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A self-encoded capsid derivative restricts Ty1 retrotransposition in *Saccharomyces*

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Abstract

Retrotransposons and retroviral insertions have molded the genomes of many eukaryotes. Since retroelements transpose via an RNA intermediate, the additive nature of the replication cycle can result in massive increases in copy number if left unchecked. Host organisms have countered with several defense systems, including domestication of retroelement genes that now act as restriction factors to minimize propagation. We discovered a novel truncated form of the *Saccharomyces* Ty1 retrotransposon capsid protein, dubbed p22 that inhibits virus-like particle (VLP) assembly and function. The p22 restriction factor expands the repertoire of defense proteins targeting the capsid and highlights a novel host-parasite strategy. Instead of inhibiting all transposition by domesticating the restriction gene as a distinct locus, Ty1 and budding yeast may have coevolved a relationship that allows high levels of transposition when Ty1 copy numbers are low and progressively less transposition as copy numbers rise. Here, we offer a perspective on p22 restriction, including its mode of expression, effect on VLP functions, interactions with its target, properties as a nucleic acid chaperone, similarities to other restriction factors, and future directions.

Keywords

retrotransposon; Gag; RNA; nucleic acid chaperone; VLP assembly; restriction factor; *Saccharomyces*

INTRODUCTION

Retrotransposons and endogenous retroviruses dominate the chromosomal landscape in many eukaryotes and comprise over 40% of the human genome (Goodier and Kazazian 2008, Jern and Coffin 2008). The *S. cerevisiae* reference genome contains long terminal repeat (LTR) retrotransposons Ty1-Ty5, yet only 3% of budding yeast's compact genome is

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comprised of Ty sequences (Kim, et al. 1998). Ty elements are effective models to understand retroviral and eukaryotic transposon biology because they inhabit the genome of a powerful model organism and are representative of a widely disseminated family of Copia/Ty1 LTR-retrotransposons that are particularly abundant in plant genomes (Lee and Kim 2014). We study Ty1 elements because they are transcriptionally active and competent for retrotransposition, can cause mutations by insertional mutagenesis or genome rearrangement, and at 32 copies, are the most abundant retrotransposon in the reference strain [see review by (Curcio, et al. 2015)]. Ty1 structure and replication cycle resembles that of retroviruses, except Ty1 transposition is not infectious (Garfinkel, et al. 1985) (Fig. 1). Ty1 is transcribed from LTR-to-LTR, forming a genomic RNA with the structural hallmarks of retroviral genomic RNA. Ty1 RNA serves as a template for protein synthesis, and reverse transcription, which occurs after packaging of the RNA into VLPs. Translation of Ty1 *POL* requires a specific +1 frameshift event near the end of *GAG* (Farabaugh 1995), resulting in a large Gag-Pol precursor (p199). Cytoplasmic foci, termed T-bodies or retrosomes (Beliakova-Bethell, et al. 2006, Checkley, et al. 2010, Dutko, et al. 2010, Malagon and Jensen 2008) contain Ty1 RNA, Gag, presumably Gag-Pol and perhaps specific cellular proteins (Doh, et al. 2014, Suzuki, et al. 2011), and are sites where VLPs assemble. Cotranslational localization of Ty1 RNA to the endoplasmic reticulum (ER) followed by Gag retrograde translocation from the ER to the cytoplasm stabilizes Gag and facilitates VLP assembly (Doh, et al. 2014). Our work suggests that Gag may encounter Ty1 RNA at an earlier step since Gag increases the stability and nuclear export of *GALI*-promoted Ty1 RNA (Checkley, et al. 2013). Reverse transcription takes place within mature VLPs following protein processing by an element-encoded protease (PR). Ty1 PR cleaves Gag-p49 near the C-terminus to generate Gag-p45 and Gag-Pol-p199 to form mature PR, integrase (IN), and reverse transcriptase (RT). Ty1 Gag is a multifunctional protein but unlike retroviral Gag is not cleaved into functionally distinct proteins such as matrix, capsid (CA) and nucleocapsid (NC), even though Ty1 Gag executes the same functions as retroviral CA and NC. A synthetic peptide containing sequences from the C-terminal region of Gag-p45 (Cristofari, et al. 2000) and recombinant Gag derivatives (Nishida, et al. 2015) display nucleic acid chaperone (NAC) activity *in vitro*, similar to NC. Like HIV-1 (Tekeste, et al. 2015, Zhu, et al. 2004), a complex formed between Ty1 RT and IN is required for reverse transcription (Wilhelm and Wilhelm 2006) and tRNA_i^{Met} primes minus-strand synthesis (Keeney, et al. 1995). A preintegration complex minimally containing IN and Ty1 cDNA returns to the nucleus via a classical import pathway that recognizes a nuclear localization signal near the C-terminus of IN (Kenna, et al. 1998, McLane, et al. 2008, Moore, et al. 1998). Ty1 usually integrates upstream of genes actively transcribed by RNA Polymerase III (Bridier-Nahmias, et al. 2015, Devine and Boeke 1996). Unlike other Ty elements, Ty1 integrates into genes transcribed by Pol II at a lower efficiency and displays a preference for promoter regions (Baller, et al. 2012, Mularoni, et al. 2012).

TY1 COPY NUMBER CONTROL (CNC)

Ty1 transposition occurs at a low rate even though most elements are transcribed (Morillon, et al. 2002) and contain intact open reading frames (Curcio and Garfinkel 1991b, Curcio and Garfinkel 1994, Kim, et al. 1998). Interestingly, *S. cerevisiae* and its close relatives such as

S. paradoxus lack eukaryotic defense mechanisms such as RNAi or host restriction factors such as the APOBEC proteins (Drinnenberg, et al. 2011, Drinnenberg, et al. 2009, Harris, et al. 2012, Malim and Bieniasz 2012, Zheng, et al. 2012), suggesting that Ty1 retrotransposition is limited by a novel mechanism. Early on, one model proposed to explain “transpositional dormancy” is that a chromosomal or Ty1 encoded inhibitor is titrated when Ty1 is overexpressed from the *GAL1* promoter on a multicopy pGTy1 plasmid (Boeke, et al. 1985, Curcio and Garfinkel 1991a, Fink, et al. 1986, Garfinkel, et al. 1985). Expression of pGTy1 elements harboring several different coding-sequence mutations, including a frameshift mutation adjacent to the *GAG* initiation codon does not change the level of genomic Ty1 transposition, suggesting that the hypothetical inhibitor is not titrated by increasing the level of Ty1 RNA (Curcio and Garfinkel 1992, Garfinkel, et al. 1985, Garfinkel, et al. 2003). Rather, the level of mature proteins IN and PR are best correlated with the massive increase in Ty1 transposition observed by pGTy1 induction, suggesting that Ty1 transposition is regulated posttranslationally (Curcio and Garfinkel 1992).

We discovered a novel form of CNC that minimizes Ty1 movement and began to explain transpositional dormancy (Garfinkel, et al. 2003). CNC is defined as a decrease in transposition when additional elements are present in a genome, and variations on this theme are observed with P-elements in *Drosophila* (Craig 1990) and the Tn10 transposon in bacteria (Simons and Kleckner 1983). Ty1 CNC can be saturated by overexpression of Ty1 and surprisingly, occurs in cells containing a multicopy pGTy1 plasmid that is repressed for transcription of Ty1 mRNA (Garfinkel, et al. 2003, Matsuda and Garfinkel 2009). The CNC region encompasses part of *GAG* as determined by mutational analyses, and the major biochemical defects in CNC⁺ cells include a low level of mature IN and reverse transcription products (Garfinkel, et al. 2003, Matsuda and Garfinkel 2009, Purzycka, et al. 2013, Saha, et al. 2015). These results suggest that a titrateable *trans*-acting factor produced by Ty1 inhibits retrotransposition and mediates CNC.

IDENTIFYING THE CNC FACTOR

Ty1 mRNA does not encode the inhibitory factor, as shown by analyses with pGTy1 summarized above (Garfinkel, et al. 2003, Matsuda and Garfinkel 2009). Therefore, subgenomic transcripts containing *GAG* sequence may encode a protein or noncoding RNA capable of inhibiting transposition in a dose-dependent manner. Ty1 antisense (AS) RNAs map within *GAG* and are implicated in transcriptional repression of Ty1 (Berretta, et al. 2008, Servant, et al. 2012). In contrast, Ty1 mRNA levels remain unchanged in the presence of the CNC factor produced from pGTy1, suggesting CNC occurs posttranscriptionally (Garfinkel, et al. 2003, Matsuda and Garfinkel 2009). Matsuda and Garfinkel (2009) provided support for the idea that Ty1AS transcripts are necessary for CNC and specifically associate with VLPs. However, additional findings suggest that the AS transcripts may not act alone, are not truly packaged into VLPs, do not anneal with Ty1 mRNA in VLPs, and may be present in lower molar amounts when compared with the mRNA in VLPs (Matsuda and Garfinkel 2009, Purzycka, et al. 2013). Together, these results fail to support a model where Ty1 mRNA is the target for the AS transcripts and invite the possibility that a Ty1 protein mediates CNC.

Genetic analyses of the CNC region demonstrated that several mutations abrogating CNC neither affect AS RNA levels nor map in the AS RNA transcription units (Saha, et al. 2015) (Fig. 2). CNC⁻ mutations *T1108C*, *A1123G*, *A1296G*, and *A1496* map downstream of two internal AUG codons (AUG1 and AUG2) about halfway into *GAG*, and all change *GAG*'s coding potential. A 5' truncated Ty1 sense RNA, termed Ty1i RNA, is present in wild type cells and Ty1i RNA is also produced by pGPOL (a CNC-proficient pGTy1 lacking most of *POL*) used in the mutational analyses. Ty1i RNA contains 5' ends 38 nts., upstream of AUG1. In addition, CNC⁻ mutations *T399C*, *238–281*, and *238–353* that map within the AS RNA transcription units (Matsuda and Garfinkel 2009) decrease the level Ty1i RNA (Saha, et al. 2015). The phenotypic screen for CNC⁻ mutations also revealed a *GAG* separation-of-function allele *A1123G* that decreases CNC but not Ty1 transposition. Remarkably, Ty1 mobility increases rather than decreases in a Ty1-less strain repopulated with Ty1*A1123G*. Together, these results strongly support the idea that an altered form of Gag encoded by Ty1i RNA underlies CNC. Although these results predict that a 22-kDa protein (p22) encoded by the C-terminal half of *GAG* should be present, as well as a smaller protein (p18) that is produced by Ty1 PR cleavage, a commonly used VLP antiserum that reacts strongly with Gag-p49/p45 fails to reproducibly detect p22 (Adams, et al. 1987, Saha, et al. 2015). Therefore, we generated a new antiserum against recombinant p18 to show that both p22 and p18 are present in wild type cells and encoded by the CNC region. Co-expression of p22/p18 and pGTy1*his3-AI* dramatically decreases Ty1 mobility 35,000-fold (Saha, et al. 2015). Thus, the self-encoded p22 restriction factor is both necessary and sufficient for Ty1 CNC, and the postulated inhibitor responsible for transpositional dormancy.

SYNTHESIS OF p22/p18

Transcription and stability of Ty1i RNA is poorly understood but may respond differently to cellular genes used to produce Ty1 mRNA (Saha, et al. 2015) (Fig. 2). For example, *SPT3* encodes a subunit of the SAGA chromatin-remodeling complex that is implicated in TATA box recognition and is required for transcription of full-length Ty1 mRNA (Grant, et al. 1997, Winston, et al. 1984). Interestingly, deleting *SPT3* does not alter the initiation site used for transcription of Ty1i RNA, and the level of Ty1i RNA increases in certain strains. These results suggest that Ty1 mRNA and Ty1i transcription responds differently to *SPT3*, and raise the possibility that there is differential recognition of the Ty1 and Ty1i promoters by SAGA. Ty1i RNA levels also increase while Ty1 mRNA slightly decreases in cells lacking *XRNI*, which encodes a 5'-3' ribonuclease involved in RNA decay associated with P-bodies (Parker 2012), RNA transcription (Berretta, et al. 2008, Haimovich, et al. 2013), and assembly of functional Ty1 (Checkley, et al. 2010, Dutko, et al. 2010) and Ty3 (Beliakova-Bethell, et al. 2006, Bilanchone, et al. 2015) VLPs. Analyses of CNC⁻ mutations within (*238–353*, *238–281*) or adjacent (*T399C*) to the 5' LTR suggest that Ty1i and Ty1 mRNA utilize similar enhancer sequences for transcription (Curcio, et al. 2015, Saha, et al. 2015).

We used ribosome footprinting (Ingolia, et al. 2009) and mutational analyses (Nishida, et al. 2015, Saha, et al. 2015) to determine whether initiation of p22 translation occurs from both AUG1 and AUG2. Ribo-seq read distribution from a published data set (Arribere and Gilbert

2013) provides evidence for initiation at AUG1 but not AUG2. However, mutational analyses using pGPOL reveals that either AUG1 or AUG2 can be used to synthesize p22 proteins capable of inhibiting Ty1 mobility (Nishida, et al. 2015). *In vitro* synthesis of p22 using a wheat germ extract confirms initiation at either AUG and also provides evidence for translation via a cap-dependent process. Together, our results suggest that translation from AUG1 involves canonical cap-dependent scanning while leaky scanning is the most plausible mechanism for initiation at AUG2. Consistent with this interpretation is the observation that AUG2 is in a better sequence context for the yeast translational apparatus than AUG1 (Cavener and Ray 1991).

Another intriguing aspect of Ty1 CNC is the presence of p18, which is formed by cleavage of p22 by Ty1-PR at the same C-terminal site used to form Gag-p45 from Gag-p49 (Saha, et al. 2015). The relative amount of p18 versus p22 is correlated with the level of PR produced from Ty1 (Tucker, et al. 2015). When PR is expressed from the full ensemble of elements present in a genome or from pGTy1 induction p18 is the predominant form, whereas in low copy strains or an *spt3* mutant only p22 is detected. Both p22 and p18 inhibit Ty1 transposition to similar extents (Nishida, et al. 2015). How p22 gains access to PR within retrosomes or during VLP assembly is an interesting question, since PR is initially part of the Gag-Pol precursor in retrosomes and cleavage events to form IN and RT from Gag-Pol likely occurs within VLPs.

MECHANISM OF RESTRICTION

Our results show that p22/p18 inhibits several steps in the process of retrotransposition prior to reverse transcription (Nishida, et al. 2015, Saha, et al. 2015, Tucker, et al. 2015), which clarifies and greatly extends earlier work (Garfinkel, et al. 2003, Matsuda and Garfinkel 2009) (Fig. 3). The challenge is to define the earliest and most inhibitory insults to the transposition process that account for the >30,000-fold decrease in mobility observed when Ty1 and p22/p18 are coexpressed in a Ty1-less strain (Saha, et al. 2015). p22/p18 associates with VLPs through binding to Gag, alters other Ty1 proteins such as IN and RT and affects VLP assembly. Ty1 Pol processing or stability and VLP yield decreases when pGTy1 and p22 are coexpressed. There is an accumulation of the *POL*-encoded precursors and less mature RT and IN. Aberrant forms of RT are detected and Gag appears to undergo more proteolysis as well. These unusual Ty1 proteins may result from altered processing by Ty1 PR, from cleavage by a cellular protease, or from differences in posttranslational modifications. Ty1 reverse transcriptase activity is lower and more broadly distributed following sucrose gradient sedimentation of cell extracts, reflecting alterations in VLP assembly as well as loss of mature IN. p22/p18 changes VLP morphology in a fraction of the particles as determined by negative staining. The restriction factor also disrupts pGTy1-induced (Saha, et al. 2015) and endogenous retrosomes (unpublished data), and colocalizes with Gag.

The functional organization of Ty1 Gag is not well understood (Fig. 4), therefore, mapping the regions responsible for Gag:p22/p18 interaction and CNC has revealed more information about *GAG*-encoded proteins (Nishida, et al. 2015, Saha, et al. 2015, Tucker, et al. 2015). GST pulldown and co-immunoprecipitation analyses reveal interactions between p22/p18

and Gag. A segment of Gag encompassing p22 and additional N-terminal sequence, termed the CTR, interacts with p18 as well as with a segment of p18 that lacks part of NAC region (sAUG1) (Nishida, et al. 2015). Interestingly, only AUG1p22/p18, AUG1p18, and sAUG1 inhibit Ty1 mobility more than 5,000-fold while expression of Gag-p45 or derivatives containing the N- and C-terminal regions of Gag produce comparable levels of protein but inhibit Ty1 mobility less than 10-fold.

To define a target for p22/p18, we characterized missense mutations in Ty1 that confer partial resistance to CNC but do not markedly alter mobility in the absence of p22 (Tucker, et al. 2015). The strongest CNC^R mutations cluster in a region of *GAG* (termed the CNC^R domain) (Fig. 4), which is present in the CTR and separate from p22 coding sequence, while other CNC^R mutations map within the Gag UBN2 domain near the C-terminus. We did not recover resistance mutations in the yeast-specific TYA domain, which corresponds to an unstructured region encoded in the 5' half of *GAG*. The CNC^R domain contains a tryptophan residue (W184) conserved in domain A of Ty1/Copia elements from disparate organisms (Peterson-Burch and Voytas 2002). When W184 is changed to alanine, Ty1 mobility is abolished and VLP assembly is defective. The behavior Ty1 Gag *W184A* and additional mutations in the adjacent UBN2 domain strongly suggest that p22 disturbs a central function of Gag during VLP assembly. Furthermore, CNC^R mutations alter the cofractionation of p22/p18 and Gag, where the restriction factor remains mostly unprocessed and is excluded from fractions containing CNC^R VLPs. Together, our results suggest that the CNC^R alleles restore productive Gag-Gag interactions in the presence of p22 during VLP assembly. Furthermore, Gag:p22/p18 interactions during VLP assembly likely represent the earliest step in Ty1 transposition altered by p22.

In addition to its function as the CA for VLPs, Ty1 Gag probably contains retroviral-like NAC functions that participate in forming dimeric Ty1 RNA, packaging RNA into VLPs, annealing the tRNA_i^{Met} primer to the RNA template, and enhancing DNA strand-transfers during reverse transcription. Both full length Gag and p22/p18 contain the NAC region originally defined by the activity of a synthetic peptide (Cristofari, et al. 2000) (Fig. 4). We analyzed the NAC activity of Gag and p18 derivatives and determined whether p18 impacts Ty1 RNA transactions necessary for retrotransposition using a variety of approaches (Nishida, et al. 2015). The CTR displays robust NAC activity comparable to that observed with mature Gag-p45 (data not shown). Interestingly, AUG1p18 and AUG2p18 proteins display different properties even though they both contain the NAC region but differ by only 10 residues. AUG1p18 shows highly reduced NAC activity but specific binding to RNA whereas AUG2p18 shows the converse behavior. However, the two forms of the restriction protein may possess additional properties when expressed together *in vivo*. AUG2p18 lacking NAC activity (sAUG2) inhibits CTR mediated annealing of tRNA_i^{Met} to Ty1 RNA, and p22/p18 antagonizes Ty1 RNA dimerization and packaging. Our results support the view that p22/p18 also inhibits essential Ty1 RNA-structural transitions through p22/p18 interactions with Gag and possibly with Ty1 RNA.

A NEW PARADIGM FOR INHIBITING GAG FUNCTION

p22/p18-mediated restriction may have evolved quickly after an ancestral *Saccharomyces* lineage lost the evolutionarily conserved RNAi pathway (Drinnenberg, et al. 2011, Drinnenberg, et al. 2009), since expression of p22 is likely all that is required to establish CNC. In contrast, other retroelement genes have been domesticated by their host to provide new cellular functions (Kaneko-Ishino and Ishino 2012). The prototypic Gag-like restriction factors Fv1 and enJS56A1 block replication of murine leukemia virus (MLV) and Jaagsiekte sheep retrovirus (JSRV), respectively, by interacting with viral proteins during infection (Arnaud, et al. 2007, Best, et al. 1996, Hilditch, et al. 2011), and share features in common with CNC of Ty1 by p22/p18. Fv1 is derived from the *GAG* gene of a member of the HERV-L family of human and murine endogenous retroviruses (Benit, et al. 1997, Best, et al. 1996, Qi, et al. 1998). Fv1 inhibits progression of the MLV life cycle following infection and reverse transcription, but prior to integration. Although the infecting viral Gag protein as well as Fv1 determines the level of restriction, an ordered assembly of Gag is required for efficient Fv1 binding (Goldstone, et al. 2014, Hilditch, et al. 2011). In contrast, p22/p18 affects VLP assembly and function whereas Fv1 inhibits a different step in the replication cycle that occurs early post-infection prior to integration. Conceptually similar to MLV-Fv1 restriction, the sheep genome harbors about 20 copies of endogenous (en) JSRVs and these sequences are homologous with exogenous JSRV virus that causes lung cancer. Certain endogenous copies have evolved a *trans*-dominant Gag protein enJS56A that like Ty1 p22 blocks replication posttranslationally. The JSRV:enJS56A interaction prevents Gag from entering into an endosome trafficking pathway, and results in aggregation and turnover by the proteasome (Arnaud, et al. 2007, Murcia, et al. 2007). Indeed, Dolly the cloned sheep contracted lung cancer caused by JSRV because she lacked enJS56A (Leroux, et al. 2007).

Retroviral studies involving sensitivity and escape from host restriction factors show similarities to Ty1 CNC^R mutants. Although Gag CNC^R mutations rescue Ty1 retrotransposition in the presence of p22, they do not interfere with Gag:p22 binding (Tucker, et al. 2015). Similarly, Mx2 restriction of HIV-1 requires Mx2:CA binding, yet known Mx2 escape mutations in the CA gene do not significantly alter binding between Mx2 and CA (Fricke, et al. 2014, Schulte, et al. 2015). It is clear in both cases that escape mutations can promote retroelement replication in ways distinct from the disruption of restriction factor-target binding. Separately, we found that artificial adjustments of Gag:p22 ratios by altering the level of expression influence the robustness of CNC. Resistance to the sheep restriction factor enJS56A1 is achieved by the provirus enJSRV26 simply by increasing proviral expression due to a mutation in the envelope glycoprotein (Armezzani, et al. 2011). Interestingly, *GAG*CNC^R mutations do not affect steady state Gag levels, although it seems logical that this evasion strategy would prove fruitful. However, it is still possible that the ratio of Gag:p22 is specifically higher within retrosomes comprised of CNC^R Gag as compared to wild type Gag.

Our results also illustrate the delicate balance between resistance to p22 and fitness of Ty1 (Tucker, et al. 2015). Since all p22 sequences are present in Gag-p49 (Fig. 4), surfaces or protein domains that interact with p22 may be the same or overlap with domains important for Gag function, and would likely constrain an “arms race” between p22 and its target

protein Gag. In fact, several CNC^R *GAG* mutations result in decreased Ty1 fitness. This is similar to studies involving HIV-1 CA resistance to the independently encoded restriction factors TRIM5 α and Mx2, which demonstrate that resistance mutations in CA can have detrimental effects on HIV fitness (Fricke, et al. 2014, Rihn, et al. 2013, Soll, et al. 2013). HIV-1 CA's sensitivity to mutation or genetic fragility is comparable to what we see with Ty1 Gag, as our CNC^R analysis supports the idea that Ty1 Gag is under strict structural constraint (Tucker, et al. 2015). Lastly, since Ty1 *GAG* or p22 sequences have not yet been detected as a domesticated gene, the graduated rate provided by CNC may benefit *Saccharomyces* and Ty1, such as extending chronological life span (VanHoute and Maxwell 2014).

FUTURE DIRECTIONS

There is much to be learned about the mechanism of Ty1/p22 restriction and whether this strategy is employed by retroelements elsewhere. Investigating VLP assembly and p22, Gag-p45, and VLP structure will help us understand how p22 alters VLP functions. In particular, Cryo-electron microscopy approaches have advanced tremendously (Bai, et al. 2015) since Ty1 VLPs were last examined (AL-Khayat, et al. 1999, Burns, et al. 1992, Palmer, et al. 1997). Further understanding of VLP assembly and CNC will be gained by identifying cellular proteins that interact with Gag or p22 and may provide clues as to whether CNC is regulated. Ty1-Gag:p22 restriction is perfectly suited for investigating how RNA structure dictates function, and reinforces the idea that both strong and weaker defects mediated by p22/p18 contribute to the severe inhibition of Ty1 movement. We are also determining how Ty1 CNC is distributed in wild *S. paradoxus* and *S. cerevisiae* isolates using functional tests and high throughput sequencing, and discovering a Ty1-like restriction factor in unrelated organisms is a reasonable expectation given the track record of the Ty/yeast paradigm.

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Figure 1.

Ty1 retrotransposition. The functional organization of Ty1 is at the top and the replication cycle is depicted below. Boxed triangles denote long terminal repeats (LTRs). Ty1 is transcribed from LTR to LTR by RNA polymerase II to form a 5.7-kb transcript, which is packaged into VLPs or translated to produce Gag-p49 (shaded blue), or Gag-Pol-p199, which requires a programmed +1 frameshift event near the end of *GAG*. Gag accumulates in the cytoplasm to become the capsid of Ty1 VLPs and also mediates nucleic acid chaperone functions during VLP assembly and reverse transcription. The Gag-Pol precursor contains the enzymes required for Ty1 transposition: protease (PR; shaded orange), integrase (IN; shaded teal) and reverse transcriptase (RT; shaded grey). Prior to VLP formation, Gag and presumably Gag-Pol form mRNA/Gag foci termed T-bodies or retrosomes, which are sites where VLPs assemble. Ty1 mRNA is specifically packaged as a dimer into VLPs. Ty1 PR cleaves Gag-p49 and Gag-Pol-p199 precursors to form mature proteins, Gag-p45, PR-p23, IN-p71, and RT-p60. Like retroviruses, an IN/RT heteromer reverse transcribes Ty1 mRNA into a linear cDNA. A pre-integration complex (PIC) minimally containing Ty1 cDNA and IN is imported into the nucleus via a bipartite nuclear localization signal present on IN. Retrotransposition is completed by IN-mediated insertion into a new location in the host genome.

Figure 2.

The CNC region and Ty1 transcripts produced from pGPOL . The CNC region covers *GAG* and the beginning of *POL*, as defined by deletion mapping. Locations of the *GAL1* promoter (hatched rectangle), LTR (solid rectangle), Ty1, Ty1i and Ty1 antisense (AS) transcripts, initiation codons present on Ty1i RNA, and CNC-defective mutations are noted. Included in these is the separation-of-function mutation *A1123G*, which affects CNC but not Ty1 transposition.

Figure 3.

Ty1 VLP functions inhibited by p22. An abbreviated Ty1 replication cycle is shown (also refer to Figure 1) highlighting steps affected by p22 (shaded green). When p22 and Ty1 are expressed, p22 colocalizes with Gag and disrupts retrovirus-like particle (VLP) foci. p22 and p18, which is derived from p22 by Ty1 PR cleavage, associates with Ty1 VLPs and alters assembly and maturation.

Figure 4.

Functional organization of Ty1 Gag. Gag-p49/p45 coding sequences are depicted at the top and show the TYA, CNCR^R, and UBN2 domains, a highly conserved W184 codon, the location of initiation codons responsible for p22 synthesis, and the nucleic acid chaperone region. Below are the restriction factor p22/p18, the Gag C-terminal region (CTR) that displays nucleic acid chaperone activity, and a short form of p18 (sAUG1) that inhibits Ty1 mobility and associates with the CTR but lacks nucleic acid chaperone activity.