

The Gag polypeptides of the *Drosophila* 1731 retrotransposon are associated to virus-like particles and to nuclei

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Abstract 1731 is a *Drosophila melanogaster* retrotransposon whose nucleotide sequence shows a proviral architecture with two long terminal repeats (LTRs) framing two internal Open Reading Frames (ORFs). The pol ORF2 of this mobile genetic element was demonstrated to code for an active Reverse Transcriptase (RT) and the ORF1 is expected to code for the structural Gag proteins of the virus-like particles (VLP). Using specific anti-Gag antibodies, we have characterized the 1731 Gag polypeptides expressed either in vitro or in Kc *Drosophila melanogaster* cultured cells. Together with the 1731 RT, the largest, likely post-translationally-modified Gag polypeptides are gathered into cytoplasmic virus-like particles. Moreover and consistent with the nuclear localization signal present in the Gag sequence, we observed that a short 1731 Gag polypeptide is associated to the cell nuclei.

Key words: 1731 retrotransposon; Virus-like particle; Gag protein; Nucleus

1. Introduction

LTR-retrotransposons are the representatives of a wide class of mobile genetic elements first isolated in *Drosophila melanogaster* and subsequently found in all eukaryotic genomes examined so far. Analyses of their nucleotide sequences has shown the striking resemblance of their structural organisation with that of the integrated form of Vertebrate retroviruses [1–4]. All these transposable elements are usually composed of an internal sequence with two or three ORFs, and of two flanking sequences, the LTRs (Long Terminal direct Repeats). Internal sequences homologous to retroviral *gag* and *pol* genes have been reported in all retrotransposons described so far. Such a canonical structure has been reported for the *Drosophila melanogaster* 1731 retrotransposon: the internal sequence should be the template for both the structural protein components of the virus-like particle (Gag proteins) and the enzymes involved in the replication of the genomic RNA, namely the protease, the integrase and the RNase H-reverse transcriptase (RT) (Pol proteins) [5]. In retrotransposons, such an internal order of the pol genes is a typical feature of the *copA*/Ty1 group [2–4,6].

It was reported that 1731 transcripts are abundantly represented in Kc *Drosophila melanogaster* cultured cells [7]. We decided to search for the existence of 1731 translation products, expecting that analysis of the production of 1731-encoded

structural and/or catalytic peptides would reveal some of the mechanisms involved in the expression control of the element, either at the translational or post-translational level. Thus, we have described the presence of an authentic RT activity encoded by the 1731 element, detected into the cytoplasmic VLPs-containing fractions prepared from Kc cells [8], suggesting that the element could be able to transpose. However, *pol*-derived enzymes are not the only proteins involved in transposition: structural Gag proteins were reported to play an active rôle in the replication/ transposition cycle of retroelements. In the yeast *Saccharomyces cerevisiae*, the importance of both Gag and Gag-Pol polyproteins on transposon replication/transposition was established through the demonstration that synthesis of Gag protein is a necessary and essential step to support the Ty1 transposition events [9–11]. Besides, the complex mechanism of the Gag processing could be one of the targets for a control strategy. Some preliminary indications in favour of such control have been already reported in the case of the Ty element [10]. Following our previous investigation on 1731 RT, we searched for the presence of the 1731 *gag* product in Kc cells, not only to examine whether this protein was assembled into VLPs, but also to probe its localization into different cellular compartments.

We presently report the existence of 1731 Gag proteins in the cellular fractions that contain the VLPs extracted from a *Drosophila melanogaster* cell line. Moreover, we observed that a 1731 Gag polypeptide is associated with the nucleus, a cell localization consistent with the presence of a putative nuclear localization signal (NLS)[12] in its sequence; a feature suggesting that, as indicated for some retroviral Gag polypeptides [13,14], 1731-encoded Gag protein could have some essential nuclear function.

2. Materials and methods

2.1. Cell lines

The *Drosophila melanogaster* Kc0 [15] and *D. hydei* DH33 cell lines [16] were used for the preparation of protein extracts [17]. Following electrophoresis, proteins were electroblotted onto nitrocellulose (Schleicher and Schuell) or Immobilon (Millipore) membrane by standard methods. Western blots were probed with preabsorbed anti-Gag antiserum (~1:1,000) and HRP-conjugated secondary antibodies (1:4,000) (Dako); then positive bands were revealed by using the DAB-CoCl₂ method or a highly sensitive chemiluminescent system (Boehringer).

2.2. Preparation of antisera against 1731 Gag recombinant protein

A Gag recombinant protein was produced in IPTG-induced RB791 *Escherichia coli* strain transformed with pRBA50, which is the pTrc99A[18] derivative obtained by in frame insertion of the 568 bp

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*Bam*HI–*Pst*I fragment corresponding to the central region of the 1731 *gag* gene, and able to produce a 23 kDa polypeptide (see Fig. 1). Plasmid pRBB8 made by an out of frame insertion of the same *gag* sequence into pTrc99B [18] was used as a negative control. Four anti-Gag antisera (GPI, II, III, IV) were obtained from guinea-pigs immunized against the Gag recombinant protein according standard protocols [19].

2.3. In vitro translation of 1731 RNAs

pAHAR1 is the pKS(+) Bluescript Vector (Stratagene) in which the pFP5c *Apa*I–*Eco*RI fragment (see Fig. 1) was inserted. After digestion by restriction enzymes, i.e. *Nru*I, *Sph*I or *Eco*RV, 1731 RNAs were produced in vitro (transcription system-Stratagene) and further translated in a rabbit reticulocyte lysate (Promega or Stratagene) in the presence of [³⁵S]methionine (30 MBq/ml)(Amersham) for 1 h at 37°C. Total synthesized ³⁵S-labelled proteins and immunoprecipitated peptides obtained with anti-Gag and anti-RT antibodies by using the protein A-Sepharose method [20] were analysed on fluorographed polyacrylamide gels. Fluorographs were scanned using a Hoefer GS300 densitometer (Hoefer Scientific Ins.)

2.4. Nuclei preparation

Drosophila cell nuclei were isolated at 4°C as previously described [21]: about 5×10^9 cells suspended in PBS were pelleted (800 $\times$ g, 5 min, 4°C) and resuspended in 10 mM HEPES (pH 7.6), 1.5 mM MgCl₂, 10 mM KCl and 0.5 mM dithiothreitol. After being allowed to stand 10 min at 4°C, cells were pelleted and resuspended in 2 volumes of the same buffer. They were disrupted by 20 strokes in a Dounce homogenizer (B-type pestle) and centrifuged (Beckman JA21 rotor, 2,500 rpm, 10 min). The supernatant was decanted and the nuclear pellet was centrifuged again through a 20% sucrose cushion (Beckman JA21 rotor-14,500 $\times$ g, 20 min). The nuclear pellet was then solubilized in sample buffer [17]. Samples were loaded onto SDS-polyacrylamide gels and electroblotted. Ponceau red staining of proteins electrotransferred onto nitrocellulose membrane monitored the presence of histones as major nuclear proteins.

2.5. VLP preparation, RT assay and immunoblotting

Drosophila cultured cells (about 3×10^9 cells) were gently detached from Falcon flasks, washed in TEN buffer (10 mM Tris, pH 8, 1 mM Na₂EDTA, 150 mM NaCl) and homogenized in the same buffer containing 10 μ g/ml of protease inhibitors (antipain, leupeptin, pepstatin and aprotinin). Following centrifugation (15,000 $\times$ g, 15 min), the post-mitochondrial supernatant [20] was layered onto a 20–30–70% sucrose step gradient in TEN buffer, spun 16 hours in a 50.2 Ti Beckman rotor (35,000 rpm, 4°C). In each gradient fraction the reverse transcriptase activity was tested, using Poly(rCm)-p(dG)(Pharmacia) as template primer [22], and proteins analyzed by immunoblotting under previously described conditions [8,12]. In some other assays the post-mitochondrial supernatant was directly spun over a 20% sucrose cushion (150,000 $\times$ g, 1 h) in order to obtain a rapid preparation of pelleted particulated forms.

3. Results

3.1. Characterization of anti-Gag antibodies

In order to test the specificity of anti-Gag antibodies raised

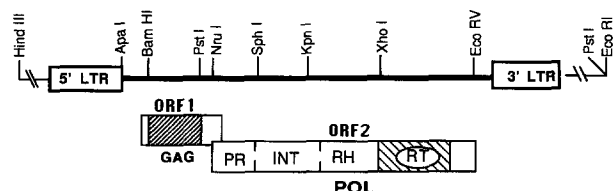


Fig. 1. Restriction map and genetic architecture of 1731. Schematic representation of the genomic 1731 element in plasmid pFP5C [5] showing the two ORFs and putative enzymatic domains of the ORF2: PR, protease; INT, integrase; RH, RNAseH; RT, reverse transcriptase. Thin line: flanking genomic sequences. Restriction sites used in this work are indicated. Hatched boxes represent parts of the genes expressed as recombinant proteins used for the preparation of antisera.

in guinea-pigs, Western blots of extracts from transformed *E. coli* cells were probed. Only a peptide of $M_r \sim 23,000$ produced in IPTG-induced RBA50 bacteria was recognized by the GPI antiserum (1:1,000 dilution) preabsorbed two times on the acetonic powder prepared from IPTG-induced RB791/RBB8 bacteria. Specificity of anti-Gag antibodies was confirmed by the fact that no protein was detected in IPTG-induced bacteria harbouring either pRBB8 or pCM294 plasmid (unpublished data). The later construct bears a 1731-ORF2 sequence and directs the expression of a 70 kDa protein, unrelated to the Gag peptide (KS construct in [8]). As described below, specificity of anti-Gag antibodies was also provided by the analysis of in vitro translation products.

3.2. Characterization of in vitro-produced Gag proteins

Fig. 2 shows the analysis of labelled polypeptides synthesized after translating 1731 RNAs produced from template DNAs containing either only a *gag* fragment or various *gag-pol* fragments. As expected, the *Apa*I–*Nru*I RNA was translated as a 27–28 kDa peptide (lane 1), corresponding to a truncated Gag peptide. However, the *Apa*I–*Sph*I RNA generated a peptide of about 32 kDa; this size is consistent with that of the entire ORF1 product, but lower than that expected for a 1.3 kb long coding sequence which was presumed to give a Gag-Protease fusion peptide (lane 2). Only in kinetic experiments and during the first 15–30 min of the translation reaction, was this expected 45 kDa polypeptide detected as a minor, weakly-labelled polypeptide representing about 5–6% of [³⁵S]methionine incorporated into the 32 kDa Gag peptide (not shown). When using the nearly entire translatable sequence of the 1731 element, contained in the *Apa*I–*Eco*RV RNA, larger peptides (M_r s from 50,000 to 110,000) and the 32,000 peptide were generated (lane 3). Taking account of the number of methionines present into the 1731 peptides, two in the Gag sequence and twenty-five in the Pol sequence, and measurements of incorporated [³⁵S]methionine, we calculated that 8 to 10 times more Gag peptide than Pol peptide were synthesized. Such a Gag/Pol ratio is usually found with other retrotransposable elements harbouring a frameshift event during the translation of the *gag-pol* sequence (see section 4).

Immunoprecipitation experiments performed with anti-Gag (GPI serum) and a previously described immunopurified antisera raised against the 1731 RT [8] allowed us to characterize both Gag and RT (pro)peptides. Two main translational products were immunoprecipitated by the anti-RT antibodies (lane 7, arrowheads): a species displaying an $M_r \sim 105/110,000$ was expected to derive from the entire Pol peptide and the other polypeptide of $M_r \sim 90,000$ could derive from processing of the Pol polypeptide. GPI anti-Gag antibodies immunoprecipitated only the radiolabelled polypeptides displaying the lower MW (M_r 32,000) translated from the tested 1731 RNAs, but no *pol*-derived peptides was recognized (lane 6). Noteworthy, these sizes fitted well to that expected for 1731 *gag* products ($\sim 30,000$ kDa). These data ensured that GP sera recognize proteins encoded by the 1731 *gag* gene (see section 4). Thereafter, GP antisera were used to probe Western blots of proteins extracted from *D. melanogaster* cultured cells and flies.

3.3. 1731 Gag protein is associated to VLPs

We previously had shown the colocalization of RT activity and 1731 RT-related polypeptides in VLPs-containing fractions

derived from a Kc cells post-mitochondrial supernatant [8]. In the present study, using the GPI antiserum, we analyzed a similar subcellular fraction extracted either from *D. melanogaster* Kc cells or from *D. hydei* DH33 cells. In Kc cells, anti-Gag antibodies detected a major band displaying an apparent size of ~42 kDa and, as expected from the absence of any 1731-related sequence in *D. hydei* genomic DNA, no immunoreactive material was revealed in DH33 cells (Fig. 3A), indicating that 1731 Gag protein is present only in *D. melanogaster* species. Moreover, after fractionation of the Kc cells post-mitochondrial supernatant over a sucrose gradient, we reproducibly detected a single immunoreactive protein band of M_r ~40,000 Da, found only in fractions displaying the strongest RT activity and as indicated by density measurement, coincident with the VLPs-containing fractions found at a density level of about 1.2 mg/ml [23] (Fig. 3B). These data demonstrate the association into the same fraction of both 1731-RT and 1731-Gag and suggest that these proteins are incorporated into the same structures.

As orderly species of Gag proteins have been described in both retroviruses and retrotransposons [24,25], the appearance of a single immunoreactive 1731 Gag protein in the gradient fractions was surprising. In order to search other forms of 1731 Gag, we concentrated cytoplasmic particles by pelleting a post-mitochondrial supernatant through a sucrose cushion, at $150,000 \times g$ for one hour. The proteinaceous pellet was solubilized and proteins separated on a 10–20% polyacrylamide denaturing gel before being transferred onto nitrocellulose. Immunoblot analysis using a sensitive chemiluminescent detection reaction revealed several GPI immunoreactive peptides, the most strongly labelled harboring M_r of ~44, ~40, ~27 and ~10 kDa (Fig. 4, lane C); the two larger forms likely correspond to the 42–40 kDa species observed in Fig. 3 and the fast migrating forms could be protease-processed forms of the Gag protein. The significance of these forms will be discussed.

3.4. A 1731 Gag-related protein is associated to the nuclear fraction

In a previous study, we have transfected *D. virilis* cultured cells with a plasmid bearing the *E. coli lacZ* gene fused to the 1731 gag gene. Some cells showed a 1731 Gag- β -galactosidase fusion protein associated with the nucleus [12], suggesting that the gag product could be directed to the nucleus. Next, nuclei from *Drosophila melanogaster* Kc0 cultured cells were purified and nuclear extract was prepared and immunoblotted (see section 2). The GPI antiserum revealed one major protein band of M_r ~22,000 Da and minor faint bands of M_r ~40,000 and ~30,000 Da (Fig. 4, lane N). The latter are similar in size to one major (40 kDa) and one minor (30 kDa) band of the $150,000 \times g$ pellet obtained from a postmitochondrial supernatant (lane C). In contrast, the band displaying an M_r of 22,000 kDa was recorded only in the nuclear extract when no corresponding immunoreactive protein was detected at this level in the cytoplasmic extract. This suggested that this 1731 Gag-related 22 kDa protein might be a nuclear-associated specific form.

4. Discussion

Analysis of the amino-acid sequence of 1731 ORF1 revealed the presence of a nucleic acid binding domain and led us to

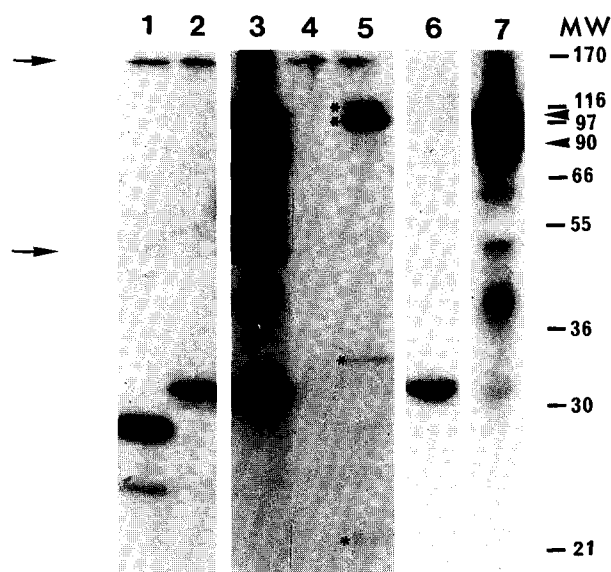


Fig. 2. In vitro translated 1731 proteins. In vitro-produced 1731 RNAs obtained from fragments of the 1731 *Apal*–*EcoRI* template inserted in pAHRI recombinant were translated in 20 μ l of rabbit reticulocyte lysate (Stratagene or Promega) in the presence of [35 S]methionine. 10 μ l of labelled translation products were analysed on a 15% polyacrylamide denaturing gel, either directly or following immunoprecipitation. The Coomassie blue-stained gel was prepared for fluorography using Na salicylate, dried and exposed on X-ray film. Lanes 1–3: translation products synthesized with RNAs obtained from the 1731 templates, *Apal*–*NruI* (1), *Apal*–*SphI* (2), *Apal*–*EcoRV* (3). Lanes 4–5: controls, no RNA (4) and BMV (Brome Mosaic Virus) RNA (5). Lanes 6–7: immunoprecipitated translation products from the *Apal*–*EcoRV* RNA, either with the GPI antiserum (6) or with the purified anti-RT antibodies (7) fixed on Protein A-Sepharose beads. The four BMV translated products (*) (20, 35, 97 and 110 kDa, lane 5) from BMV RNA (Promega) and reference proteins (Combitex-Boehringer) were used as size markers. Arrows on the left indicate the presence of endogenous synthesized peptides. Arrowheads: major immunoprecipitated peptides (lane 7).

consider it as a putative retroviral-like Gag protein [5] which is expected to be the main structural component of 1731 VLPs. Following insertion of the *E. coli lacZ* gene into the *NruI* site of 1731 (see fig. 1), we had obtained the first evidence for the existence of 1731 Gag-like proteins; indeed, 1731 Gag- β -galactosidase fusion proteins were found into VLPs-containing, RT-active fractions extracted from *Drosophila virilis* transfected cells [12]. As Gag proteins from both retrotransposons and retroviruses have been repeatedly proven to be difficult to characterize when only the M_r was considered [24,25], we prepared antibodies directed against the central part of the 1731 Gag protein, assuming that they would allow recognition of gag products in cells and/or in organisms.

4.1. Antibody specificity

The specificity of antibodies was demonstrated through several analyses: (i) in *E. coli*, the p23 recombinant Gag polypeptide is the only peptide recognized by the preabsorbed antisera; (ii) immunoprecipitation experiments showed that the Gag and Pol products were clearly and unambiguously distinguished by antibodies directed against each peptide (Fig. 2, compare lanes 6–7); (iii) as expected, comparison of VLPs-containing fractions extracted from both *Drosophila melanogaster* and *D. hydei* cultured cells demonstrated that 1731 Gag is detectable only in

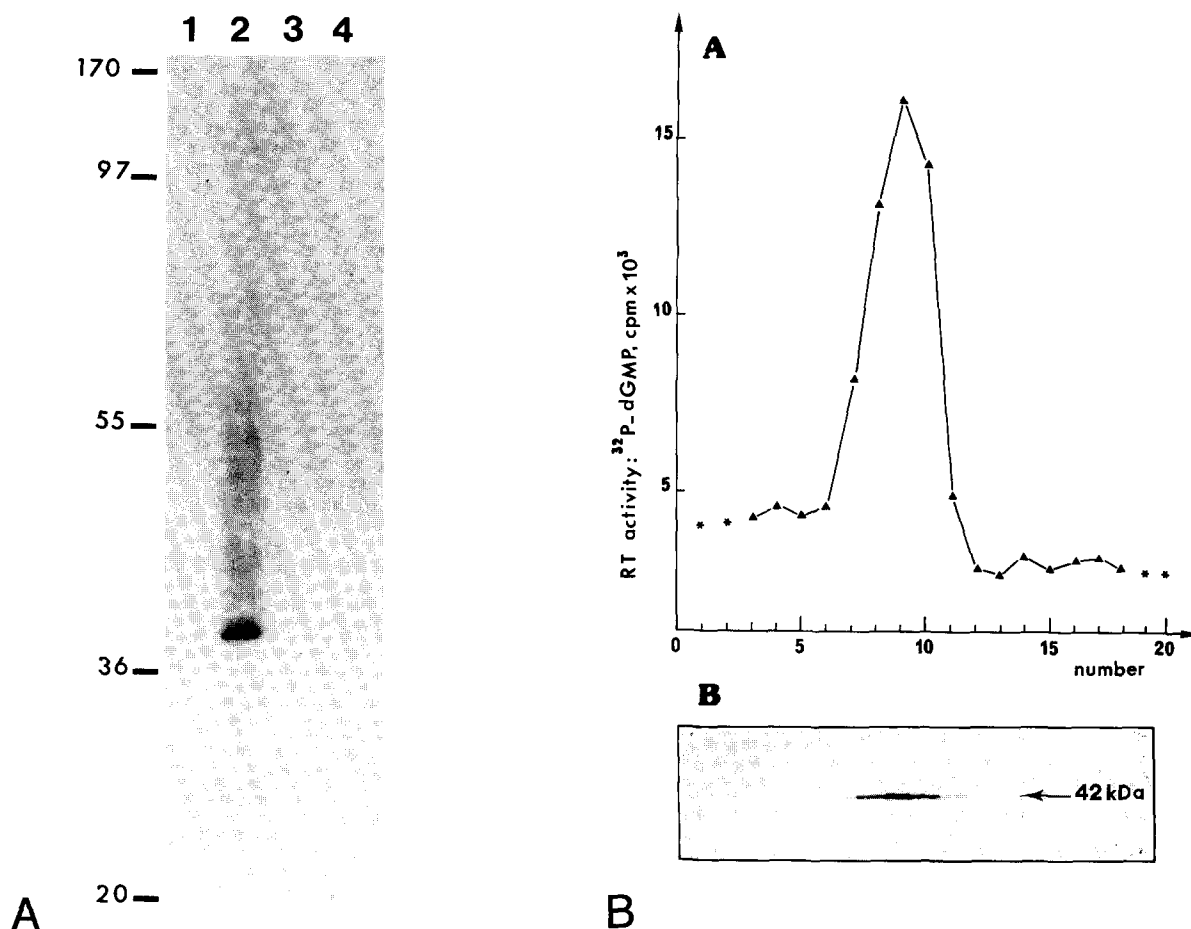


Fig. 3. 1731 Gag protein in the cytoplasmic fraction of *D. melanogaster* cultured cells (A) *absence of 1731 Gag in D. hydei cells*. Post-mitochondrial cytoplasmic extracts of *D. melanogaster* and *D. hydei* cells were centrifuged over a sucrose gradient; proteins from 150,000 $\times g$ pellets and supernatants were separated on a 12.5% polyacrylamide gel and immunoblotted with anti-Gag antiserum. Lanes 1–2: Kc0 *D. melanogaster* cells; 3–4: *D. hydei* cells; 1–3: 150,000 $\times g$ supernatants; 2–4: 150,000 $\times g$ pellets. Size markers are indicated on the left side of the figure. (B) *presence of 1731 Gag in VLP-containing fractions* (A) Upper part: RT activity in gradient fractions. Abscissa: fraction number. Ordinate: cpm of incorporated [^{32}P]dGMP. (B) Lower part: Western blot of the corresponding fractions. Arrow indicate the 1731Gag protein positioned at ~42 kDa. Cytoplasmic extract from Kc0 cells was fractionated on a sucrose step gradient (see section 2). From each 0.8 ml fraction, 20 μl were tested for their RT activity and 50 μl were analysed by immunoblotting of the proteins separated on a 12.5% polyacrylamide gel. The blot was probed with the GPI preabsorbed serum (1:1000 estimated concentration); the second antibody was used at a 1:4000 dilution and HRP activity revealed using the Boehringer chemiluminescence Western blotting reagents. Size markers were from Boehringer (Combitex) and from Novex USA (Mark 12).

Drosophila melanogaster cells (Fig. 3A). All these assays showed that these antisera can recognize their target proteins even if those are produced in various systems, i.e. in vitro systems or cells. Moreover, the colocalization of both 1731 RT-specific and 1731 Gag immunoreactivities along the step gradient fractionation is further circumstantial evidence for the specificity of the antisera raised against the recombinant 1731 Gag protein. Strengthening for this evidence came from the observation that immunoreactive fractions also displayed the strongest VLPs-associated RT activity (Fig. 3B).

4.2. Comparison of in vitro and in vivo products

4.2.1. Translational frameshift. Study of various retroelements had shown that the ORF1-derived Gag proteins are both the main translational products and components of viral or viral-like particles, whereas the amount of Gag-Pol products resulting from a translational frameshift represents only few percent of the former [26,27]. From 1731 sequence analysis it was expected that 1731 Gag-Pol precursor should be produced

by a +1 frameshift and consequently would be a transient and minor form of translated products. Indeed, in the in vitro assays, the final level of *pol*-derived peptides was about 8–10% of that found for the *gag* products. This result is in agreement with those obtained either for other retrotransposons, especially Tys in the yeast (reviewed by Farabaugh [28]), or in experiments using 1731 *pol*-CAT constructs which produced a CAT activity ten times lower than the activity generated by the 1731 5'LTR-CAT construct (C. Coulondre and Best-Belpomme, unpublished results). Surprisingly, in early in vitro translation experiments, no protein species fitting with the expected 1731 Gag-Pol fusion protein were detected (see Fig. 2, lanes 2, 3, 7). As an example, in addition to the p32 Gag peptide, the *ApaI*-*SphI* RNA was expected to generate a 45 kDa peptide containing the putative protease (Fig. 2, lane 2). Exclusively, in kinetic experiments, a weakly labelled peptide displaying such a size was recorded transiently during the first 15–30 min of the translation experiment. We hypothesise that this peptide corresponded to a 1731 Gag-Protease-containing peptide and was

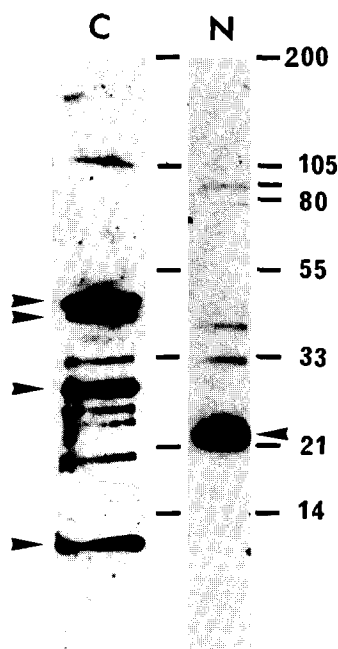


Fig. 4. Cytoplasmic and nuclear-associated species of 1731 Gag Proteins of the post-mitochondrial cytoplasmic and nuclear fractions prepared from Kc0 cultured cells (see section 2) were analysed on a 10–20% polyacrylamide gradient gel, and immunoblotted on the same nitrocellulose membrane. Immuno detection was made with GPI absorbed anti-Gag antiserum and HRP-conjugated secondary antibodies revealed by using a chemiluminescence system (Boehringer). Lane C: 1731 Gag in pelleted ($150,000 \times g$ - 1 h - $4^{\circ}C$) proteins extracted from a Kc0 cytoplasmic fraction. Lane N: 1731 Gag in Kc0 nuclear fraction. Size markers: 'Rainbow' colored markers (Amersham). Arrowheads indicate the major peptides described in the text.

detectable only during a short period preceding the proteolytic processing which allows the separation of the Gag and Pol moieties. This rapid processing could be attributed to either an instability of the truncated peptide or an *in vitro* enhanced protease activity and might explain why we were unable to detect the large Gag-Pol fusion peptides in our *in vitro* experiments. Interestingly, in some tissues of the fly, we have recently characterized a 1731 Gag-Pol polyprotein of around 140–145 kDa, recognized by both anti-Gag and anti-RT antibodies (in preparation). All these data suggest that in one hand a frameshift usually occurs in about 8–10% of translated 1731 RNAs and on the other hand, in most *Drosophila* tissues, the Gag-Pol polyprotein is rapidly processed by the protease.

4.2.2. The *in vivo* synthesized gag protein is larger than the *in vitro* translated products. 1731 ORF1 was expected to produce a polypeptide displaying a size in the 30 kDa range. By *in vitro* translation of 1731 RNAs (Fig. 2 lanes 1–3), a Gag polypeptide showing an M_r of 32 kDa was formed, consistent with the predicted size and immunologically identified by anti-Gag antibodies. This product, which is not post-translationally modified, is presumed to be the precursor peptide really synthesized *in vivo*.

In contrast, in Kc cell extract, the 1731 Gag protein was found in VLP-containing fractions, as a single species of about 40–42 kDa (Fig. 3). Its apparent molecular weight is quite similar to that found in fly extracts (unpublished result), but higher than the predicted 32 kDa or the Gag polypeptide syn-

thesized *in vitro* (Fig. 2). The p32 species is thought to be the primary, unmodified form of the Gag protein and the p40–42 should correspond to a post-translationally modified p32. Such apparent larger sizes of Gag proteins are frequently encountered and were reported for the two yeast retrotransposons Ty1 and Ty3 [25, 28–30]. However, the nature of the post-translational modification of 1731 Gag is unknown. For retroviral Gag proteins, addition of hydrophobic side chains as myristic or palmitic acid on some particular amino-acid positions is known to induce abnormal migration during electrophoresis. As for RSV Gag protein [31], it can be speculated that the presence of a glycine residue in the penultimate place of the amino-terminal side of the 1731 Gag protein could be involved in acylation modification; such hypotheses will be tested by using a mutated 1731 *gag* gene. It is noteworthy that such modifications are believed to play a rôle in localizing the retroviral Gag proteins to the plasma membrane during virus assembly [31]; conversely one can suppose that non-myristoylated Gag proteins are immobilized in the cytoplasm.

Analysis of the VLPs-containing fractions was fulfilled by testing a rather crude preparation (Fig. 4, lane C): four major bands were recognized by anti-Gag antibodies. The MW of the two largest polypeptides (40–45 kDa) are similar to those already observed in VLP-containing fractions and could correspond to the Gag polypeptide doublet described for other retroelements [29,30]. The significance of the peptides displaying lower MWs (27, 10 kDa) is hypothetical: in absence of any possibility to distinguish immunologically the amino or carboxy-end of the Gag protein, it can be proposed that they are cleavage products of the p40–45, similar to capsid and nucleocapsid peptides described for other retrotransposons [28].

4.3. Nuclear-localization of a Gag species

Conceptual translation of 1731 *gag* sequence revealed a polypeptidic sequence, SAKKRKDV which is similar to the Nuclear Localization Signal (NLS) present in proteins conveyed through the nuclear membrane from cytoplasm to nucleoplasm [33]. Consistently, we observed that some 1731 Gag- β -galactosidase fusion protein was associated to the nucleus of *Drosophila virilis* transfected cells [12]. Therefore, following the analysis of Kc cells cytoplasmic fractions, we tested a nuclear extract for the presence of 1731 Gag polypeptides. An unexpected prominent product in the M_r 22,000 range was immunologically revealed: no corresponding band was found neither in the postmitochondrial pellet nor in VLP extract, i.e. in cytoplasmic extracts (Fig. 3, 4N). As both extracts have been treated in the same way and separated on the same gel, it is clear that the p22 nuclear-associated species belongs exclusively to this fraction and that other Gag species are essentially of cytoplasmic origin. This nuclear localization could be attributable to the presence of a karyophilic signal which might be unmasked and functional or accessible only in this p22 form of 1731 Gag. Indeed, we described previously a putative karyophilic signal or NLS in the C-terminal end of the 1731 Gag amino sequence (nucleotide position: 1075–1099 in [5]). We hypothesised that such a sequence was brought by the p22 peptide, enabling its nuclear localization. This polypeptide is expected to derive from the C-terminal moiety of the 1731 Gag protein and to possess not only the NLS but also a nucleic acid binding site (nucleotide position: 1099–1147 in [5]). It is now well established that proteolytic control is involved in the mechanism of

nuclear addressing of some transcription factors [34]. Similarly, a specific proteolysis of the Gag precursor would be required to generate the p22 nuclear-associated species.

Among other retroviral elements, a NLS-containing Gag protein was reported for HIV-1 [13]; it was suggested that this NLS is able to induce an accumulation of non-myristoylated Gag products into the nuclei of infected cells and of *Spodoptera* insect cells transfected by a HIV-1 mutated *gag* gene [35]. Nuclear localization was also reported for *copia* Gag proteins overexpressed in yeast cells [36]. We can now propose that this direction to the nucleus could be monitored by a NLS-like sequence (nucleotide position: 1079 to 1102 in the cDm2055 plasmid - see [36]); this sequence is found close to the nucleic acid binding site (nucleotide position 1123 to 1165), suggesting similar structural organisations in the Gag products of both *copia* and 1731. It has also been reported that the Gag product of Moloney Leukemia Virus was associated with the nucleoprotein complex involved in the process of transposition [37]. In addition the localization of Gag protein of foamy viruses was found to be nuclear, a localization due to the presence of glycine-arginine rich boxes (NLS-like) in its sequence [14].

Altogether, these observations strongly suggest that during the life cycle of the retrotransposon, 1731 Gag products might have a more complex role than building up the VLP's wall, likely by directing some retrotranscription products to the nucleus. Compared to the behaviour of retroviral Gag peptides which are usually driven to the plasma membrane, the purely intracellular localization of the retrotransposon's VLPs could actually be due to some mutation located on the Gag product which impedes the budding process, and in turn allows an enhancement of targeting to the nucleus. In such a context, the putative functional role of the 1731 p22 Gag protein in the retrotransposon life cycle, either through a targeting of reverse transcripts to the nucleus or through an unknown regulatory function only operative in the nucleus, has now to be investigated.

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