Volume 244, number 2, 307-310 FEB 06819 February 1989

Retrotransposon transposition intermediates are encapsidated into virus-like particles

T.V. Gorelova, N.L. Resnick and N.G. Schuppe

N.I. Vavilov Institute of General Genetics, Gubkin str. 3, 117809 Moscow B-333, USSR

Received 16 December 1988

Virus-like particles (VLPs) possessing reverse transcriptase activity are persistently present in *Drosophila melanogaster* cultured cells and are formed in yeast induced for transposition. Different retrotransposon transposition intermediates consistent with those expected from the model of reverse transcription pathway of retrotransposon transposition have been detected during the analysis of nucleic acids isolated from VLPs. These data indicate that the act of reverse transcription takes place in VLPs which may be considered as functional intermediates of transposition.

Retrotransposon; Transposition intermediate; Virus-like particle; Reverse transcription; (*Drosophila* cultured cell, Yeast)

1. INTRODUCTION

Retrotransposons are a particular class of eukaryotic transposable elements which are organized like retroviruses. The *copia*-like elements in *Drosophila* and Ty elements in yeast fall into this class of transposons [1-3]. Data that retrotransposons have many features diagnostic of retroviruses indicate the possibility that reverse transcription may be involved in the process of retrotransposon transposition. Evidence supporting this proposition has been accumulating during the last few years. Firstly, different transposition intermediates consistent with the reverse transcription pathway of transposition were isolated from *Drosophila* cultured cells [4-7]; secondly, it was shown that Ty elements transpose via an RNA intermediate [8]; thirdly, complete sequence analysis of some *Drosophila* and yeast retrotransposons has revealed the regions that apparently encode a reverse transcriptase [9-11]; and fourthly, transpositions of retrotransposons proved to result in the formation of virus-like particles (VLPs) containing reverse transcriptase activity [12-14].

However, several questions related to the synthesis of VLP remain obscure. It is not yet clear where VLPs are synthesized in the cells. Shiba and Saigo [14] found that VLPs are restricted to nuclei of *Drosophila* cultured cells. Earlier, Heine et al. [15] isolated evidently the same VLP from the cytoplasm of the cells. The Ty VLPs, in yeast are restricted entirely to the cytoplasm [12,13]. All the authors note that only the RNA of corresponding retrotransposons is detected in VLPs [12-15], therefore it remains unclear whether the act of reverse transcription takes place within the VLPs.

This paper reports the composition of nucleic acids isolated from VLPs persistently present in cultured *Drosophila* cells, as well as from VLPs which are formed in yeast induced for Ty transposition. It has been shown that all of the transposition intermediates that might be expected from a retroviral model of retrotransposon transposition are revealed in the VLPs.

2. EXPERIMENTAL

2.1. *Cells*

The 67j25D cultured *D. melanogaster* cells were grown at

Correspondence address: N.G. Schuppe, N.I. Vavilov Institute of General Genetics, USSR of Sciences, Gubkin str. 3, 117809 Moscow B-333, USSR.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

26°C in KS-10 medium with 2% heat-inactivated Gibco foetal calf serum. The cells were harvested for experiments on the sixth day of cultivation. *Saccharomyces cerevisiae* strains DBY746 (MATα leu2-3, 112 his3-1 ura3-52 trp1-289) and F808 (MATa GAL$^+$ leu2-3, 112 his4-519 ade1-100 ura3-52) were used.

2.2. *Plasmid construction and yeast transformation*

The *Xho*I fragment of Ty was isolated from the plasmid pIns32 [16] and then inserted into the *Xho*I site of the expression plasmid YpLPT29. The resulting plasmid, pPTX, drives Ty expression from the promoter of the yeast phosphoglycerate kinase (PGK) gene. The authenticity of pPTX was inspected by restriction analysis. The second Ty-expression plasmid was pGTyH3 (a gift from G.R. Fink) described in [8]. Yeast was transformed via the LiOAc procedure of Ito et al. [17].

2.3. *Fractionation of* Drosophila *cells*

Drosophila cells were fractionated into nuclear and cytoplasmic fractions by the method of either Mayrand and Pederson [18] or Shiba and Saigo [14].

2.4. *VLP isolation and analysis*

VLPs from the nuclear fraction of *Drosophila* cells were isolated according to Shiba and Saigo [14]. The cytoplasmic fraction was centrifuged firstly at 12 000×*g* (20 min at 2°C) to remove cell debris, cytoplasmic VLPs then being precipitated by high-speed centrifugation at 100 000×*g* (90 min at 2°C). Nuclear and cytoplasmic VLPs were then purified by centrifugation in a 25–60% (w/v) sucrose gradient in 10 mM Tris-HCl buffer (pH 7.5), containing 5 mM $MgCl_2$ and 100 mM NaCl. The peak fractions corresponding to maximum reverse transcriptase activity were collected and used for analysis.

VLPs from yeast transformed by plasmid pPTX were isolated and analyzed as in [12]. VLPs from yeast transformed by pGTyH3 were isolated as in [13].

Reverse transcriptase activity of *Drosophila* VLPs was tested according to [15] and yeast VLPs analysed as in [13].

pDm58 plasmid was used as mdg1 probe and pDm86 as mdg3 probe [6], pDm111 as gypsy probe [7], and the 252 bp *Eco*RI fragment of mdg1 LTR cloned in pUC19 (gift from V.A. Gvozdev) was used as LTR-specific probe and for strand-specific probes.

Nucleic acids from VLPs were isolated by phenol extraction in 20 mM Tris-HCl buffer (pH 8.0), containing 5 mM EDTA, followed by ethanol precipitation. Plasmid isolation, restriction analysis, gel electrophoresis, strand separation, blotting, nick-translation, and hybridization analysis were performed by standard procedures [19].

3. RESULTS

3.1. *Reverse transcriptase activity of VLPs*

VLPs isolated from nuclear and cytoplasmic fractions of *Drosphila* cells and from the yeast transformed by pPTX or pGTyH3 possess marked reverse transcriptase activity (table 1). As shown in separate experiments this activity had the cation and template primer responses expected for the reverse transcriptase. The presence of VLPs in nucleic and cytoplasmic fractions is not cell-specific: experiments with the Kc line and Schneider-2 line gave the same results (not shown). In yeast VLPs appeared only in transformed cells, control strains or strains transformed by the vector plasmids alone containing very few, if any, VLPs, and, correspondingly, reverse transcriptase activity.

Table 1

Reverse transcriptase activity of VLPs isolated from *Drosophila* cultured cells and yeast

Source of VLPs	Activity (cpm)	
	Exogenous	Endogenous
Nuclear VLPs (*D. melanogaster*)	6350	1250
Cytoplasmic VLPs (*D. melanogaster*)	5970	1200
Cellular VLPs (*D. hidei*)	530	470
Yeast VLPs (*D. virilis*)	560	490
Yeast VLPs (YpLPT29)	560	520
Yeast VLPs (pPTX)	9800	2950
Yeast VLPs (pGTyH3)	6655	NT
Rauscher MuLV	49249	6990

Reverse transcriptase activity of *Drosophila* VLPs was assayed according to Heine et al. [15] and yeast VLPs as in [12]. Poly(rC)/oligo(dG) primer-template complex was used in exogenous reactions. The same amounts of protein were taken in reactions. Activity was measured as [α-^{32}P]dGTP incorporated above the background level (reaction without template was at maximum 500 cpm). Rauscher MuLV was taken as positive control. NT, not tested

Endogenous activity provides that the VLPs also contain the substrates for the reverse transcriptase reaction and exogenous template is not an obligatory implement of reaction.

3.2 *Nucleic acid composition VLPs*

Fig.1A, B illustrates the analysis of nucleic acids isolated from *Drosophila* nuclear and cytoplasmic VLPs, separated by electrophoresis under denaturing conditions. The odd lanes represent the transfer of total nucleic acids from VLPs, and the even lanes the samples which were pretreated with NaOH before analysis to remove RNA, these lanes therefore representing the DNA entity of VLP nucleic acids.

If VLP DNA was probed by plasmid, carrying only the sequence of the long terminal repeat (LTR) of mdg1 (fig.2, lane 1), a wide band of the

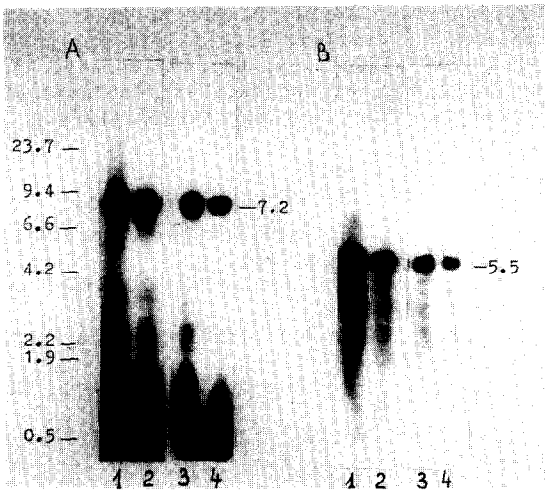

Fig.1. Blot analysis of nucleic acids from *Drosophila* VLPs, separated under denaturing conditions and probed by mdg1 (A) or mdg3 (B) Lanes: (1,2) (both panels) nuclear VLPs; (3,4) cytoplasmic VLPs. Odd lanes, transfer of total nucleic acids from VLPs; even lanes, samples pretreated with NaOH to remove RNA.

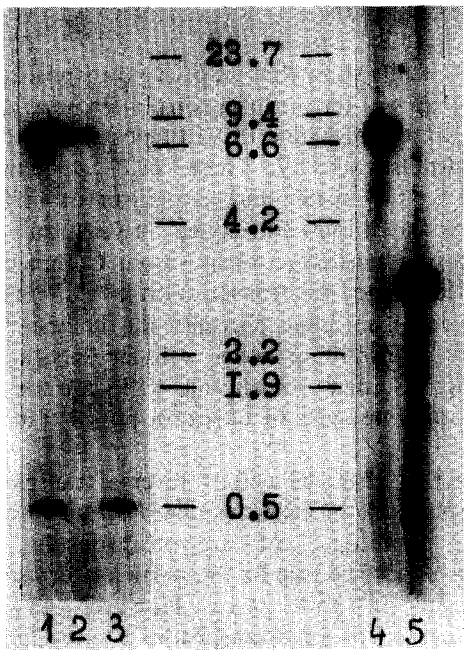

Fig.2. Intermediates of mdg1 isolated from *Drosophila* VLPs. Samples in lanes 1-3 were separated under denaturing conditions and pretreated with NaOH to remove RNA. Lanes: (1) probed by LTR DNA; (2) plus strand; (3) minus strand of LTR. Samples in lanes 4,5 were separated under native conditions. Lane 4, 5 μg VLP nucleic acids probed with mdg1 DNA; lane 5, 10 μg VLP nucleic acids pretreated with RNase in low salt and probed as in lane 4.

size of full-length mdg1 DNA and a band corresponding to the length of the LTR were revealed. If the plus strand of LTR was used as probe only full-length DNA was resolved (fig.2, lane 2), hybridization with the minus strand revealing only the LTR (fig.2, lane 3). Electrophoresis under native conditions showed that VLP nucleic acids primarily consist of DNA-RNA hybrid molecules. Treatment of VLP nucleic acids either with RNase at low ionic strength or NaOH increases the mobility of the DNA components of hybrid molecules, and hence the band corresponding to the mobility of single-stranded DNA appeared after hybridization (fig.2, lanes 4,5), Thus, analysis of VLP nucleic acids showed that all of the retrotransposon transposition intermediates isolated earlier from intact cells [6,7] might also be detected in VLPs. No double-stranded DNA was detected in *Drosophila* VLPs for all retrotransposons studied (mdg1, 412, mdg3, *gypsy* and *copia*).

Yeast VLPs appearing after transformation of cells by plasmids pPTX or by pGTyH3 also contain both RNA and DNA (fig.3A). However, yeast VLPs, as revealed after RNase treatment, also contain the full-length, linear, double-stranded DNA (fig.3B).

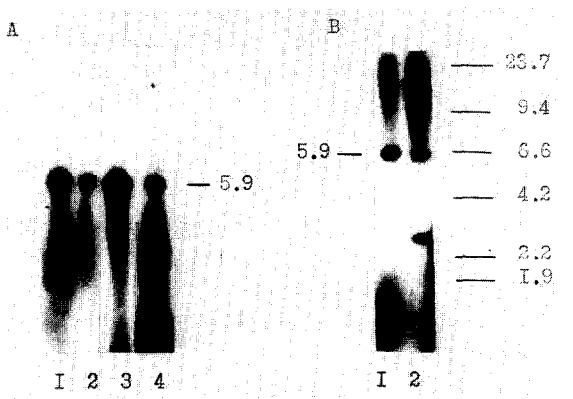

Fig.3. Blot analysis of nucleic acids from yeast VLPs. (A) VLPs were isolated from yeast transformed by plasmid pPTX (lanes 1,2) or pGTyH3 (lanes 3,4). Nucleic acids were separated under denaturing conditions and probed by Ty DNA. Samples in lanes 2,4 were pretreated with NaOH to remove RNA. (B) Yeast VLP nucleic acids were separated under native conditions and probed by Ty DNA. Lane 1, 1 μg untreated nucleic acids; lane 2, 3 μg nucleic acids pretreated with RNase in low salt and probed as in lane 1.

4. DISCUSSION

Our results show that all of the transposition intermediates of retrotransposons in *Drosophila* and yeast are encapsidated in VLPs possessing reverse transcriptase activity. These VLPs permanently reside in the nuclei and cytoplasm of different *Drosphila* cell lines and appear in yeast only after they are induced for transposition. Compartmentalization of reverse transcription in VLPs may be the mechanism that prevents the enzyme from reverse transcribing other cellular RNAs. Our findings also demonstrate that VLPs are the functional intermediates in retrotransposon transposition.

The fact that the act of reverse transcription takes place in the particles demonstrates the very close homology of retrotransposons and retroviruses. The role of the particles seems to be analogous in both cases. However, to date, transposition of retrotransposons is considered to be solely intragenomic, whereas retroviruses have an essentially intergenomic transposition cycle. Therefore, retrotransposons could be retroviruses that lack an extracellular phase and the possibility of forming infectious particles. To gain an understanding of the obvious, though still generally unclear, evolutionary relationship of retroviruses and retrotransposons, it is necessary to investigate the molecular events that regulate the formation of VLPs and, correspondingly, the transposition of retrotransposons. First of all, the role of the putative proteins encoded by retrotransposons in this regulation must be elucidated. Such evidence has been accumulated thus far for Ty elements 20–22 but very little is known about *Drosophila* retrotransposons. We believe that for attaining success in the study of *Drosophila* retrotransposons, a system similar to those used for yeast must be developed for *Drosophila* cells.

Acknowledgements: We are grateful to K. Sasnauskas, V. Gvosdev, Y. Chernoff and D. Gordenin for plasmids. We especially thank G.R. Fink for sending us the plasmid pGTyH3. The plasmid pPTX was constructed in collaboration with S. Cheperegin (Institute of Molecular Genetics, Moscow).

REFERENCES

[1] Finnegan, D.J. and Fawcett, D.H. (1986) Oxford Surveys on Eukaryotic Genes 3, 1–62.
[2] Fink, G.R., Boeke, J.D. and Garfinkel, D.J. (1986) Trends Genet. 2, 118–123.
[3] Adams, S.E., Kingsman, S.M. and Kingsman, A.J. (1987) BioEssays 7, 3–9.
[4] Flavell, A.J. and Ish Horowicz, D. (1981) Nature 292m 591–594.
[5] Flavell, A.J. (1984) Nature 310, 514–516.
[6] Arkhipova. I.R., Gorelova, T.V., Ilyin, Y.V. and Schuppe, N.G. (1984) Nucleic Acids Res. 12, 7533–7548.
[7] Arkhipova, I.R., Mazo, A.M., Chercasova, V.A., Gorelova, T.V., Schuppe, N.G. and Ilyin, Y.V. (1986) Cell 44, 555–563.
[8] Boeke, J.D., Garfinkel, D.J., Styles, C.A. and Fink, G.R. (1985) Cell 70, 491–500.
[9] Hauber, J., Nelbock-Hochstetter, P. and Feldman, H. (1985) Nucleic Acids Res. 13, 2745–2758.
[10] Mount, S.M. and Rubin, G.M. (1985) Mol. Cell. Biol. 5, 1630–1638.
[11] Stucka, R., Hauber, J. and Feldman, H. (1986) Curr. Genet. 11, 193–200.
[12] Mellor, J., Fulton, A.M., Dobson, M.J., Wilson, W., Kingsman, S.M. and Kingsman, A.J. (1985) Nature 313, 243–246.
[13] Garfinkel, D.J., Boeke, J.D. and Fink, G.R. (1985) Cell 42, 507–517.
[14] Shiba, T. and Saigo, K. (1983) Nature 302, 119–124.
[15] Heine, C.W., Kelly, D.C. and Avery, R.J. (1980) J. Gen. Virol. 49, 385–395.
[16] Chernoff, Y.O. and Gordenin, D.A. (1987) Genetica 23, 30–40.
[17] Ito, H., Fukuda, Y., Murata, K. and Kimura, A. (1983) J. Bacteriol. 153, 163–168.
[18] Mayrand, S. and Pederson, T. (1983) Mol. Cell. Biol. 3, 161–171.
[19] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[20] Adams, S.E., Mellor, J., Gull, K., Sim, R.B., Tuite, M.F., Kingsman, S.M. and Kingsman, A.J. (1987) Cell 49, 111–119.
[21] Youngren, S.D., Boeke, J.D., Sanders, N.J. and Garfinkel, D.J. (1988) Mol. Cell. Biol. 8, 1421–1431.
[22] Boeke, J.D., Eichinger, D., Castrillon, D. and Fink, G.R. (1988) Mol. Cell. Biol. 8, 1432–1442.