Identification of autonomous IAP LTR retrotransposons mobile in mammalian cells

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Mammalian genomes contain two main classes of retrotransposons, the well-characterized long and short interspersed nuclear elements, which account for ~30% of the genome, and the long terminal repeat (LTR) retrotransposons, which resemble the proviral integrated form of retroviruses, except for the absence of an envelope gene in some cases. Genetic studies confirmed mobility of the latter class of elements in mice, with a high proportion of phenotypic mutations consequent to transposition of the intracisternal A particle (IAP) family of LTR retrotransposons¹. Using the mouse genome sequence and an efficient *ex vivo* retrotransposition assay, we identified functional, master IAP copies that encode all the enzymatic and structural proteins necessary for their autonomous transposition in heterologous cells. By introducing mutations, we found that the three genes *gag*, *prt* and *pol* are all required for retrotransposition and identified the IAP gene products by electron microscopy in the form of intracellular A-type particles in the transfected cells. These prototypic elements, devoid of an envelope gene, are the first LTR retrotransposons autonomous for transposition to be identified in mammals. Their high rates of retrotransposition indicate that they are potent insertional mutagens that could serve as safe (noninfectious) genetic tools in a large panel of cells.

To identify IAP elements encoding the proteins necessary for retrotransposition, we first devised an *ex vivo* assay in heterologous cells (Fig. 1), which combined a reporter IAP element marked with an indicator gene for retrotransposition and expression vectors for full-length genomic IAP elements. The marked reporter is derived from an internally deleted IAP (a naturally occurring type IΔ1 element¹ that recently transposed within the gene *Il3* and is still competent but not autonomous for transposition²), which we marked with the *neo*^{TNF} indicator gene for retrotransposition³ (described in Fig. 1b,c). The full-length IAP elements were sorted by an *in silico* search on the available sequence of the mouse genome, by imposing that the three retroviral genes *gag*, *prt* and *pol* be fully coding. A search on the nr database in GenBank (2% of the mouse genome, May 2001 release) identified 18 full-length elements, of which 9 had three canonical open reading

Figure 1 Structure of IAP elements and description of the assay for retrotransposition. (a) Genomic organization of full-length IAP elements. The LTRs (U3-R-U5) border three ORFs homologous to the retroviral genes *gag*, *prt* (protease) and *pol* and are separated from one another by a -1-nt frameshift at the indicated heptameric slippery sites⁴. The transcription start site is marked with an arrow, and the signals necessary for retroviral reverse-transcription are indicated. PBS, primer binding site; PPT, polypurine tract. (b) Schematic representation of an IAP marked with the reporter gene *neo*^{TNF} (light gray), in which *neo* placed in reverse orientation with its own promoter (Pr) is rendered inactive by the presence of a forward intron (top), which should be spliced out in the transposition RNA intermediate (middle), resulting in an active *neo* gene in the *de novo* IAP insertion (bottom). (c) Experimental procedure for detection of IAP retrotransposition. Cells were transfected either with a *neo*^{TNF}-marked defective IAP and an expression vector for the selected IAPs or with a full-length *neo*^{TNF}-marked IAP alone. Cells were amplified for 1 wk and retrotransposition events detected after G418 selection.

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Figure 2 Mobilization of a marked IAP reporter using expression vectors for cloned genomic IAPs. **(a)** The *neo*^{TNF}-marked IAP reporter was assayed for retrotransposition in heterologous feline cells after cotransfection with a control plasmid (pCMV β) or an expression vector for the cloned IAP elements (BAC clone number indicated, structural organization schematized). Results of the G418^R selections are illustrated (in the plates shown, the G418^R foci were fixed and stained). The number of foci is given for each construct as the mean $\pm$ s.e. of 2–4 independent experiments, carried out with at least 4×10^6 cells. The resulting retrotransposition frequencies are also shown. **(b)** G418^R clones are associated with retrotransposition. Genomic DNA of individual clones from **a** was assayed by PCR for the presence of retrotransposed copies with the intron of the *neo*^{TNF} indicator spliced out (using primers bracketing the intronic domain as indicated), and the PCR products were separated by electrophoresis in agarose gels stained with ethidium bromide. All clones tested had a band corresponding to the size of an intronless copy of the *neo* gene (intron-containing and intron-less PCR size markers indicated for each gel), and no band was detected with mock-transfected cells (control).

frames (ORFs) separated from one another by a -1 frameshift⁴ (**Fig. 1a**). In addition, their two LTRs shared a high degree of identity (0 or 1 nucleotides (nt) different), consistent with recent integration of these elements. The search also identified copies with three intact ORFs but with a 1-nt insertion into the *prt-pol* frameshift signal resulting in fusion of these two genes. We retained a series of six full-length IAP elements for which BAC clones could be obtained, including four canonical elements with the -1 frameshift and two 'mutant' elements

with *prt-pol* fusion (**Fig. 2**). For the assay *in trans*, we subcloned these elements into a unique expression vector, in which we inserted the 5' U5-*gag-prt-pol-3'* U5 IAP proviral fragment in place of the corresponding region of the internally deleted *Il3* IAP (whose LTR has strong promoter activity, possibly owing to the presence of flanking *Il3* enhancer sequences²).

We cotransfected heterologous cells with the IAP reporter and the expression vector for each of the cloned full-length IAP proviruses and

Figure 3 IAP gene products required for retrotransposition. **(a)** Assay for IAP transposition was done as in **Figure 2a**, with expression vectors (schematized on the left) encoding either the wild-type IAP 440N1 (WT) or the same element rendered defective for *gag*, *prt* (in-phase deletion so as not to alter translation of the downstream ORFs) or *pol* (insertion of a premature termination codon). Transcript levels for each IAP construct (determined by northern blots) are shown. The retrotransposition frequencies obtained for each construct are given as the mean $\pm$ s.e. of 2–4 independent experiments, carried out with at least 4×10^6 cells. In the plates shown, the foci were fixed and stained. **(b)** Western-blot analysis for cleavage of the IAP Gag polyprotein. Lysates of cells transfected with a control plasmid (mock) or an expression vector for either the wild-type IAP 440N1 (WT) or the same element rendered defective for the *prt* gene (*prt*⁻) were separated by electrophoresis, blotted and hybridized with a rabbit antiserum directed against the capsid domain of the Gag protein. Both IAP expression vectors drive the synthesis of the full-length Gag protein (black arrowhead, 73 kDa) with a few degradation products in common (bracket), whereas only the wild-type *prt* ORF results in the occurrence of two additional specific products (empty arrowheads) of ~ 35 and 24 kDa (prolonged exposure shown below) corresponding to a partial processing of the Gag protein.

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then selected G418-resistant clones (Fig. 2a). These assays resulted in a high number of clones (frequency in the range of 10^{-4}) for the four canonical IAP elements but very few or no clones for a control vector or for the two mutant IAP copies with the *prr-pol* fusion. To confirm that the G418^R clones resulted from retrotransposition of the marked element, we verified that the intron present on the indicator gene in the initial marked copy was spliced out in the transposed IAPs by PCR analysis of genomic DNA for a series of randomly selected G418^R clones (Fig. 2b).

We then selectively inactivated each of the three IAP genes, by deletions in either *gag* or *prr* (in-phase so as to preserve translation of the downstream genes) and by introducing an early termination codon in *pol*. None of these modifications altered the RNA level of the transfected IAPs (Fig. 3a). All three mutants had a much lower rate of transposition ($<10^{-7}$ versus 10^{-4} for the wild-type element), indicating that the three genes are necessary for transposition. It has been reported that the Gag precursor remains uncleaved^{1,5}, which seems to contradict our observation that the protease gene is required for transposition. We therefore carried out western-blot experiments with cells transfected with the wild-type element and the *prr* mutant. Comparison of the two profiles (Fig. 3b) indicates that partial process-

ing of the Gag precursor specifically occurred in the presence of a full-length *prr* ORF (with evidence for cleavage products of ~35 kDa and ~24 kDa), consistent with the functional data for retrotransposition.

We then tried to rescue the two IAP elements with a *prr-pol* fusion: we deleted the 1-nt insertion that places *prr* and *pol* in-frame in these elements and assayed the 'corrected' copies for retrotransposition (data not shown). For the 262J21 element, this single mutation increased retrotransposition efficiency by a factor of ~10, suggesting that, as for Ty1 elements⁶⁻⁸, the ratios of the various 'viral' polypeptides, which depend on the frameshift signals, are essential for retrotransposition. The same correction made on the 31B18 IAP element did not restore any activity, indicating that in this case, other mutations impair the function of the encoded proteins. The first and most extensively studied full-length IAP element (IAP14; refs. 4,5,9) was defective in this assay for unknown reasons (data not shown).

We next determined whether the four canonical IAP elements described above could promote their own mobilization, that is, whether they also contained the signals required in *cis* for their efficient autonomous retrotransposition. We recloned each IAP element as a complete LTR-*gag-prr-pol*-LTR provirus, marked them with the indicator gene downstream of the coding sequences (Fig. 4a) and

Figure 4 Assay for autonomous IAP retrotransposition and characterization of the *de novo* insertions. **(a)** Autonomous IAP retrotransposition. Top and middle sections: each of the four IAP elements encoding functional proteins (copy name indicated) was cloned as a complete provirus with the *neo^{TNF}* indicator inserted downstream of the IAP ORFs as schematized and tested for its autonomous retrotransposition in heterologous feline (G355.5) and human (HeLa) cells as described in Figure 1c; retrotransposition frequencies are the mean $\pm$ s.e. of 2–3 independent experiments. The marked L1-2B human LINE element used as a control (data not shown) yielded transposition frequencies of 1.5×10^{-3} and 1.1×10^{-2} in G355.5 and HeLa cells, respectively. Bottom section: LTR-luciferase activities of the corresponding IAPs in both cell types. Values expressed in arbitrary units are the mean $\pm$ s.e. of 2–3 independent experiments. **(b)** Structure and chromosomal localization of nine transposed IAPs. The complete characterization of transposed IAP copies and insertion sites was done using the genomic IAP clone 92L23 marked as indicated in **a** and assayed for transposition in HeLa cells. A *de novo* IAP insertion and the corresponding empty site is schematized. The sequences of nine insertions are given below, with the flanking DNA in uppercase and the proviral sequences in lowercase; target-site duplications (TSD, shaded) are found in all nine cases, associated with complete LTRs. The GenBank accession number of each insertion site is given, together with its chromosomal localization (indicated with an arrowhead; R bands are shown in white and G bands in dark gray); seven of nine insertions are in R bands.

Figure 5 *Cis* preference for IAP retrotransposition. Assays for retrotransposition were carried out in feline G355.5 cells transfected with (top) an autonomous marked IAP element (0.75 μ g of DJ33/440N1 *neo*^{TNF} and 0.75 μ g of control pCMV β) or (bottom) the unmarked IAP element acting in *trans* on the IAP marked with *neo*^{TNF} and rendered defective for the IAP proteins (premature stop codon in *gag* indicated by the asterisk), using 0.75 μ g of each construct. In the plates shown, the foci were fixed and stained. The corresponding retrotransposition frequencies (mean $\pm$ s.e. of 2–4 independent experiments) are also shown. The higher transposition rates compared with those in **Figure 4a** are caused by the use of a strong LTR promoter (also used in **Fig. 2a**).

assayed their autonomous retrotransposition in heterologous cells. Three of the elements had high retrotransposition activity (**Fig. 4a**). We found that these three active, autonomous copies belong to the previously described PC subfamily¹⁰, whereas the nearly inactive one is a member of the LS subfamily¹¹. Because this classification was based on a few diagnostic positions in the LTR (notably in the TATA box), we tested the promoter activity of each 5' LTR using LTR-luciferase constructs. These assays confirmed (**Fig. 4a**) that the LTR promoter efficiency of the LS element was 3–30 times lower than that of the PC elements; this probably accounts for its lower rate of autonomous retrotransposition. For the three PC elements, transposition rates measured in the *cis* assay do not correlate perfectly with their relative LTR promoter strength, probably because of subtle differences in their enzymatic transposition machinery. This is supported by their different transposition rates in the assay in *trans* where they are placed under the control of the same promoter (**Fig. 2a**) but could not be directly inferred from a comparison of their amino acids sequences.

We confirmed canonical retrotransposition for the most active IAP copy by characterizing (by inverted PCR) several transposed copies and their insertion sites (**Fig. 4b**). These newly inserted elements had perfect 6-bp target-site duplications as well as the canonical structure of endogenous and *de novo* transposed IAPs^{1,2}. In six of nine cases, insertion occurred in actively transcribed domains (as inferred from an expressed-sequence tag-based analysis using the University of California Santa Cruz Genome Bioinformatic site; data not shown), consistent with the integration preference of retroviral elements¹².

We next investigated whether IAP retrotransposons had a *cis* preference effect similar to that reported for non-LTR LINE retrotransposons, in which a coding mRNA is a far better substrate for retrotransposition than a noncoding one complemented in *trans*^{13,14}. We compared the retrotransposition rate of a full-length autonomous marked IAP with that of the same marked IAP rendered defective for protein synthesis (premature stop codon in the *gag* gene) and complemented in *trans* by an expression vector for the IAP ORFs. All three

Figure 6 Electron microscopy of the viral-like particles generated by a retrotransposition-competent IAP. G355.5 feline cells were transfected with the IAP element in **Figure 5** (wild-type in **a–d**, *prt* mutant in **e**) and observed 48 h after transfection. **(a)** Representative low-magnification image of a transfected cell, with numerous particles grouped in the cisternae of the endoplasmic reticulum. The nucleus (N) is indicated. **(b)** High-magnification image of a representative particle, with two concentric shells surrounding an electron-lucent core. **(c)** Example of merged particles (a doublet in the present view), as frequently observed in the assay. **(d)** Example of particles budding into the cisternae of the rough endoplasmic reticulum. **(e)** Particles obtained with the expression vector for the protease mutant; morphology is similar to that of wild-type particles, and merged particles (as in **c**) are also observed. Scale bars: **a**, 500 nm; **b–e**, 100 nm.

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constructs are derived from the same provirus (440N1), placed (as in Fig. 2a) under the control of the same strong LTR promoter (from *Il3* IAP). G418^R clones were 30 times more numerous when the IAP proteins were encoded by the marked mRNA itself than when they were provided in *trans* (Fig. 5).

The identification of functional retrotransposition elements provides a unique opportunity to visualize and characterize the IAP gene products. Transfection of cells with a functional IAP yielded numerous viral-like particles in the cisternae of the endoplasmic reticulum (Fig. 6a), whereas cells transfected with a control vector did not. The particles were ~90 nm in diameter with an A-type, immature-like morphology (Fig. 6b) and were not substantially different from particles spontaneously expressed in mouse tumor cells¹. Some of them were associated in doublets (Fig. 6c), probably resulting from fusion of individual particles (due to overexpression in the transient transfection). Budding could be detected at the endoplasmic reticulum membrane (Fig. 6d), and no particles were found outside the cells. Close examination of a large (>100) set of particles did not show any obvious heterogeneity among them. Transfection of cells with the IAP protease mutant vector (Fig. 6e) resulted in a similarly large number of intracisternal particles undistinguishable from those produced by the wild-type IAP. These observations are consistent with the low level of protein cleavage shown by western-blot analysis (Fig. 3b). Although we cannot exclude the possibility that the functional particles (mediating retrotransposition) underwent a complete cleavage and had a mature morphology too rare to be observed, it is more likely that all the particles were subjected to a limited, protease-dependent processing that did not change their morphology but yielded a sufficient amount of *pol* gene enzymatic products to drive reverse-transcription and integration.

We identified, for the first time in mammals, LTR retrotransposons that encode all the proteins required for their mobility and that transpose autonomously in heterologous cells with a high efficiency. Transposition rates (up to 10⁻³) were similar to those of L1 LINEs^{3,15-17}, which are non-LTR retrotransposons responsible for most of the insertional mutations observed in humans¹⁸. We found that all three genes *gag*, *prt* and *pol* are required for transposition and that transposition of coding IAP elements is more efficient than that of defective IAPs complemented in *trans* by an active element. This result is somewhat unexpected for a retroviral-like element but reminiscent of the strong *cis* effect observed for the non-LTR LINE retrotransposons^{13,14}. This result could be interpreted, as for LINEs, by assuming that the coding transcript during translation is a preferred target for the retrotransposon nascent proteins. This would ensure that active copies are preferentially transposed, preventing transposon extinction. Close examination of the mouse genome shows that most IAPs (~700 of the 1,000 genomic copies) are full-length elements, and computations from our *in silico* analysis indicate that more than half of them contain three canonical ORFs. Our functional analysis of six such randomly selected elements suggests that a large fraction of these full-length coding IAPs (possibly ~300) could be transposition-competent, autonomous elements.

Finally, the identified autonomous IAP copies could be used as safe (noninfectious) insertional mutagens in a large series of cells owing to their high transposition rate, being possibly useful complements to the Mariner DNA-transposon¹⁹ as well as to LINEs. Because they are LTR retrotransposons, IAPs might insert with distinct target site specificities¹². In addition, IAPs are only present in some rodents, and their mobility therefore might not be restricted by specific 'epigenetic' taming processes (e.g., homology-dependent gene silencing) as observed for transposons in several organisms²⁰⁻²³, making them appropriate mutagenic agents.

METHODS

Plasmids. We derived full-length IAP-containing plasmids from commercial BACs (BACPAC Resources). GenBank accession numbers, positions of the first and last nucleotides of the IAP proviruses and strand orientation were as follows: RP23-231P12: AL590616, 87,256-94,327, +; RP23-262J21: AL450406, 135,142-142,227, +; RP23-31B18: AC074106, 150,810-143,692, -; RP23-324C9: AC090431, 15,424-22,549, +; RP23-440N1: AC087891, 163,007-155,853, -; RP23-92L23: AC012382, 161,601-168,684, +.

Constructs for the assay in *trans*. We constructed the marked defective IAP element (DJ33 *neo*^{TNF} reporter plasmid) by removing the *SalI* fragment corresponding to *neo*^{RT} from DJ33 (ref. 2) and replacing it by a blunt-ended *HindIII-SalI* fragment from pSV*neo*^{TNF} (ref. 3) containing the indicator gene *neo*^{TNF}. We obtained the expression vectors for the six IAP elements by introducing the corresponding *MluI-MluI* IAP internal fragment (6.9 kb extending from the end of 5' LTR to the end of 3' LTR) excised from the BACs into the IAP-DJ33 reporter digested by *MluI*. We constructed the *gag*⁻, *prt*⁻ and *pol*⁻ IAP mutants using the IAP 440N1 expression vector by either in-frame deletions (*gag*⁻: 813-nt deletion, 891-1,703; *prt*⁻: 411-nt deletion, 2,410-2,820) or insertion of a linker containing multiple stop codons (for the *pol*⁻ mutant) at the unique *PmlI* site (nucleotide position 3,417).

Construction of the autonomous marked IAPs. We amplified the 5' LTRs of the four functional IAPs on the corresponding BAC by PCR and cloned them into the polylinker of the pGL3 Basic Vector (Promega; pGL3/LTR IAP plasmids). We excised the *MluI* IAP internal fragments from the BACs, inserted them into the unique *MluI* site of the corresponding pGL3/LTR IAP plasmids and inserted the *neo*^{TNF} reporter gene at the *SalI* site (IAP nucleotide position 5,744) of the resulting plasmids as a PCR fragment (*SalI* is located 36 nt upstream of the *pol* stop codon, and *pol* was restored by introducing the lacking sequence in the primer).

Constructs for the *cis-trans* assay. We constructed the expression vector for the functional marked IAP (DJ33/440N1*neo*^{TNF}) used in the test in *cis* by inserting the *neo*^{TNF} indicator gene at the *NdeI* site (downstream of *pol*, nucleotide position 6,566) of the IAP-440N1 expression vector. This marked IAP was additionally rendered defective by introducing a 2-nt frameshift in *gag* (by religating the *ClaI* opened and blunt-ended vector), thus resulting in a premature stop codon.

Cells, transfection and transposition assays. We grew G355.5 feline and human HeLa cells in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum (Gibco BRL), 100 g ml⁻¹ streptomycin and 100 U ml⁻¹ penicillin. The day before transfection, we seeded 5 × 10⁵ cells per 60-mm dish. For transfection, we used 12 l of lipofectamine, 8 l of Plus Reagent (Gibco BRL) and 1 g (single transfection) or 1.5 g (cotransfection) of DNA for the G355.5 cells or 4 g of DNA for the HeLa cells, following the manufacturer's instructions. To assay for retrotransposition, we expanded transfected cells for 6 d, seeded them at 5 × 10⁵ cells per 100-mm dish and allowed them to settle for 24 h before adding G418 (560 g ml⁻¹, Gibco-BRL). After selection for 10-15 d, we fixed, stained and counted or individually pipetted and plated G418^R foci. We extracted genomic DNA of some clones and subjected it to a PCR analysis for the presence of a spliced copy of the *neo* reporter gene as described¹⁷, using primers *neo*12 and *sv3* (sequences available on request). We carried out northern-blot analysis as described¹⁷ using the *PmlI-SalI* IAP fragment as a probe.

Characterization of retrotransposed copies and flanking sequences. We determined integration sites by inverted PCR, essentially as described¹⁷. We digested 5 g of each clone with *DraI*, *SspI* or a mix of *BamHI* and *BclI*. After removing the enzymes, we diluted and self-ligated the DNA overnight. We precipitated it with ethanol, resuspended it in 20 l of 10 mM Tris and subjected it to one round (or in some cases two rounds) of PCR with sets of divergent primers (selected for the amplification of either the 5' or 3' proviral ends). We gel-purified, cloned and sequenced the amplified DNAs, yielding insertion sites. We further analyzed the corresponding inserted IAP copies by direct PCR amplification (and sequencing), using primers derived from the flanking sequences and the *neo* reporter gene. All primer sequences are available on request.

Gag antiserum and western-blot analysis. We cloned a *HpaI*-*NotI* blunt-ended fragment from the MIA14 IAP genome (nucleotide positions 1,547–1,914) corresponding to the 3' half of the putative Gag capsid domain into the pET-19b plasmid (Novagen) opened by *XhoI* and blunt-ended, to allow prokaryotic expression as a histidine-tagged protein. We then purified the recombinant protein on a HiTrap column (Amersham) loaded with NiSO_4 and used it to immunize rabbits. We affinity-purified the antiserum (Agrobio, France) before use. For analysis of Gag cleavage, we lysed cells transfected with the expression plasmids for the wild-type IAP, the *prt*[−] mutant or a control in Laemmli buffer 48 h after transfection. We separated whole-cell lysates by SDS-PAGE using gels containing 12% polyacrylamide (37.5:1 ratio of acrylamide to N,N-methylenebisacrylamide). We transferred proteins to nitrocellulose membranes (Schleicher & Schuell) and incubated them with the affinity-purified rabbit antiserum (1:500 dilution). We used goat antibody to rabbit IgG conjugated to horseradish peroxidase (Amersham) as the secondary antiserum.

LTR promoter activity. To assay for IAP LTR activity, we transfected feline G355.5 cells and human HeLa cells with an equal mass of pGL3-LTR IAP plasmid and pRL-TK vector (Promega) to normalize transfection efficiencies. We recovered cell lysates 48 h after transfection and tested them for activities of both luciferases with the Dual Luciferase Reporter Assay System (Promega) following the manufacturer's instructions.

Electron microscopy. For morphological studies, we grew G355.5 cells in monolayer and fixed them *in situ* for 1 h at 4 °C with 1.6% glutaraldehyde (Taab Lab Equip Ltd) in 0.1 M Sørensen phosphate buffer, pH 7.3. We scraped the cells from the plates during fixation and centrifuged them at 4 °C. We rinsed the fixed pellets for 2 h in ice-cold phosphate buffer, post-fixed them with 2% aqueous osmium tetroxide (Pellane Instruments) and dehydrated them in increasing concentrations of ethanol before embedding them in Epon. We collected ultrathin sections on 200-mesh grids coated with Formvar and carbon, stained them with uranyl acetate and lead citrate and observed them with a Zeiss EM902 transmission electron microscope.

URL. We determined the chromosomal localization of the transposed IAP insertions using Ensembl (available at http://www.ensembl.org/Homo_sapiens/blastview).

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Kuff, E.L. & Lueders, K.K. The intracisternal A-particle gene family: structure and functional aspects. *Adv. Cancer Res.* **51**, 183–276 (1988).
2. Heidmann, O. & Heidmann, T. Retrotransposition of a mouse IAP sequence tagged with an indicator gene. *Cell* **64**, 159–170 (1991).
3. Esnault, C., Casella, J.F. & Heidmann, T. A Tetrahymena thermophila ribozyme-based indicator gene to detect transposition of marked retroelements in mammalian cells. *Nucleic Acids Res.* **30**, e49 (2002).
4. Fehrmann, F., Welker, R. & Kräusslich, H.G. Intracisternal A-type particles express their proteinase in a separate reading frame by translational frameshifting, similar to D-type retroviruses. *Virology* **235**, 352–359 (1997).
5. Welker, R., Janetzko, A. & Kräusslich, H.G. Plasma membrane targeting of chimeric intracisternal A-type particle polyproteins leads to particle release and specific activation of the viral protein. *J. Virol.* **71**, 5209–5217 (1997).
6. Xu, H. & Boeke, J.D. Host genes that influence transposition in yeast: the abundance of a rare tRNA regulates Ty1 transposition frequency. *Proc. Natl. Acad. Sci. USA* **87**, 8360–8364 (1990).
7. Kawakami, K. *et al.* A rare tRNA-Arg(CCU) that regulates Ty1 element ribosomal frameshifting is essential for Ty1 retrotransposition in *Saccharomyces cerevisiae*. *Genetics* **135**, 309–320 (1993).
8. Balasundaram, D., Dinman, J.D., Wickner, R.B., Tabor, C.W. & Tabor, H. Spermidine deficiency increases +1 ribosomal frameshifting efficiency and inhibits Ty1 retrotransposition in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **91**, 172–176 (1994).
9. Mietz, J.A., Grossman, Z., Lueders, K.K. & Kuff, E.L. Nucleotide sequence of a complete mouse intracisternal A-particle genome: relationship to known aspects of particle assembly and function. *J. Virol.* **61**, 3020–3029 (1987).
10. Lueders, K.K., Fewell, J.W., Morozov, V.E. & Kuff, E.L. Selective expression of intracisternal A-particle genes in established mouse plasmacytomas. *Mol. Cell. Biol.* **13**, 7439–7446 (1993).
11. Mietz, J.A., Fewell, J.W. & Kuff, E.L. Selective activation of a discrete family of endogenous proviral elements in normal BALB/c lymphocytes. *Mol. Cell. Biol.* **12**, 220–228 (1992).
12. Bushman, F.D. Targeting Survival. Integration Site Selection by Retroviruses and LTR-Retrotransposons. *Cell* **115**, 135–138 (2003).
13. Esnault, C., Maestre, J. & Heidmann, T. Human LINE retrotransposons generate processed pseudogenes. *Nat. Genet.* **24**, 363–367 (2000).
14. Wei, W. *et al.* Human L1 retrotransposition: cis preference versus trans complementation. *Mol. Cell. Biol.* **21**, 1429–1439 (2001).
15. Moran, J.V. *et al.* High frequency retroposition in cultured mammalian cells. *Cell* **87**, 917–927 (1996).
16. Naas, T.P. *et al.* An actively retrotransposing, novel subfamily of mouse L1 elements. *EMBO J.* **17**, 590–597 (1998).
17. Dewannieux, M., Esnault, C. & Heidmann, T. LINE-mediated retrotransposition of marked Alu sequences. *Nat. Genet.* **35**, 41–48 (2003).
18. Ostertag, E.M. & Kazazian, H.H.J. Biology of mammalian L1 retrotransposons. *Annu. Rev. Genet.* **35**, 501–538 (2001).
19. Plasterk, R.H., Izsvak, Z. & Ivics, Z. Resident aliens: the Tc1/mariner superfamily of transposable elements. *Trends Genet.* **15**, 326–332 (1999).
20. Jensen, S., Gassama, M.-P. & Heidmann, T. Taming of transposable elements by homology-dependent gene silencing. *Nat. Genet.* **21**, 209–212 (1999).
21. Ketting, R.F., Haverkamp, T.H., van Luenen, H.G. & Plasterk, R.H. Mut-7 of *C. elegans*, required for transposon silencing and RNA interference, is a homolog of Werner syndrome helicase and RNaseD. *Cell* **99**, 133–141 (1999).
22. Tabara, H. *et al.* The rde-1 gene, RNA interference, and transposon silencing in *C. elegans*. *Cell* **99**, 123–132 (1999).
23. Bernstein, E., Denli, A.M. & Hannon, G.J. The rest is silence. *RNA* **7**, 150–1521 (2001).