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TY3 *GAG3* protein forms ordered particles in *Escherichia coli*

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Abstract

The yeast retrovirus-like element Ty3 *GAG3* gene encodes a Gag3 polyprotein analogous to retroviral Gag. Gag3 lacks matrix, but contains capsid, spacer, and nucleocapsid domains. Expression of a Ty3 Gag3 or capsid domain optimized for expression in *Escherichia coli* was sufficient for Ty3 particle assembly. Virus-like ordered particles assembled from Gag3 were similar in size to immature particles from yeast and contained nucleic acid. However, particles assembled from the CA domain were variable in size and displayed much less organization than native particles. These results indicate that assembly can be driven through interactions among capsid subunits in the particle, but that the nucleocapsid domain, likely in association with RNA, confers order upon this process.

Keywords: Retrotransposon; Retrovirus; Virus-like particles; Capsid; *Escherichia coli*; Yeast Ty3

Introduction

Gag, the major structural component of retrovirus cores, typically contains matrix (MA), capsid (CA), nucleocapsid (NC), and one or more spacer (SP) domains as well as other virus-specific domains. Within the Gag context, MA functions in membrane targeting; CA mediates Gag interaction; and NC packages RNA and promotes Gag interaction (Adamson and Jones, 2004; Scarlata and Carter, 2003). NC is a small domain rich in basic residues that typically contains one or two copies of a $CX_2CX_4HX_4C$ zinc-binding motif. After particle assembly and budding, these domains are separated by proteolytic cleavage and characteristic cores are formed. Expression of Gag in heterologous systems, such as baculovirus and *Escherichia coli*, has shown formation of virus-like particles (VLPs). In the case of HIV, depending on salt and pH conditions CA can form tubes, spheres, and cones *in vitro*. Inclusion of the NC domain and RNA enhances formation of sheet-like structures or cylindrical forms (Ehrlich et al., 1992; Ehrlich et al., 2001; Campbell and Vogt, 1995; Ganser et al., 1999; Johnson et al., 2002; Yu et al., 2001). Formation of spheres approximating immature particle forms occurs when regions amino-terminal to CA are included. This effect is attributed to constraint of the amino terminus of CA which rearranges during maturation and is engaged in an internal salt bridge in the mature form (von Schwedler et al., 1998; Gross et al., 1998; Joshi and Vogt, 2000; Rumlova-Klikova et al., 2000; Campbell and Vogt, 1997).

The budding yeast retrotransposon Ty3 has a Gag3 domain comprised of CA, SP3 and NC domains (Fig. 1) (Sandmeyer et al., 2002). Initial assembly of Ty3 results in a roughly spherical VLP which remains in a similar form even after proteolytic maturation (Kuznetsov et al., 2005). The Ty3 precursor differs from the retroviral precursor in that it lacks the amino-terminal extension of CA into MA, as well as the conserved amino-terminal proline of CA. In this work, we determined that expression of Ty3 Gag3 was sufficient to form spherical particles in a bacterial expression system. The organization of particles was greatly enhanced by inclusion of the NC domain.

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Results

Assembly of recoded GAG3

Because Ty3 Gag3 protein is comprised only of CA, SP, and NC domains, it was of interest to determine whether its expression was sufficient to support spherical particle formation in a heterologous system. A synthetic Ty3 *GAG3* gene optimized for bacterial protein expression produced significant levels of Gag3 (Fig. 2, lanes 1–3). Little if any protein expression was observed from the native yeast Ty3 *GAG3* gene (Fig. 2, lanes 4–6). About 40% of the recoded Gag3 was soluble (Fig. 3A). The nature of the insoluble protein fraction was not explored, but it could have contained Gag3 inclusion bodies or aggregates.

Atomic force microscopy analysis of E. coli cell lysates expressing Gag3

Approximately 500 μg of cleared lysate from cells expressing recoded Gag3 was fractionated over a 20/30/70% sucrose step gradient as previously described (Kuznetsov et al., 2005). Gradient fractions were analyzed by denaturing polyacrylamide gel electrophoresis. A protein of the same size as Gag3 that reacted with anti-CA antibody was the major component of fractions 7 and 8 (Fig. 3B); this is the position of migration of native Ty3 VLPs produced in yeast (Kuznetsov et al., 2005 and data not shown). A 5 μl aliquot of the 70/30% interface fraction was used for AFM imaging as described previously. Imaging of the interface fraction showed particles, ranging from 30 to 50 nm in diameter (Figs. 4A–C). These were of comparable size to protease mutant Ty3 VLPs produced in budding yeast, which had a mean of 49 nm ± 2.3 nm (Kuznetsov et al., 2005 and data not shown). A small proportion of VLPs appeared to be joined, mostly in pairs (e.g. Fig. 4C). Similar associations were observed in AFM imaging of wt Ty3 VLPs from yeast (Kuznetsov et al., 2005). Overall, VLPs displayed ordered capsomeres and were generally similar to those previously observed. Tapping disruption of the particles resulted in release of fibrous material from some which resembled RNA (Figs. 4G–H), suggesting that similar to retroviruses, Ty3 Gag3 can package heterologous RNA in the absence of its native genome.

Expression of CA and AFM analysis of E. coli expression lysates

In order to determine the contribution of SP and NC domains to VLP structure, the CA-coding domain was amplified using

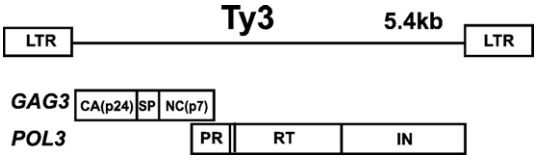

Fig. 1. Ty3 organization. Ty3 ORFs *GAG3* and *POL3* are shown with protein domains capsid (CA), spacer (SP), nucleocapsid (NC), protease (PR), reverse transcriptase (RT), and integrase (IN). PR and RT domains are separated by a domain of unknown function of approximately 10 kDa. Gag is a 290 aa polyprotein which is processed into 207 aa CA, 26 aa spacer, and 57 aa NC.

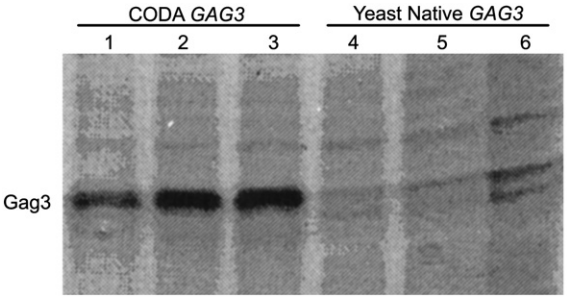

Fig. 2. Recoded Gag3 is expressed at a higher level in *E. coli* than native yeast Gag3. DH5α cells were induced to express Gag3 from pET3a-CODA-GAG (lanes 1–3) and pET3a-yeast-native-GAG3 (lanes 4–6) for 1, 2, and 3 h, respectively. Protein extracts of 50 μl of cells expressing Ty3 Gag3 were fractionated on SDS 10% polyacrylamide gels and stained with Coomassie Brilliant Blue R-250 as described in Materials and methods.

recoded *GAG3* sequence as the template. Expression of this domain from the IPTG-inducible T7 promoter was efficient, but failed to produce sufficient levels of soluble protein for analysis (data not shown). The construct was recloned into an arabinose-inducible expression vector (pBAD-CODA-CA) where expression was controlled to optimize solubility (data not shown). Under these optimal conditions, much less CA protein was produced than Gag3, but the proportion of soluble protein was comparable to that in the Gag3 expression system (Figs. 3A and C). Approximately 500 μg of cleared lysate from cells expressing pBAD-CODA-CA was fractionated over a 20/30/70% sucrose step gradient and 5 μl of the 30/70% interface fraction was used for AFM imaging as

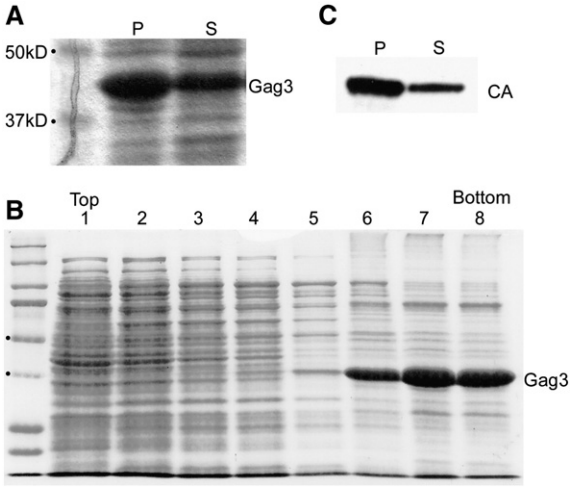

Fig. 3. Expression of Gag3 and CA in *E. coli*. DH5α cells were induced to express pET3a-CODA-GAG3 (A) and pBAD-CODA-CA (C) as described in Materials and methods. Whole cell extracts were centrifuged and pellets were resuspended in a volume equal to the supernatant. Equal aliquots of pellets (P) and supernatant (S) were fractionated on SDS 10% polyacrylamide gels. Gag3 (A) was visualized by staining with Coomassie Brilliant Blue R-250. (B) Clarified whole cell extract prepared from cells expressing pET3a-CODA-GAG3 as described in Materials and methods was fractionated on a 20%30%/70% sucrose step gradient and analyzed by SDS 10% gel electrophoresis. Fractions 7 and 8, corresponding to the 30%/70% interface and below, contained Ty3 Gag3 protein. (C) CA, which was expressed at a lower level than Gag3, was visualized by immunoblot analysis. Size markers were included in all gels, and relevant markers are indicated with dots in A and the same markers are indicated with dots in B.