaced after 6 h with supplemented DMEM containing 100 µg of penicillin and 100 µg of streptomycin per ml. Cells were analyzed by fluorescence microscopy at 24 and 48 h and by immunoblotting at 48 h after transfection.

Protein gel electrophoresis and immunoblot analysis. Cells were collected 48 h after transfection, washed three times in phosphate-buffered saline (PBS) buffer, and resuspended in extraction buffer (50 mM Tris HCl, pH 6.8; 0.15 M NaCl; 2 mM EDTA; 0.5% NP-40). The cell suspensions were incubated with 0.2 µg of RNase A (Sigma-Aldrich)/ml on ice for 1 h; an equal volume of 2× protein sample buffer was added (60 mM Tris HCl, pH 6.8; 100 mM dithiothreitol; 2% sodium dodecyl sulfate [SDS]; 0.01% bromphenol blue; and 20% glycerol), and samples were heated to 100ºC for 5 min. Proteins were resolved by SDS-polyacrylamide gel electrophoresis and transferred to polyvinyl dibluoride membranes. The fusion proteins were visualized by immunoblotting with anti-GFP antiserum from guinea pig (20) and secondary anti-guinea pig antibody conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories, Inc.). To visualize untagged HeT-A Gag, we raised an antibody against a peptide, amino acids 17 to 30, from the amino-terminal end of the protein sequence. Immunoblot analysis confirmed that this antibody recognized both untagged protein and the GFP-tagged protein.

Microscopy. Transfected SL2 cells were dropped onto slides at 24 and 48 h after transfection. Cells were allowed to settle for 20 min and were then fixed with 3.7% formaldehyde in PBS-Tween buffer (1× PBS and 0.1% Tween 20) for 30 min. The cells were washed three times for 5 min in PBS-Tween and were stained with 0.2 µg of 4′,6-diamidino-2-phenylindole (DAPI)/ml in 20 mM Tris HCl, pH 7.4, for 1 min. The slides were destained in water and were mounted in 1× PBS-50% glycerol. Cytological analysis was performed with a Nikon ECLIPSE E 600 microscope equipped with a charge-coupled device camera. Images were taken by using Spot RT version 3.0 and were processed with Adobe Photoshop 5.5 (Adobe Systems, Mountain View, Calif.).

RESULTS

Expression of GFP-tagged Gag constructs and deletion derivatives in SL2 cells. In this study, we have compared the intracellular localizations of deletion derivatives of the Gag proteins from HeT-A and TART. (These are depicted in Fig. 2.) Each coding sequence was cloned under the same promoter and was tagged with GFP at the 3′ end. This allowed us to compare the subcellular localizations of the retrotransposon Gag proteins and their deletion derivatives independent of other element-specific influences, like promoter strength and signals in the 5′ and 3′ untranslated regions. The polypeptide constructs were expressed in transiently transfected cultured SL2 cells. Western blot analysis with an antibody against GFP showed that each construct yielded a tagged polypeptide of the expected size (Fig. 3). While there is some variation in the amount of protein from each construct, it is clear that the major protein detected by the GFP antibody is the desired polypeptide. Furthermore, most, if not all, of the other material recognized by the GFP antibody represents cross-reacting SL2 proteins that do not fluoresce and thus do not interfere with our cytological assays.

The distinctive localizations that have been observed for HeT-A and TART Gags (21) suggest that these proteins contain signals for multiple interactions with other cellular components and with each other. To examine the contributions of different regions of HeT-A Gag protein to its unique final localization, we have made a selected set of partially overlapping deletion constructs that dissect the entire length of the full-length protein (Fig. 2). These constructs have identified regions in both Gags that have the ability to drive particular steps in the localizations and interactions of the two proteins. At least two regions of HeT-A Gag and one in TART Gag are capable of directing localization to the nucleus. The polypeptide containing residues 1 to 265 (hereafter designated HeT-A:265-381) was completely localized to the nucleus but spread through the nucleoplasm, avoiding the nucleolus (Fig. 2 and 4D). Comparison of these results suggests that sequences necessary for nuclear localization lie between amino acids 265 and 382, although amino acids 1 to 265 may also contribute to either localization or retention in the nucleus. A second region capable of directing nuclear localization is found near the C-terminal end: the derivative HeT-A:589-921 moved into the nucleus. At least some of the sequences necessary for this localization must lie between residue 690 and the end of the protein, because HeT-A:482-689, which includes the MHR and the three zinc knuckles, remained in the cytoplasm (Fig. 2 and 4E).

In contrast to HeT-A, only the N-terminal region of TART contains sequences that directed localization to the nucleus. These sequences lie within the first 536 amino acids, because TART:1-536 localizes entirely to the nucleus. Because TART:157-970 also localizes entirely to the nucleus, the simplest interpretation is that the region directing this localization lies between amino acids 157 and 536, although we have not ruled out more complex scenarios. The TART:157-536 region is at a position roughly equivalent to region 266-381 in HeT-A Gag discussed above. None of the TART constructs lacking this region showed nuclear localization. In summary, both HeT-A and TART Gags contain N-terminal regions capable of nuclear localization; HeT-A also has C-terminal sequences with similar capability. The HeT-A C-terminal fragment also includes a region that has little amino acid sequence similarity to TART.
Gag. The amino acid differences in this region of the two proteins are reflected in functional differences; proteins containing only the C-terminal half of TART Gag did not enter the nucleus (Fig. 2 and 4H).

**Regions in the C-terminal half of HeT-A and TART Gags are required for protein clustering.** The polypeptides from the N-terminal region of both Gags spread evenly throughout the cell (HeT:1-265 and TART:1-190) or in the nucleus (HeT:1-381, HeT:1-534, and TART:1-536), thus showing no evidence suggestive of self-association. In contrast, polypeptides containing the C-terminal regions of Gags formed clusters of various sizes and shapes, regardless of whether they entered the nucleus or remained in the cytoplasm (Fig. 2). The C-terminal half of the proteins contains the MHR, the zinc knuckles, and a leucine zipper-like region. Because each of these motifs has been implicated in protein associations in other molecules, any or all

might be responsible for the clustering. Therefore, we tested the effects of deleting these regions. For HeT-A Gag, both the polypeptide lacking the zinc knuckles and the polypeptide lacking the leucine zipper-like region localized entirely to the nucleus, where they formed loose and irregular clusters (Fig. 2 and 4F). Each of these regions could be deleted from HeT-A Gag without destroying the ability of the polypeptide to form clusters. Although there are 22 amino acids (589 to 610) common to all six constructs that cluster, we interpret the data in Fig. 2 to show that the regions involved in cluster formation are redundant: either the MHR (e.g., HeT-A:1-625, HeT-A:1-880, or HeT-A:Δ610-697) or the zinc knuckles plus leucine zipper-like region (HeT-A:589-921) are sufficient for cluster formation.

The ability of TART Gag deletion derivatives to form clusters is similar to that of HeT-A Gag derivatives, although TART Gag derivatives lacking amino-terminal sequence formed cytoplasmic rather than nuclear clusters (TART:533-793, TART:533-970, and TART:734-970). The clusters of any of these derivatives are distinctly different from the large, well-defined dots formed by the intact HeT-A Gag protein.

Formation of discrete nuclear Het dots requires the entire HeT-A Gag protein. The experiments discussed above show an apparently simple distribution of polypeptide regions contributing to the localizations and cluster formation of HeT-A and TART Gags. In contrast, a complex set of amino acid sequences, distributed throughout the HeT-A Gag protein, is required for the final targeting to telomeres. Although several of the tested deletion derivatives could enter the nucleus and form loose and irregular clusters (HeT-A:1-635, HeT-A:1-695, HeT-A:1-880, and HeT-A:Δ610-697), none could form the definitive Het dots. We conclude that these structures require contributions from the complete protein sequence or at least from many regions of that sequence.

To exclude a possible effect of the GFP tag on the formation of Het dots, we used an antibody raised against amino acids 17 to 30 of the HeT-A Gag protein to detect the untagged protein in cytological preparations. Untagged, full-length HeT-A Gag formed nuclear Het dots apparently identical to those formed by the GFP-tagged protein (data not shown).

The MHR and zinc knuckle regions are required for both heterologous and homologous interactions. The difference in localization of HeT-A and TART Gags, seen when the proteins are expressed separately, is eliminated when the proteins are coexpressed (22). HeT-A Gag localizes to its characteristic Het dots. In contrast, TART Gag does not show its characteristic distribution; instead, it completely colocalizes with HeT-A Gag in the nuclear Het dots (Fig. 5A). These experiments show that the HeT-A protein controls the localization of TART Gag. The association between the two proteins is strong enough to withstand centrifugation (22). The complete colocalization suggests that HeT-A and TART Gags interact, either directly or indirectly, possibly by forming heteromers.

To identify protein regions involved in these interactions, we have extended these experiments by including a set of deletion derivatives (Table 1). In heterologous cotransfections, each of the full-length Gag proteins caused some of the deletion derivatives of the other Gag protein to colocalize in the nuclear pattern of the full-length partner (Fig. 5). Full-length HeT-A Gag moved partners into Het dots; full-length TART Gag moved partners into smaller and more numerous clusters in the nucleus. All of the deletion derivatives that were colocalized into the partner’s pattern contain both the zinc knuckle region and the MHR, suggesting that these regions are important for nuclear colocalization.

Polypeptides from the C-terminal half of TART Gag, TART:533-793, TART:533-970, and TART:734-970 did not enter the nucleus when expressed alone (Fig. 2). When coexpressed with full-length HeT-A Gag, the two constructs carrying the MHR colocalized with Het dots (Fig. 5B) while TART:734-970 remained in the cytoplasm (Fig. 5C). When the same constructs, TART:533-793 and TART:533-970, were cotransfected with the HeT-A zinc knuckle deletion construct, HeT-A:Δ610-697, none were carried into the nucleus (Fig. 5D). Full-length TART Gag transported HeT-A:482-689 into the nucleus into TART-like clusters (Fig. 5E).

Whenever full-length HeT-A Gag is coexpressed with derivatives containing the interacting regions of either Gag, the other partner can be detected in the Het body. (Derivatives with only the N-terminal ends do not appear in the Het body.) The coexpressed deletion derivative is not efficiently gathered in the Het body; much is left dispersed through the cytoplasm, whether the protein is one that is not transported into the nucleus or one that moves to the nucleus into Het dots. Full-length TART Gag appears to be different; any protein remaining in the cytoplasm is efficiently moved into the Het body.

Deletion of the leucine zipper-like regions near the C terminus of either HeT-A or TART Gags did not eliminate transport of the heterologous polypeptides into the nucleus, indicating that these regions are not required for the observed interaction.

We have not found evidence for Gag-Gag associations in the N-terminal regions of the Gag proteins. Neither TART:1-190 nor TART:1-536 showed altered distribution in cotransfection experiments with full-length HeT-A Gag (Fig. 5F). For that
matter, HeT:A-Gag did not change its localization in the presence of full-length TART Gag.

In addition to providing evidence for heterologous interaction, coexpression experiments with deletion derivatives demonstrated that each of the Gag proteins interacts with its homologous peptides (Table 1). Full-length HeT-A Gag but not HeT:Δ610-695 transported HeT:482-689 into the nuclear localizations. Full-length TART Gag transported TART:533-793 and TART:533-970 but not TART:734-970. Thus, it appears that the two Gags use the same regions (the MHR and the zinc knuckle region) for both homologous and heterologous interactions. In spite of evidence that deletion derivatives colocalize with full-length HeT-A Gag in Het dots, coexpression of HeT:1-880 with HeT:589-921 or TART:533-970 did not result in

FIG. 4. Intracellular localization of HeT-A Gag, TART Gag, and deletion derivatives. Fluorescence micrographs of SL2 cells transiently transfected with constructs encoding HeT-A and TART proteins shown schematically in Fig. 2. Left panel shows GFP image. Right panel shows merged GFP, DAPI, and differential interference contrast. DNA in all cells is stained with DAPI (false-colored red). The nucleus is defined by the differential interference contrast image and is slightly larger than the area of brightly stained DAPI. Transfectants shown: (A) HeT:1-921, Het dots in cell with Het body, Het dots appear to be different sizes because the micrograph is an optical section, and the apparent size changes as focus is changed; (B) TART:1-970, small clusters in nucleus and a very small amount of cytoplasmic protein; (C) HeT:1-265, diffuse through cell; (D) HeT:1-381, diffuse through nucleus; (E) HeT:482-689, clusters in cytoplasm; (F) HeT:Δ610-697, irregular clusters in nucleus; (G) TART:1-927, irregular clusters in nucleus; and (H) TART:533-970, clusters in cytoplasm.
Our experiments have shown that, with one exception, deletion derivatives containing analogous regions of the two proteins are localized to similar regions of the cell. The exception is a second region directing nuclear localization found in HeT-A but not TART Gag.

HeT-A and TART Gags, like Gags of retroviruses and other retrotransposons, show marked sequence variation. Proteins of two adjacent HeT-A elements cloned from a single chromosome end have been found to differ by 20% in amino acid sequence (19). TART Gags have been less studied but appear to have similar sequence variation. As mentioned previously, both proteins have a region of length polymorphism near the amino-terminal end. This raises the question of whether the sequences analyzed here are representative of the targeting potential of other HeT-A and TART Gags. We believe that they are; all proteins maintain the same general features. The similarities in localization of analogous regions in the HeT-A and TART Gags studied here argue that the same similarities exist within each family, and there is some experimental support for this hypothesis. We have tested the targeting of Gag from a second HeT-A element that differs from the sequence used here by a 55-amino-acid substitution and found that it displayed exactly the localization pattern seen for the sequence studied here (21).

**Requirements for formation of Het dots and the Het body.** Our deletion analyses allow us to determine requirements for the formation of Het dots. The results presented here suggest that these requirements are stringent. The Het dot formation requires cooperation of several parts of the intact protein. Many of the HeT-A Gag deletion derivatives entered the nucleus very efficiently, but even the smallest alterations tested destroyed the ability of the protein to form these structures. It is interesting that some of the peptide derivatives with very small deletions in HeT-A Gag localized in clusters similar to those of TART Gag. Although deletion derivatives can interact and cotransport into the nucleus, we have not seen these derivatives complement each other in trans to form Het dots.

Like the nuclear Het dots, the Het body is a distinctive cytoplasmic structure seen only in cells expressing the full-length HeT-A Gag protein. We have not yet been able to show that it associates with a particular cytoplasmic organelle. Because it has been reported that human foamy virus initially targets to the centriole (24), we looked for but did not find preferential association of Het bodies with centrioles. In addition, we have detected Het bodies at various distances from the nucleus, another indication that they are not centriole associated.

We believe that Het bodies are a response to overexpression of the protein. If this is true, it must indicate that the cell is either measuring or responding to the overexpressed HeT-A Gag differently from the other proteins in this study. None of the HeT-A Gag deletion derivatives forms this distinctive structure, although they can localize to Het bodies when coexpressed with full-length HeT-A Gag.

**HeT-A–TART Gag colocalization.** Although we have not yet determined whether this interaction involves direct binding, the suggestion that HeT-A Gag interacts very effectively with TART Gag is supported by our observations that full-length HeT-A Gag and some of its deletion derivatives redirect localization and/or cotransport TART Gag and some of its deriva-

**FIG. 5. Intracellular localization of proteins in cotransfections.** Fluorescence micrographs of coexpressed proteins, tagged with either CFP or YFP. Proteins are shown schematically in Fig. 2. Panels show merged YFP, CFP, DAPI (false-colored red), and differential interference contrast. Inserts show CFP and YFP images alone. (A) HeT-A:1-921 (YFP) and TART:1-970 (CFP), colocalization to Het dots in a cell with no Het body; (B) HeT-A:1-921 (YFP) and TART:533-970 (CFP), colocalization to Het dots and Het body; (C) HeT-A:1-921 (CFP) and TART:734-970 (YFP), HeT-A Gag in Het dots and TART Gag derivative in cytoplasmic clusters; (D) HeT-A:610-697 (CFP) and TART:533-970 (YFP), HeT-A Gag derivative in irregular nuclear clusters and TART Gag derivative in cytoplasmic clusters; (E) TART:1-970 (YFP) and HeT-A:482-689 (CFP), both proteins colocalize in TART-like clusters in nucleus; and (F) HeT-A:1-921 (CFP) and TART:1-536 (YFP), HeT-A Gag in Het dots and Het body and TART Gag derivative spread through nucleus.
4. No change was detected for telomere-targeting function, while the speci

city of the relationship between the telomeric retrotransposons do not interact with Gag proteins from other Drosophila retrotransposons (22), although those proteins also have sequence similarities in these regions (19). It appears that the Gags of the telomeric retrotransposons does not interact with Gag proteins from other retrotransposons. This discrimination is reminiscent of the selectivity of retroviral Gags in forming heteromultimers (1, 7). The specificity of the relationship between HeT-A and TART Gag may be important for their roles in maintaining telomeres. Our results support the hypothesis that these elements have a symbiotic relationship in which HeT-A supplies the telomere-targeting function, while TART provides the reverse transcriptase activity needed for transposition of both elements, since HeT-A does not encode this enzyme.

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