A prion-like domain in Gag capsid protein drives retrotransposon particle assembly and mobility
Pascale Lesage, Patrick Maxwell

To cite this version:
Pascale Lesage, Patrick Maxwell. A prion-like domain in Gag capsid protein drives retrotransposon particle assembly and mobility. Proceedings of the National Academy of Sciences of the United States of America, 2023, 120 (35), 10.1073/pnas.2311419120. hal-04258672

HAL Id: hal-04258672
https://hal.science/hal-04258672
Submitted on 14 Nov 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License
A prion-like domain in Gag capsid protein drives retrotransposon particle assembly and mobility

Pascale Lesage¹ and Patrick H Maxwell²

¹Université Paris Cité, IRSL, Inserm, U944, CNRS, UMR7212, 75010 Paris, France
²Biology Department, Siena College, Loudonville, NY, USA

Prion domains are diverse amino acid sequences that acquire alternative conformations that become self-propagating. Transformation of proteins into prions is generally accompanied by a propensity to aggregate proteins into oligomers. Some prion proteins cause fatal neurodegenerative disorders while other can perform cellular functions (1). In this issue of PNAS, Beckwith et al. demonstrate that Ty1, a retrovirus-like retrotransposon in budding yeast, encodes a prion-like domain (PrLD) within the capsid protein. The study describes the prionogenic activity of the Ty1 PrLD and its essential roles in virus-like particle morphology and assembly and in retrotransposition (2).

Retrotransposons propagate in genomes by reverse transcription of an RNA intermediate and integration of the resulting complementary double strand DNA (cDNA) into a new genomic location. Retrotransposons play a major role in genome evolution. Recent work has shown that intermediate steps in retrotransposon replication can have significant health impacts, regardless of whether cDNA is introduced into the host genome, highlighting the importance of understanding all steps of retrotransposon replication. Cytoplasmic cDNA production from human Alu or L1 retrotransposons triggers innate immune responses contributing to macular degeneration or cell aging, respectively (3, 4). Furthermore, retroviral particles formed by endogenous retroviruses can contribute to senescence and tissue aging (5).

Long terminal repeat (LTR)-retrotransposons are structurally and functionally related to retroviruses, collectively known as retroelements, except that LTR-retrotransposons lack an envelope gene and are not infectious. The Ty1 and Ty3 LTR-retrotransposons of the yeast S. cerevisiae have served as excellent models for retroelement replication and regulation (6, 7). Ty1 has a classic LTR-retrotransposon organization with LTRs flanking
GAG and POL open reading frames (Figure 1). The GAG gene encodes a structural protein with RNA-binding domains that forms virus-like particles (VLPs) to encapsidate and protect Ty1 RNA. The POL gene encodes the enzymatic activities protease (PR), integrase (IN) and reverse transcriptase ribonuclease H (RT-RH), required for retrotransposition. Cytoplasmic VLPs play an essential role in the replication cycle, keeping Ty1 RNA and the enzymatic activities required for replication in close proximity. They are the sites of RNA packaging, Gag-Pol precursor maturation and reverse transcription of RNA into cDNA. Membraneless cytoplasmic foci, where retrotransposon RNA and proteins are assembled, have been identified for Ty1 and Ty3 and may represent an essential step in VLP formation (8–10). However, it was not understood how these foci, known as retrosomes, transition into mature VLPs.

Beckwith et al. characterized the N-terminal portion of Ty1 Gag with the broader goal of learning more about retrosome and VLP formation. No function had previously been identified for this Gag region, which was predicted to be unstructured. The authors used bioinformatic tools to show that a 71 amino acid region near the Ty1 Gag N-terminus has an amino acid composition very similar to that of yeast prion proteins, which they call the prion-like domain (PrLD) of Gag. The authors used a well-established prionogenesis assay to detect prion protein self-aggregation, developed for the yeast translational terminator prion protein Sup35, to demonstrate that the Ty1 Gag PrLD could functionally substitute for the Sup35 prion domain to drive aggregate formation. PrLD deletion blocks Ty1 retromobility, which includes retrotransposition to non-homologous sites and insertion of cDNA by recombination with genomic Ty1. In a clever way, the authors used a two-plasmid system, one encoding Ty1 proteins and the other providing a minimal RNA template for reverse transcription, to clearly show that the inhibition of retromobility was due to deficiency in Gag function as opposed to loss of RNA sequences necessary for Ty1 RNA packaging in VLPs. In fact, the yeast Sup35 prion domain could functionally substitute for the Gag PrLD to support wild-type Ty1 retromobility, the mouse PrP prion domain supported a low level of retromobility, while the yeast Ure2 prion domain and the human amyloid beta peptide Aβ1-42 failed to do so. The discovery that a prion domain performs an important function in retromobility is new and unexpected. One might have expected the Gag protein to contain organized structural domains that ensure Gag-Gag
interactions to scaffold the VLP, but the involvement of a prion-like domain raises new questions about the regulation of VLP assembly, especially since all prion sequences did not impact Ty1 retromobility in the same way.

The authors assessed the impact of the Ty1 Gag PrLD on microscopically visible Gag foci and VLP formation. When they expressed a plasmid copy of Ty1 Gag fused to GFP, most wild type cells exhibited multiple Gag-GFP foci. Although the authors did not provide evidence that Ty1 RNA is associated to Gag-GFP, these foci could be retroosomes or their precursors. Replacing the Gag PrLD with the yeast Sup35 prion domain led to virtually the same pattern of Gag-GFP foci, while the mouse PrP prion domain and human Aβ1-42 resulted in a mixture of abnormal and normal foci. In contrast, substitution with the yeast Ure2 prion domain or deletion of the Gag PrLD caused a diffuse Gag-GFP signal in nearly all cells (Figure 1). The association of a variety of Gag:GFP foci phenotypes with different prion domains suggests that Gag PrLD influences Gag coalescence and assembly into VPLs or pre-VLP assemblies. However, not any prion sequence can induce Gag self-assembly and the mere formation of Gag foci is not sufficient for retromobility.

To investigate a connection between the PrLD and VLP assembly, the authors showed that Gag protein is predominantly present in higher density sucrose gradient fractions in extracts from cells expressing wild type Ty1 or Ty1 with either the Sup35, PrP, or Aβ1-42 domains in place of Gag PrLD (Figure 1). In contrast, deletion of PrLD or replacement with the Ure2 domain resulted in Gag predominantly being present in low density fractions. Ty1 VLPs are heterogeneous in size, with diameters ranging from 30-80 nm, and by transmission electron microscopy (TEM) appear as electron dense shells around lighter cores. The surface of the porous VLP shell is formed by the Gag N-terminus, including PrLD, with the C-terminal end of Gag facing towards the core (7). TEM data showed that replacement of PrLD with the Sup35 or PrP domains resulted in wild-type VLP morphology, while replacement by Aβ1-42 formed electron-dense VLPs, lacking a central lumen (Figure 1). No VLPs were detected following deletion of PrLD or its replacement by the Ure2 domain. Only the Sup35 substitution supported efficient proteolytic processing of Ty1 RT and IN domains by PR in VLPs. While VLPs can be formed in the absence of PR activity (10), it is intriguing that replacement of the prion-like domain by the PrP
sequence appears to support assembly of normal VLP structures, but results in very low PR activity as well as low retromobility. Hence, the Ty1 PrLD is not uniquely required for VLP assembly but it might also induce subtle changes in VLP structure or surface interactions that may have substantial impacts on subsequent steps of Ty1 replication or have other functions in Ty1 replication.

The cellular environment contains various membraneless compartments, also known as biomolecular condensates. These are often formed by liquid-liquid phase separation (LLPS), a thermodynamic process whereby distinct, coexisting liquid phases are formed to minimize free energy, so that macromolecular complexes do not mix with the surrounding cellular environment (11). Prion-like RNA binding proteins have the properties to form such condensates (12). Gag PrLD might also function by phase separation to compartmentalize Ty1 components in the cytoplasm. Phase separation could take place during the coalescence of Gag and Ty1 RNA into retrosomes, followed by assembly into VLPs, or VLP assembly could be the point at which LLPS happens, if indeed it is occurring. Whatever the biophysical characteristics of these condensates, the study of Beckwith et al. represents an important finding beyond the Ty1 model, as it shows for the first time that a prion-like domain in Gag is required for VLP assembly, a step in retrotransposition that is still poorly understood in general. Moreover, the observation that heterologous prion domains are sufficient to drive retroelement particle assembly and propagation opens up novel ways to dissect the mechanism of particle assembly and validates the use of Ty1 as a powerful platform to test simply and rapidly in yeast, the ability of an unstructured sequence to form condensates in vivo.

Formation of biomolecular condensates is a common phenomenon for diverse viruses and TEs (13). In the case of the human adenovirus, a nuclear-replicating DNA virus, phase separation of an intrinsically disordered region of the viral 52-kDa protein has a critical role in the coordinated assembly of infectious progeny particles (14). 52K protein is not a bona fide capsid protein but it organizes viral capsid proteins into nuclear biomolecular condensates, which are a prerequisite for the assembly of complete, packaged particles containing viral genomes. The human L1 non-LTR retrotransposon ORF1p is also not a capsid protein, but forms a ribonucleoprotein particle necessary for retrotransposition, and
also forms condensates with or without RNA in vitro (15, 16). Many viruses and TEs, including retrotransposons, are activated or inhibited by a variety of stress conditions. Interestingly, phase separation can provide a dramatic response to small changes in environmental conditions (17). Phase separation or other means of forming biomolecular condensates could therefore provide a common contribution to the sensitivity of retrotransposons and viruses to various environmental stresses and be a trigger for viral replication activation (18). In this respect, it would be interesting to determine whether the Gag-PrLD can be a stress sensor regulating Ty1 retrotransposition.

This work once again demonstrates the relevance of the Ty model for discovering new fundamental properties of the replication of retroelements and exploring new avenues of their regulation.

References.
2. Beckwith et al. PNAS 2023, in press.

**Figure 1.** Ty1 replication cycle. An endogenous Ty1 element (purple rectangle bracketed by two triangles representing the LTRs) is transcribed in the nucleus and the Ty1 RNA (wavy purple lines) is exported to the cytoplasm. The RNA is translated into Gag and Gag-Pol proteins and associates with these proteins to form membraneless ribonucleoprotein particles or retrosomes. Retrosomes give rise to virus-like particles (VLPs), in which Gag and Pol proteins are cleaved by protease (PR) to form mature Gag, PR, integrase (IN), and reverse transcriptase ribonuclease H (RT-RH) proteins. Following VLP maturation, Ty1 RNA is reverse transcribed into cDNA by RT. IN binds to the cDNA to form the pre-integration complex, which is imported into the nucleus. IN integrates Ty1 cDNA into the yeast genome. A table below the replication cycle schematically summarizes data from the study by Beckwith et al. for Ty1 with its normal Gag prion-like domain (PrLD), a heterologous prion domain substitution (Sup35, PrP, Human Aβ, or Ure2), or a deletion of PrLD (None).

**Acknowledgments**
PL's research is supported by Fondation pour la Recherche Médicale (FRM-EQU202203014635), Institut National du Cancer (INCA PLBIO-2020-312) and intramural funding from CNRS, Université de Paris and INSERM. PM's research is supported by institutional funds from Siena College.