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Presence of the *gypsy* (MDG4) retrotransposon in extracellular virus-like particles

Boris V. Syomin[a], Konstantin V. Kandror[b], Andrej B. Semakin[a], Vladimir L. Tsuprun[c] and Alexander S. Stepanov[b]

[a]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, 117984, Russian Federation,* [b]*Bakh Institute of Biochemistry, Russian Academy of Sciences, Moscow, 117071, Russian Federation* and [c]*Shubnikov Institute of Crystallography, Russian Academy of Sciences, Moscow, 117033, Russian Federation*

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As a first step to investigate the functional activity of *gypsy* virus-like particles (VLPs) we explored the possibility of preservation of its VLP in extracellular form. The preparations containing extracellular *gypsy* VLP from *Drosophila melanogaster* and *D. virilis* were obtained. Full-length polyA$^+$ RNA and polyA$^+$ RNA–DNA complexes of *gypsy* were observed in both preparations. The polypeptides with some specificity to *gypsy* nucleic acids were identified in the obtained VLP preparations. These data accompanied by morphological characteristics of samples testify the presence of intact *gypsy* VLP in cultured media both from *D. melanogaster* and *D virilis* cultivated cells.

Retrotransposon; Virus-like particle; *gypsy*

1. INTRODUCTION

It has been shown that retrotransposons are the part of genome of many (if not all) eukaryotic organisms. The structure of these mobile elements resembles the structure of proviral forms of vertebrate retroviruses, known to be causative agents of tumors and leukemias in rodents and birds and recently have been shown to cause human adult t-cell leukemia.

Drosophila retrotransposons resemble the vertebrate retroviruses structurally [1] and possibly functionally because there are some findings showing that the main part of the RNAs of retrotransposons are involved in the formation of VLP [2–4]. However, the question about the functional properties of VLP and particularly infectivity is obscured. Nucleotide sequence analysis of *Drosophila* retrotransposons shows that the majority of retrotransposons are not infectuous because they do not encode an *env*-like function.

The *gypsy* element [5] is of particular interest, as it is one of only four known retrotransposons containing a long third ORF downstream from the *pol*-like ORF. There are a number of theoretical considerations which allow to propose that the *gypsy* third ORF may represent a functional homolog to the *env* gene of vertebrate retroviruses [6,7]. Moreover *gypsy* was found in genomes of different species of *Drosophila*: *D. melanogaster* and *D. virilis* [8] and was shown to be functionally active in *D. hydei* (Yu. Ilyin, pers. comm). Thus, there are data which allow to consider the possibility of *gypsy* horizontal transmission and therefore to examine *gypsy* VLP infectivity. As a first step to investigate the *gypsy* infectivity we explored the possibility of preservation of its VLP in extracellular form.

Correspondence address. B.V. Syomin, Department of Genome Mobility, Engelhardt Institute of Molecular Biology, Vavilov Str. 32, Moscow 117984, Russian Federation. Fax: (7) (095) 135 14 05.

2. MATERIALS AND METHODS

2.1. *Source of VLPs*

The media from growing suspension cells (1×10^7 cells/ml) of *D melanogaster* 67j25D [9] and *D virilis* 79f9 [10] were kindly provided by Vitaliy Kakpakov.

2.2. *Isolation of extracellular virus-like particles*

Extracellular virus-like particles were isolated from cultured media according to the technique of Shibo and Saigo [2] with some modifications [11].

2.3. *Isolation of nucleic acids*

The Poly A$^+$ fraction from phenol-extracted nucleic acids of VLP were isolated by chromatography on polyU-Sepharose 4B (Pharmacia). Alkaline treatment was carried out by 0.5 NaOH at 37°C for 30 min.

2.4. *Construction of a plasmid for in vitro RNA synthesis*

The pGEMgyp plasmid was obtained by insertion of the *gypsy* *Xho*I fragment from the Dm111 plasmid [5] into the *Sal*GI site of the pGEM-1 (Promega) polylinker.

2.5. *Production of radioactive RNA and DNA probes*

The pGEMgyp plasmid DNA was linearized with *Bam*HI restriction endonuclease. (+)strand *gypsy* RNA was synthesized by SP6 polymerase under standard conditions [13]. The labelled precursor was [^{32}P]ATP; 0.1 mCi per 1 reaction. DNA was labelled using the Amersham multiprime DNA labelling kit.

2.6. *Blot-hybridization*

Nucleic acids were electrophoresed on 1% agarose gels under denaturation conditions Northern-blot analysis was carried as described [12].

2.7. *Nucleic acids-proteins binding experiments*

These were carried out as described elsewhere [11].

2.8. *Electron microscopy*

The samples on carbon-covered collodion films were negatively stained with 1% uranyl acetate and examined in a Philips EM400 electron microscope operating at 80 KV with a magnification of 50,000 ×.

3. RESULTS AND DISCUSSION

A considerable quantity of endogenous copies of *gypsy* retrotransposon were discovered in genomic DNA both in *D. melanogaster* and *D. virilis* species [5,8]. That is why media from cultivated cells of these species of *Drosophila* were chosen for investigation.

Retrotransposons VLPs pellets were purified from cultivated media by fractioning in sucrose gradients as described [11]. Fractions were assayed for reverse transcriptase activity [2]. The peak of this activity was detected at a density of 1.22 g/ml as described previously [2,11]. Fractions with a maximum of reverse transcriptase activity were further investigated. Polyadenylated nucleic acids isolated both from *D. melanogaster* and *D. virilis* preparations demonstrated practically identical patterns of hybridization with the major band corresponding to the full-length *gypsy* RNA (Fig. 1, lanes 1 and 3). Treatment by NaOH before fractionation practically did not change the pattern of hybridization in the band corresponding to the full-length *gypsy* RNA (Fig. 1, lanes 2 and 4). This fact indicates that at least some of the full-length *gypsy* RNAs synthesized (−) DNA strands [4,14].

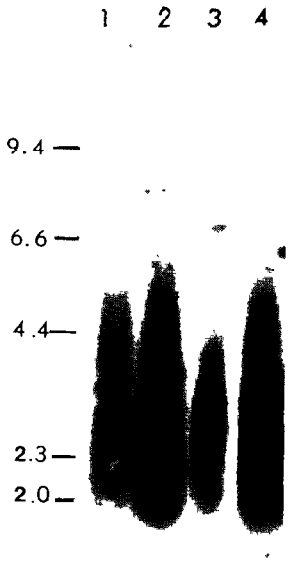

Fig. 1. Northern-blot analysis of polyadenylated nucleic acids isolated from extracellular VLP of *D. melanogaster* (lanes 1 and 2) and *D. virilis* (lanes 3 and 4) probed with *gypsy* from Dm 111. In lanes 2 and 4 probes were NaOH-treated before electrophoresis The full-sized transcript of *gypsy* is marked by an arrow

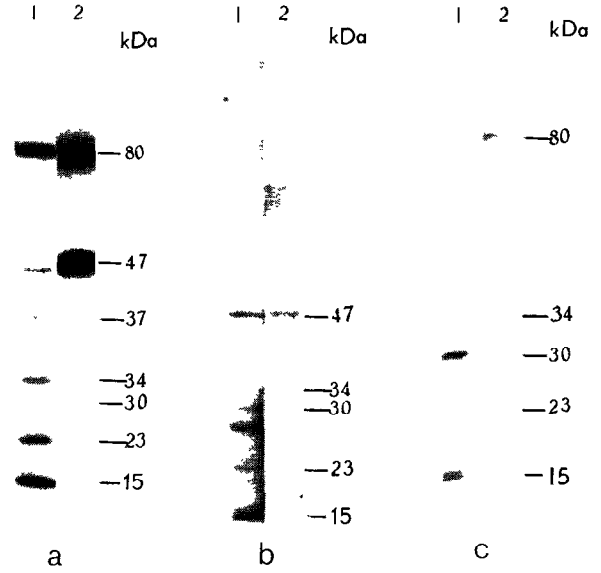

Fig. 2. Identification of *D melanogaster* (a) and *D virilis* (b) VLP polypeptides binding *gypsy* RNA (lane 1) and heterologous RNA (lane 2). (c) represents *gypsy* DNA binding polypeptides isolated from *D. virilis* (1) and *D melanogaster* (2) VLP preparations.

In a next stage we attempted to identify the proteins which can specifically bind to *gypsy* RNA. Ligand blotting was used for this purpose [11]. ^{32}P-labelled (+)strand *gypsy* DNA was incubated with nitrocellulose-immobilized polypeptides of VLP fractioned by size at a 1000-times excess of unlabeled ribosomal RNA. The results are shown in Fig. 2a and b, lanes 1. Polypeptides p47 (common for both VLP preparations) and p80 are effectively bound to any nucleic acid as is shown in the control lanes (Fig. 2a and b, lanes 2). Binding bands in the range of 15 kDa to 34 kDa practically coincide in both preparations and correspond to p15, p23, p30 and p34. Polypeptides of this group show different affinity to *gypsy* DNA (Fig. 2c). Polypeptide p37 displays most affinity to *gypsy* RNA. This polypeptide is found only in VLPs preparation from *D. melanogaster* (in Fig. 2a marked by arrow) and is not displayed when we used other probes: heterological RNA obtained by SP6 polymerase transcription of plasmid pGEM1, *E. coli* ribosomal RNA (Fig. 2a, lane 2, results were identical) and *gypsy* DNA (Fig. 2c, lane 2).

Negatively stained VLP preparations of *D. melanogaster* and *D. virilis* are shown in Fig. 3. Morphologically similar particles of approximately the same size were found in both preparations. Particles of about 70–75 nm in diameter were predominant on the support. The particles have two shells: the outer shell of about 7.5 nm thick; sometimes the outer shell is partially absent revealing the inner shell (marked by arrows). Some of the particles are hollow, some are stained inside,

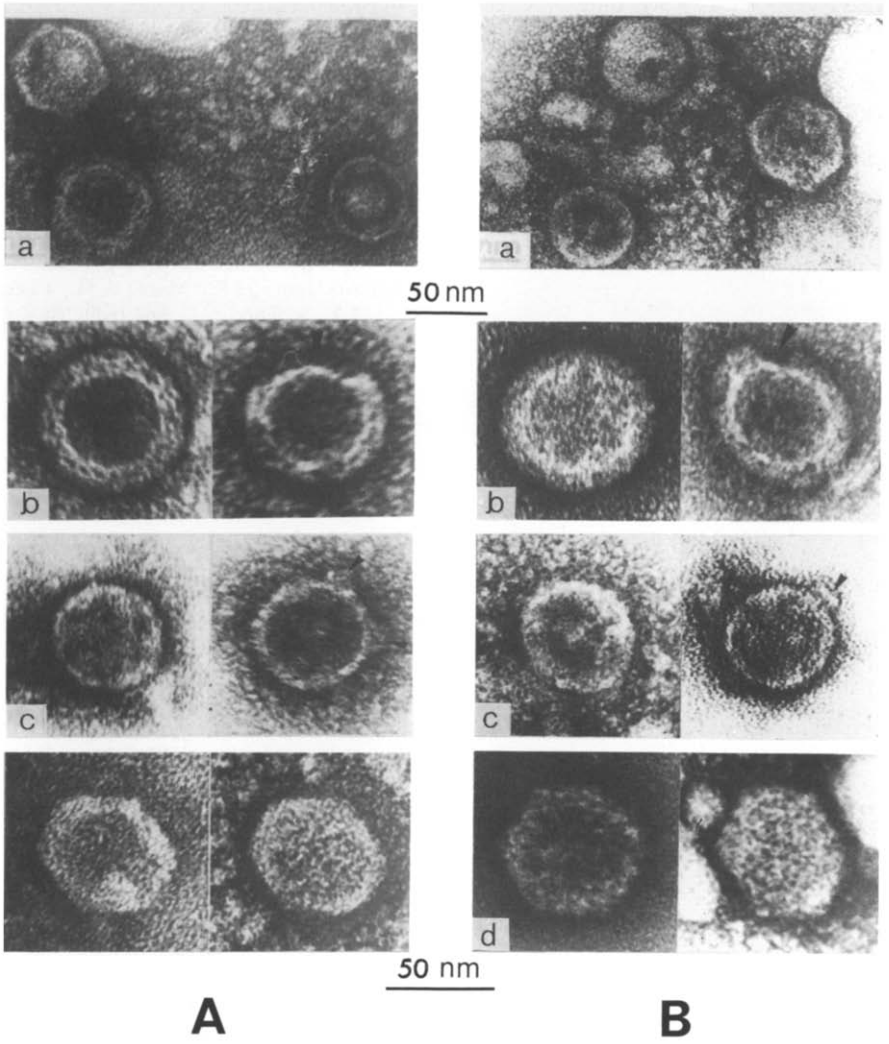

Fig. 3. Types of VLPs (A, from *D melanogaster*; B, from *D virilis*) (a) Electron micrograph of negatively stained preparations. (b–d) Images of characteristic types of VLP. (b) Round double-shelled particles; (c) Single-shelled particles. Arrows indicate the sites of partial lack or appearance of the second shell. (d) Hexagonal particles with icosahedral symmetry and visible protein capsomers

possibly indicating the presence of nucleoprotein. The small spherical particles (of the second type) are about 50 nm in diameter which corresponds to the diameter of the outer part of the inner membrane of the particles of the first type. Small areas of the shell of particles of the second type are covered by another shell. It is possible that particles of this type are an internal part of particles of the first type. We also found in both preparations a small quantity of hexagonal particles with typical icosahedral symmetry and visible protein capsomers of the shell.

Without using immunological techniques we cannot exactly define to which of these morphological types *gypsy* VLPs belong. However, it can be supposed that there are *gypsy* VLPs among the observed particles. Additional support for this suggestion is the similarity of patterns of polypeptides and nucleic acids which are homologous to *gypsy* in both preparations (other *D. melanogaster* retrotransposons do not show considerable hybridization to nucleic acids of *D. virilis* (unpublished data). In any case, the described results show that endogenous *gypsy* of different species of *Drosophila* is conserved in extracellular form. The first explanation of this fact is based on the similarity between the *gypsy* genome organization and proretroviruses. In this case it can be suggested that the mechanism of *gypsy* VLP maturation is similar to that of retroviruses. On the other hand, it can be suggested that *gypsy* VLPs are found in culture media due to the disruption of some part of the cells during cultivation.

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