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Production of a unique multi-lamella structure in the nuclei of yeast expressing *Drosophila copia gag* precursor

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Drosophila retrotransposon *copia* produces virus-like particles (VLPs) in the nuclei of cultured *Drosophila* cells. The VLPs contain *copia* RNA and reverse transcriptase activity, and thus, play a major role in *copia* replication. Here we have expressed the *copia gag* polyprotein precursor in yeast. The precursor, which includes *copia* protease itself, showed correct autoprocessing to produce a unique multi-lamella structure in the nuclei of the yeast cells. This expression system should be useful for the analysis of nuclear localization of the major *copia* VLP protein, and furthermore, would provide important information concerning the mechanism of *copia* VLPs formation.

Autoprocessing; Electron microscopy; *gag* Precursor; Heterologous expression; *Saccharomyces cerevisiae*; Retrotransposon *copia*

1. INTRODUCTION

The *Drosophila* transposable element *copia* is structurally related to retroviral proviruses [1–3]. It is 5 kb in length with long terminal repeats of 276 bp [4–6]. Major transcripts of *copia* are a full-length 5 kb and a 2 kb RNAs in cultured *Drosophila* cells [7], and the smaller one is generated by splicing of the 5 kb RNA [8,9]. Nucleotide sequence analyses indicate that both the 5 kb [10,11] and 2 kb [8,9] RNAs contain open reading frames (ORFs) capable of encoding 1409 and 426 amino acid polyproteins, respectively. Translation products of these ORFs seem to be similar to retroviral *gag-pol* and *gag* polyprotein precursors, respectively [8,9,11]. And *copia* protease is mapped in the *gag* precursor as in the case for avian retroviruses [8,12].

Based on many observations such as *copia* RNA directed DNA synthesis by a methionine tRNA [13] and the finding of an unusual genomic *copia* which lacks the region corresponding to the intron of the 2 kb RNA [14], *copia* is believed to replicate by a mechanism similar to that of retroviral replication. The similarity of *copia* and retroviruses has been further strengthened by the finding of virus-like particles (VLPs) containing *copia* RNA and reverse transcriptase activity in the nuclei of cultured *Drosophila* cells [15]. Our previous study [8] demonstrated that the 2 kb *copia* RNA contains sufficient information to make the VLPs, probably through autoprocessing of the *copia gag* precursor, in cultured *Drosophila* cells. Although the VLPs play an important role for *copia* replication, the precise mechanism of the VLPs formation has not been known. As a first step to solve the problem, we have now established the expression system of the *copia gag* precursor in yeast.

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2. MATERIALS AND METHODS

2.1. *Plasmid construction*

Expression plasmid pYC1 was constructed using the plasmids pEC1 [16] and pAA7 (a kind gift of T. Fukazawa). pEC1 was digested with *Nco*I and filled in with Klenow fragment. The released 1.4 kb fragment contains the entire region of the ORF encoded by the 2 kb *copia* RNA (termed ORF2). pAA7 was digested with *Bgl*II and *Sal*I, filled in with Klenow fragment, and the released 8 kb fragment, which contains yeast *GAL7* promoter and the 2 μm plasmid replication origin, was ligated with the 1.4 kb fragment to give rise to pYC1.

2.2. *Strains and transformation of yeast cells*

Saccharomyces cerevisiae, CG380 (*MAT* a/α, *ADE5/ade5, HIS7/his7, leu2/leu2, trp1/trp1, ura3/ura3*; A. Fujita, unpublished), was used for yeast transformation with the plasmid pYC1. The plasmid was propagated in *E. coli* JM109 [17].

The lithium acetate method was employed for transformation of yeast cells [18]. Ura$^+$ transformants were selected on 2% agar plates containing minimal medium (0.7% yeast nitrogen base, 2% glucose, leucine (360 μg/ml) and tryptophan (240 μg/ml)).

2.3. *Yeast cell growth for protein expression*

Recombinant yeast cells were grown at 30°C in YPD (1% yeast extract, 1% polypeptone, 2% glucose) until early-log phase (OD_{660}=0.3–0.4). Then, cells were collected and resuspended in YP-

galactose (1% yeast extract, 1% polypeptone, 2% galactose). The cells were grown at 30°C for an appropriate period. For electron microscopy the *copia gag* gene was induced for 10 h. As a negative control, the recombinant yeast cells were grown at 30°C in YPD without changing the medium with YP-galactose at the early-log phase. The incubation periods were the same with those of the yeast cells grown in YP-galactose.

2.4. *Other methods*

Western blot analysis and electron microscopy were described previously [8,16].

3. RESULTS AND DISCUSSION

To express the *copia gag* precursor in yeast, the entire region of ORF2 (the ORF encoded by the 2 kb copia RNA) was placed downstream of yeast *GAL7* promoter. The resultant plasmid was designated pYC1 (Fig. 1). Expression of the *gag* precursor is, therefore, controlled by galactose.

Immunological analysis of yeast strain containing pYC1 (termed YC1) was performed and the result is shown in Fig. 2. When YC1 was grown in the presence of galactose, two major polypeptides were detected using anti-VLP serum. One is a 48 kDa polypeptide and the other one is a 33 kDa polypeptide. The sizes of these polypeptides correctly correspond to those of the *copia gag* precursor and the major *copia* VLP protein, respectively. Both the 48 kDa and 33 kDa polypeptides appeared 4 h after the *gag* expression was induced. The 33 kDa polypeptide increased with time. In contrast, no specific band was detected using anti-VLP serum when YC1 was grown in the absence of galactose (data not shown). These results indicate that the *gag* precursor expressed in yeast produces the major VLP protein through autoprocessing as in the case for in vitro [8] and in *E. coli* [16].

Furthermore, we analyzed the yeast YC1 using electron microscopy. When YC1 was grown in the presence of galactose, a unique multi-lamella structure was found in the nuclei of the cells (Fig. 3a and b). Since the multi-lamella structure was not detected in YC1 grown without galactose (Fig. 3c), the structure should be the

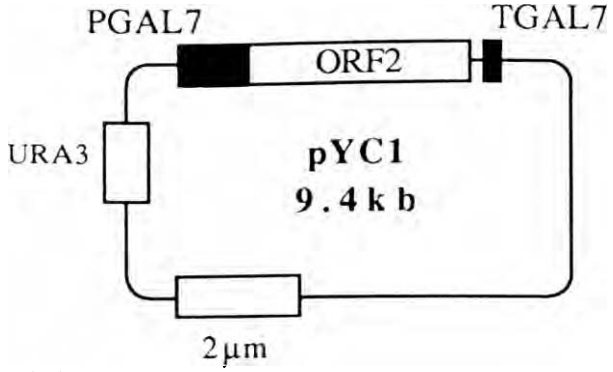

Fig. 1. Structure of the expression plsmid pYC1. The closed boxes indicate the yeast *GAL7* promoter (PGAL7) and terminator (TGAL7) regions. The open boxes indicate yeast *URA3* gene (URA3) and the 2 μm plasmid replication origin (2 μm). Transformants with the plasmid pYC1 were selected for Ura⁻ phenotype.

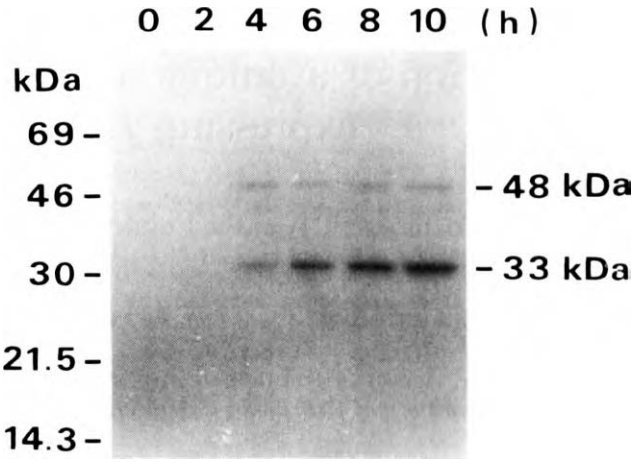

Fig. 2. Western blot analysis of the *copia gag* precursor expressed in yeast YC1. At the times indicated, cells (approximately 2×10^6) were suspended in 10 μl of the lysis buffer, consisting of 50 mM Tris-HCl pH6.8, 2% SDS, 5% glycerol, 5% 2-mercaptoethanol, 0.01% Bromophenol blue. The proteins were separated on a 12.5% SDS-polyacrylamide gel, transferred to nitrocellulose, and subjected to immunoblot analysis using anti-VLP serum. Mol.Wt. markers are given on the left.

assembled product of the 33 kDa polypeptide. The structure is, however, quite different from that of *copia* VLPs in cultured *Drosophila* cells, and also somehow different from a laminate structure [16] detected in *E. coli* expressing the *copia gag* precursor. These observations and the fact that *copia* VLPs formation may need molecular chaperone, and the presence of different structures in each organism may reflect the difference of molecular chaperone.

To date, it has not been known whether the assembly of the major *copia* VLP protein occurs in cytoplasm or in nucleus. The yeast expression system presented here should be useful for the analysis of nuclear localization of the major VLP protein, and furtheremore, may provide informative data concerning the mechanism of *copia* VLPs formation.

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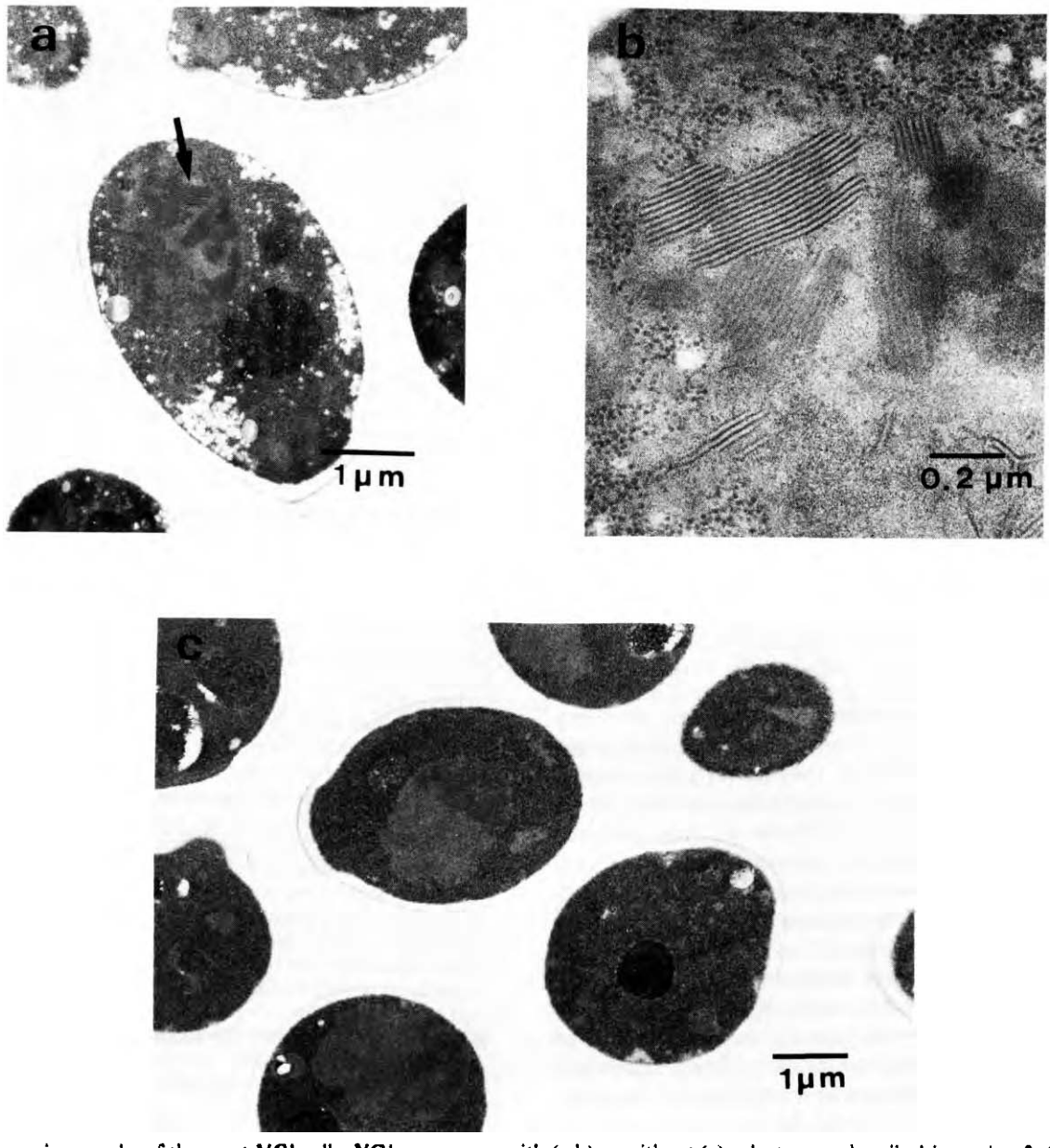

Fig. 3. Electron micrographs of the yeast YC1 cells. YC1 was grown with (a,b) or without (c) galactose as described in section 2. Specimens for electron microscopy were prepared as described previously [16]. The arrow indicates the multi-lamella structure. The scale bars are 1 μm (a), 0.2 μm (b) and 1 μm (c).

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