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LINE-1 ORF1 protein enhances Alu SINE retrotransposition

Nicholas Wallace [1], Bradley J. Wagstaff [1], Prescott L. Deininger, Astrid M. Roy-Engel *

Tulane Cancer Center SL-66, Deparment of Epidemiology, Tulane University Health Sciences Center, 1430 Tulane Ave., New Orleans, LA 70112, United States

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ABSTRACT

Retroelements have contributed over one third of the human genome mass. The currently active LINE-1 (L1) codes for two proteins (ORF1p and ORF2p), both strictly required for retrotransposition. In contrast, the non-coding parasitic SINE (Alu) only appears to need the L1 ORF2p for its own amplification. This requirement was previously determined using a tissue culture assay system in human cells (HeLa). Because HeLa are likely to express functional L1 proteins, it is possible that low levels of endogenous ORF1p are necessary for the observed tagged Alu mobilization. By individually expressing ORF1 and ORF2 proteins from both human ($L1_{RP}$ and LRE3) and rodent ($L1_{A102}$ and $L1_{spa}$) L1 sources, we demonstrate that increasing amounts of ORF1 expressing vector enhances tagged Alu mobilization in HeLa cells. In addition, using chicken fibroblast cells as an alternate cell culture source, we confirmed that ORF1p is not strictly required for Alu mobilization in our assay. Supporting our observations in HeLa cells, we find that tagged Alu retrotransposition is improved by supplementation of ORF1p in the cultured chicken cells. We postulate that L1 ORF1p plays either a direct or indirect role in enhancing the interaction between the Alu RNA and the required factors needed for its retrotransposition.

1. Introduction

The human *S*hort *IN*terspersed *E*lement (SINE), Alu, is a non-autonomous retroelement of approximately 300 bp that requires enzymatic assistance from *L*ong *IN*terspersed *E*lement-1 (LINE-1 or L1) to retrotranspose. L1 retroelements exhibit a *cis*-preference, whereby the L1 proteins preferentially use the L1 RNA from which they were encoded as the transcript for reverse transcription and integration (Wei et al., 2001; Kulpa and Moran, 2006). However, L1 proteins also work *in trans* to mobilize other cellular RNAs, such as SINEs (Dewannieux et al., 2003; Dewannieux and Heidmann, 2005), SVA (Ostertag et al., 2003; Wang et al., 2005), and processed pseudogenes (Esnault et al., 2000). Alu and L1 are by far the most abundant human non-LTR retroelements, contributing to approximately 11% and 17% of human genome sequence mass, respectively (Lander et al., 2001). The abundance and continuing retrotransposition potential of Alu and L1 are major contributors to human genomic instability (Kazazian and Moran, 1998; Kazazian, 2004; Xing et al., 2007). Both elements have been implicated in multiple instances of human disease, whether by triggering deletion events through mediating non-allelic, homologous recombination events or from *de novo* insertions (Deininger and Batzer, 1999; Chen et al., 2005).

L1 contains two open reading frames (ORF1 and ORF2) that are translated from a single bi-cistronic transcript (Scott et al., 1987), though various splice products diversify the potential mechanisms of translation (Perepelitsa-Belancio and Deininger, 2003; Belancio et al., 2006). Both proteins are necessary for L1 retrotransposition. ORF2p is a multifunctional protein consisting of an N-terminal endonuclease domain (Feng et al., 1996), a central reverse transcriptase domain (Mathias et al., 1991), and a C-terminal cysteine-rich domain of unknown function (Fanning and Singer, 1987). Non-LTR retroelements and their non-autonomous parasites replicate via target-primed reverse transcription (TPRT) of RNA intermediates (Luan et al., 1993; Cost et al., 2002), which result in signature target site duplications upon integration. During retrotransposition, a semi-conserved T-rich DNA target site is cleaved on one strand by the ORF2p endonuclease (Feng et al., 1996; Cost and Boeke, 1998). The free 3′-hydroxyl is thought to serve as a primer by annealing to the polyA tail of the L1

Abbreviations: LINE, Long INterspersed Element; LTR, Long Terminal Repeat; G418R, G418 resistant; ORF, Open Reading Frame; PCR, Polymerase Chain Reaction; Pol II, RNA Polymerase II; Pol III, RNA Polymerase III; SINE, Short INterspersed Element; snRNA, short nuclear RNA; TET, tetrahymena intron; TPRT, Target Primed Reverse Transcription.

* Corresponding author. Tulane Cancer Center, SL66, Tulane University Health Sciences Center, 1430 Tulane Ave., New Orleans, LA 70112, United States. Tel.: +1 504 988 6316; fax: +1 504 988 5516.
E-mail address: aengel@tulane.edu (A.M. Roy-Engel).

[1] NW and BW contributed equally to this research.

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(Luan et al., 1993; Cost et al., 2002). The reverse transcriptase then uses the L1 RNA as a template to synthesize the first strand of the retrotransposed product. By analogy to observed enzymatic functions of the non-LTR R2 retrotransposon (Kurzynska-Kokorniak et al., 2007), second strand cleavage and synthesis are also likely functions of the ORF2p. However the exact details and the potential cellular factors involved in these final insertion steps remain unclear.

ORF1p consists of an N-terminal coiled-coil domain, a middle domain of unknown function, and C-terminal conserved domain (Januszyk et al., 2007). The ORF1 protein binds single-stranded L1 RNA to form a ribonucleoprotein particle and is thought to serve as a nucleic acid chaperone (Martin et al., 2000; Martin and Bushman, 2001). Mutations that affect the chaperone activity of ORF1p also abolish L1 retrotranspositional capability (Martin et al., 2005). A potential L1 ORF1p function in TPRT has been suggested (Martin and Bushman, 2001) and may exist in other non-LTR elements with very different ORF1 proteins (Matsumoto et al., 2006), but a direct role has not been established. Although ORF1p is essential for L1 retrotransposition and likely aids trans-mobilization of other elements such as U6 (Garcia-Perez et al., 2007) and processed pseudogenes (Esnault et al., 2000; Wei et al., 2001), Alu retrotransposition in cultured HeLa cells is observed when supplemented with L1 elements lacking functional ORF1p (Dewannieux et al., 2003; Hulme et al., 2007). While these experiments suggest that ORF1p is not essential for Alu retrotransposition, the endogenously expressed ORF1 in HeLa cells, leaves the possibility that low levels of ORF1p are required for Alu mobilization. We explore the role of ORF1p in Alu retrotransposition. By measuring retrotransposition efficiency of tagged Alu constructs with varying amounts of cotransfected ORF1, we show that ORF1p is able to increase Alu mobilization in HeLa cells. We further demonstrate that Alu mobilization remains possible in the absence of L1 ORF1p through experiments in chicken cells lacking endogenous ORF1 sources.

2. Materials and methods

2.1. Plasmids

A schematic of the basic Alu and L1 tagged vectors is shown in Fig. 1.

JM101/L1.3 referred to "L1-tag" contains a full-length copy of the L1.3 element and the *mneo*I indicator cassette cloned in pCEP4 (InVitrogen) (Dombroski et al., 1993; Sassaman et al., 1997).

JM101/L1.3 no tag, referred as "L1 no tag" contains a full-length copy of the L1.3 element cloned in pCEP4 (InVitrogen) (Wei et al., 2001).

JM101/L1.3 and JM101/L1.3 no tag were a kind gift of Dr. John Moran.

The open reading frames of the different L1 elements were all cloned into the expression vector pBudCE4.1 (InVitrogen), under control of the CMV promoter:

pBudORF2opt (Gasior et al., 2006) and pBudORF1opt (Wallace, et al. unpublished) were created using a partially codon optimized ORF2 or fully codon optimized ORF1 of $L1_{RP}$.

$pBudORF2_{spa}$ and $pBudORF1_{spa}$ contain the coding sequences of the $L1_{spa}$ (Naas et al., 1998); $pBudORF1_{A102}$ and $ORF2_{A102}$ contain the coding sequences of the $L1_{A102}$ (Goodier et al., 2001); $pBudORF1_{LRE3}$ and $pBudORF2_{LRE3}$ contain the coding sequences of the $L1_{LRE3}$ (Brouha et al., 2002). Plasmids containing the $L1_{A102}$ and $L1_{LRE3}$ sequences were kind gifts from Dr. John Goodier.

$pBudORF2_{syn}$ and $pBudORF1_{syn}$ contain the $L1_{spa}$ codon optimized sequences from plasmid psmL1 (a kind gift from Dr. Jef Boeke) (Han and Boeke, 2004).

AluYa5-*neo*^{TET} contains a 7SL upstream enhancer region —AluYa5 followed by the *neo*^{TET} self-splicing indicator cassette and 44 A-stretch followed by a pol III terminator was a kind gift from Dr. Thierry Heidmann (Dewannieux et al., 2003).

*p*AluYa5-*neo*^{TET}, contains a larger amount of the upstream pol III enhancer sequence of the 7SL gene (113 bp) and theAluYa5 consensus sequence from $p^{7SL}Ya5^{BC1}$ (Roy et al., 2000).

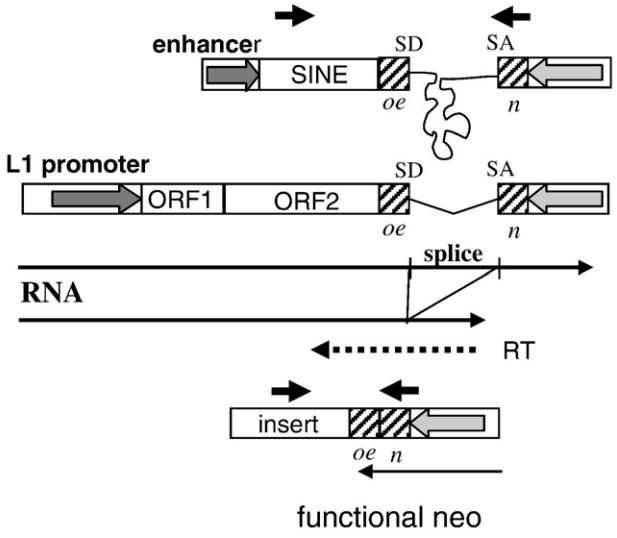

Fig. 1. Schematic of the LINE and SINE assay. RNA transcription is performed by L1 5′UTR promoter or the internal pol III promoter of the SINE enhanced by the 7SL upstream sequence. An intron (a self-splicing in the SINE vector) interrupts the neomycin (neo) resistance gene (hatched box) and promoter present in an inverted orientation. Because of orientation, the intron will splice out only from the transcripts generated by the retroelement's promoter. The RNA is reverse transcribed, followed by integration of the cDNA into the genome. The new insert contains a functional neomycin gene.

pIRES2-EGFP (BD Biosciences Clontech) was used as the $G418^R$ expression plasmid for toxicity control.

All plasmid DNA was isolated by alkaline lysis and twice purified by cesium chloride buoyant density centrifugation. Plasmids were independently purified in triplicate. DNA quality was also evaluated by the visual assessment of ethidium bromide stained agarose gel electrophoresed aliquots to evaluate purity (RNA) and quality (nicking of the supercoiled plasmid). All new constructs were sequence verified.

2.2. LINE and SINE assays

Transient L1 (Moran et al., 1996) or Alu (Dewannieux et al., 2003) retrotransposition assays were performed as previously described with some minor modifications. Briefly, HeLa cells (ATCC CCL2) or chicken embryo fibroblast cells (ATCC CRL-12203), were seeded in T-75 flasks at a density of 5×10^5 and 1.5×10^6 cells/flask, respectively. The chicken fibroblasts were grown at their optimal temperature of 39 °C. Transient transfections were performed the next day using a Lipofectamine and Plus cocktail (InVitrogen) following the manufacturer's protocol. All plates within an experiment were transfected with identical total amounts of DNA to avoid potential variations in transfection efficiency. When experimental amounts of plasmid, such as ORF1-expression plasmids were varied between points, empty expression plasmid was used to normalize them all to the same total mass of plasmid DNA in each plate. The plasmid concentrations were standardized to achieve a linear range and avoid Alu colony saturation levels for each experiment. Cells were grown under selection media containing 400 μg/ml Geneticin (Fisher Scientific), also known as G418, for 14 days. Colonies were fixed, stained and visually scored as $G418^R$-resistant colonies.

2.3. Northern blot analysis

RNA extraction and poly(A) selection was performed as previously described (Perepelitsa-Belancio and Deininger, 2003) with the following modifications. The polyadenylated RNA species were separated in a 2% agarose-formaldehyde gel and transferred to a Hybond-N nylon membrane (Amersham Biosciences). A PCR product using the following primers T7neo(−): 5′-TAATACGACTCACTATAAGGACGAGGCAGCG-3′ and Neo

northern(+): 5′-GAAGAACTCGTCAAGAAGG-3′ was used a DNA template to generate a riboprobe complementary to the 3′ region of the neomycin gene. The results were analyzed using a Typhoon PhosphorImager (Amersham Biosciences) and the ImageQuant software.

2.4. DNA isolation and PCR analysis

After the two week selection, the G418^R colonies obtained were pooled, reseeded in 100×15 mm dishes and grown to confluency. DNA was extracted from the cells using the DNA Easy kit (Qiagen) following the manufacturer's recommended protocol. PCR amplification was performed with primers designed to amplify from the Alu sequence (5′-GAGGCGGCGGATCACGAGG-3′) to the 3′ region of the neomycin cassette (5′-ACTGGGCACAACAGACAATCGCC-3′); the primer annealing sites are shown as arrows in Fig. 1.

3. Results

3.1. ORF1p enhances mobilization of a tagged Alu in HeLa cells

Previous data demonstrate that supplementation of L1 ORF2p is sufficient for Alu retrotransposition to occur in HeLa cells (Dewannieux et al., 2003). However, L1 ORF1p implication in the augmentation of U6 sn RNA pseudogene formation (Garcia-Perez et al., 2007) supports the idea that L1 ORF1p may also enhance Alu retrotransposition. To further characterize the effect of L1 ORF1p on Alu retrotransposition, we studied the retrotransposition of a marked Alu driven by L1 ORF2p (ORF2) in the presence of varying amounts of an L1 ORF1 expression vector in HeLa cells. We utilize previously described tissue culture assay systems (Fig. 1) designed to allow for the specific detection of newly inserted retroelements (Moran et al., 1996; Dewannieux et al., 2003). In this assay, the generation of a functional neomycin gene only occurs if the tagged SINE or L1 RNA is spliced, reverse transcribed and inserted into the genome. Thus, the newly inserted SINE or LINE will carry a functional neomycin gene that will generate cell colonies after selection with G418. Alu retrotransposition was enhanced by the addition of L1 ORF1 in a dose dependent manner with a slope of 95.12±11.92. Alu retrotransposition driven by ORF2p was increased up to 5 fold (Fig. 2A) when supplemented with L1 ORF1p. To determine if this enhancing effect was specific to Alu retrotransposition, we also measured the retrotransposition rate of a tagged L1 (L1-tag) in the presence of various amounts ORF1. As expected, L1 retrotransposition appears to be unaffected by exogenous supplementation of ORF1p (showing a negative slope of −12.70±3.55) probably due to its *cis*-preference (Wei et al., 2001). Thus, L1 ORF1p expressed *in trans* appears to enhance Alu retrotransposition, while not affecting L1 retrotransposition.

We evaluated the possibility that ORF1p may affect Alu-tag transcription and/or splicing and thus altering the total amount of RNA that will generate neomycin colonies. Northern blot analysis showed no significant effect of L1 ORF1p on the Alu RNA levels (Fig. 2B), implicating a more downstream role of ORF1p on Alu retrotransposition.

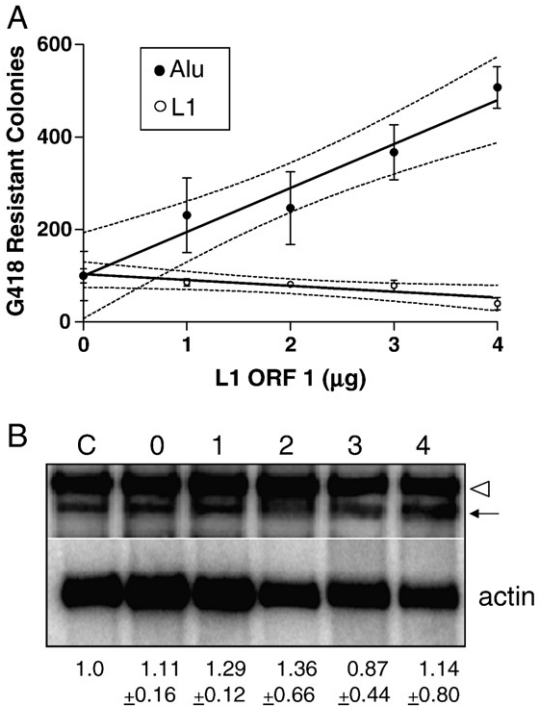

Fig. 2. L1 ORF1p enhances retrotransposition of a tagged Alu but not L1. A. L1 ORF1p gradient and retrotransposition activity of L1 and Alu (driven by ORF2$_{RP}$) in HeLa cells. Cells were transfected with L1-tag (open circle) or Alu*neo*^{TET} plus ORF2 (black circle) and supplemented with increasing concentrations of ORF1. The mean G418^R colonies for each retroelement are plotted as a solid line, where error bars represent the standard deviation and the flanking discontinuous lines represent the 95% confidence interval. The calculated slope for Alu is 95.12±11.92 and for L1 is −12.70±3.55 and shown to be significantly different as determined by Least squares regression analysis, $p=0.00013$ $n=3$. B. Alu-tag RNA transcription and processing is unaffected by the supplementation of ORF1p. Representative northern blot analysis of polyA selected RNA from transfected cells with the tagged Alu vector alone (C, control), or supplemented with ORF2p plus different amounts of ORF1p (0–4 μg). The unspliced (open arrowhead), spliced RNA (small arrow) and actin bands are indicated. Four separate transfections and northern blot analyses were performed for the quantitative evaluation. The numbers below represent the relative mean±SD of Alu-tag spliced (β-actin corrected) relative to the control (C) which was arbitrarily defined as "1". No significant differences between the amounts of the spliced Alu-tag RNA of any of the experimental conditions relative to the control (Student paired *t*-test $p\geq0.23$).

3.2. Different ORF1p sources from humans and rodents consistently enhance tagged Alu mobilization

We next sought to determine whether or not ORF1p enhanced Alu mobilization was restricted to ORFs derived from human L1$_{RP}$. We chose one additional human L1 source (LRE3) from the eight recognized "hot L1s" (Brouha et al., 2003) and the rodent L1$_{A102}$ and L1$_{spa}$ (Goodier et al., 2001). We find that cotransfection of human or rodent ORF2p from any of these elements is sufficient to mobilize the tagged Alu in HeLa cells (Fig. 3). Supplementation with the "partner" ORF1p significantly increased Alu mobilization rates in all three cases (Fig. 3). Thus, our observations from both human and rodent L1 sources suggest that ORF1p consistently enhances the mobilization of tagged Alu elements.

3.3. An exogenous source of L1 ORF2p is sufficient to mobilize tagged-SINE elements in cells devoid of ORF1p

While L1 retrotransposition requires both expression of functional ORF1p and ORF2p, previous tissue culture experiments in HeLa cells using an in-phase deletion of ORF1 sequence suggest that Alu retrotransposition is dependent only upon L1 ORF2p (Dewannieux et al., 2003). However, these data do not rule out the possibility that low levels of endogenous ORF1p, contributed to the observed mobilization, because HeLa cells are known to express full-length L1 mRNA (Belancio et al., 2006) and ORF1p (Harris Soifer, personal communication).

To further evaluate the requirement of ORF1p in Alu retrotransposition, we selected an alternate cell line devoid of endogenous LINE-1. LINE-1 is present throughout mammalian genomes. However, bird genomes contain only a very distantly-related family of non-LTR retroelement termed CR-1 (Burch et al., 1993; Haas et al., 1997; Kajikawa et al., 1997). Furthermore, analysis of the chicken genome reveals a paucity of new SINEs or retropseudogenes, strongly indicating that CR1 proteins may not efficiently mobilize *in trans* other "A-tail" containing transcripts *i.e.* mRNAs and SINEs (Hillier

et al., 2004). These reasons make a chicken cell line a reasonable option to further investigate the role of L1 factors in Alu mobilization.

We evaluated the mobilization of tagged tagged-SINE elements in a chicken embryo fibroblast cell line. Chicken cells transfected with the Alu-tag supplemented only with the human ($L1_{RP}$) or mouse ($L1_{spa}$) ORF2 expression vectors generated multiple G418 resistant colonies (Fig. 4A). No colonies were ever observed without the expression of L1 proteins (control). To confirm the validity of these inserts as retrotransposed Alu-tag inserts, DNA extracted from pooled $G418^R$ colonies was evaluated by PCR to confirm the absence of the intron within the neomycin resistance gene (a signature of a genuine retrotransposed sequence). We determined that the colonies obtained from the transfected chicken cells represented *bona fide* retrotransposed inserts of the marked Alu transcript (Fig. 4B). These data from transient transfections demonstrate that Alu mobilization is possible in a cellular environment devoid of L1 ORF1 protein.

3.4. The human ORF1p enhances the mobilization of Alu in chicken cells

As performed in previous experiments using HeLa cells, we evaluated the effect of supplementation with increasing amounts of ORF1p on Alu retrotransposition in the chicken fibroblasts. Inserts from these colonies were also evaluated and confirmed to represent true tagged Alu retrotranspositions (Fig. 4B). Cotransfection of the ORF1p was able to enhance mobilization of a tagged Alu in chicken cells (Fig. 4C). The effect tapered off at the higher concentrations of ORF1 expression vector, though not decreasing to the low level of efficiency observed for transfections with no added ORF1. Similar effects were also observed when using HeLa cells (data not shown). This reduction in $G418^R$ colony numbers is likely due to the inhibitory or "toxic" effects of ORF1p overexpression on cellular viability and colony formation capability (Supplemental Figs. 1 and 2).

4. Discussion

Previous studies using human cells have suggested that SINEs might not require, or even be able to use, the L1 ORF1p for mobilization. Our data clearly confirm that Alu can be efficiently mobilized in tissue culture without the assistance of L1 ORF1p. However, supplementation of L1 ORF1p can increase Alu retrotransposition up to 5-fold. This enhancement appears to be conserved between rodents and humans, as ORF1p from different human and mouse L1 subfamilies similarly aid Alu retrotransposition. In mammals, ORF1 proteins contain a conserved C-terminal basic domain and a less conserved coiled-coil N-terminal domain.

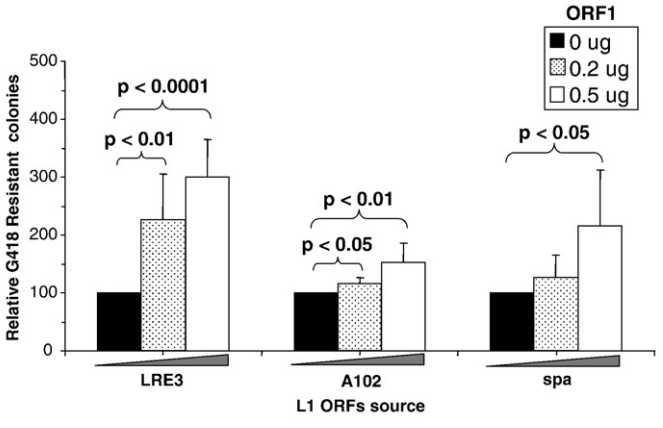

Fig. 3. Both human and rodent ORF1p enhance ORF2 driven Alu retrotransposition. HeLa cells were transfected with the tagged Alu supplemented with an ORF2p expression vector from the human $L1_{LRE}$ element or the rodent $L1_{A102}$ or $L1_{spa}$ elements ($n=5$). Each Alu-ORF2p set was cotransfected with different amounts of the corresponding human/rodent ORF1p expression vector or empty vector for the "0 μg" or ORF2 only control. The relative G418R colonies were graphed using the ORF2 only control (black column) reference, which was arbitrarily assigned as 100. The *p*-values (Student paired *t*-test) for significant differences are indicated above the columns.

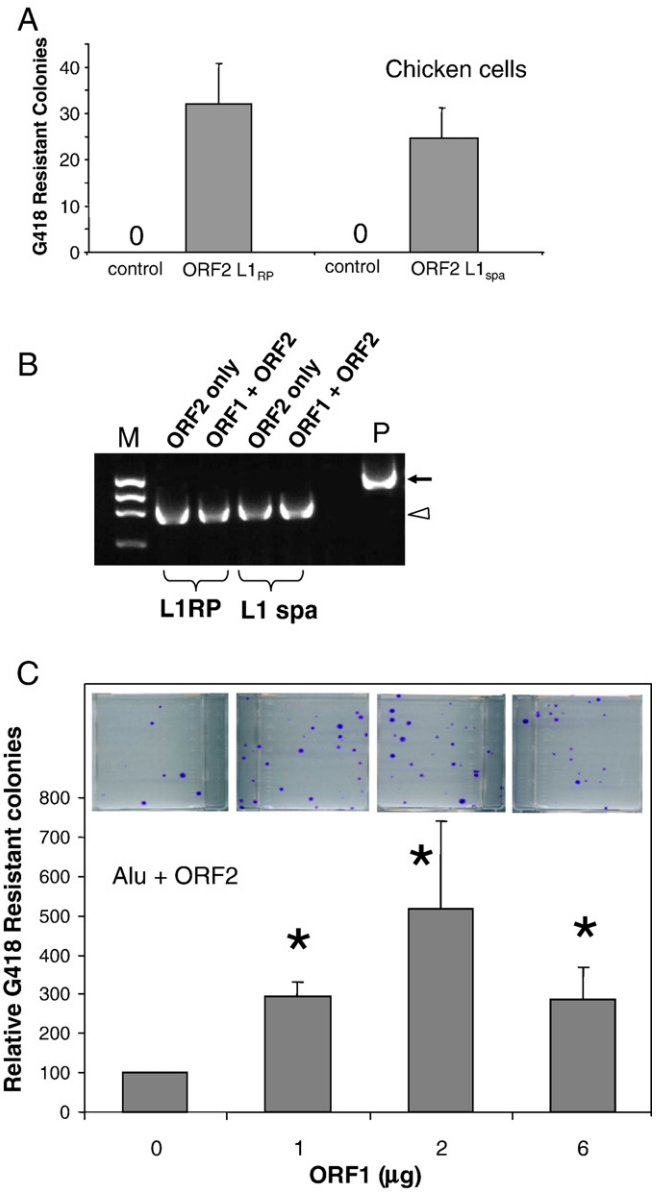

Fig. 4. A ORF2p is sufficient to mobilize SINEs in chicken cells. A. Retrotransposition activity of tagged Alu supplemented with the synthetic ORF2s from the human $L1_{RP}$ or the mouse $L1_{spa}$ in chicken embryo fibroblast cells. The mean of the total number of G418 resistant colonies for each time point is shown ($n=5$). No colonies were ever observed for the Alu-tag cotransfected with the empty vector (control) indicated by "0". B. Evaluation of the Alu inserts from the chicken cells. Alu inserts were evaluated by PCR analysis using primers to the Alu sequence and the neomycin gene sequence (shown as bold arrows in Fig. 1). DNA was recovered from pooled G418 resistance colonies generated in the chicken embryo fibroblast cells transfected with the tagged Alu vector plus the synthetic versions of either human ($L1_{RP}$) or mouse ($L1_{spa}$) ORF2 with and without supplementation of the corresponding ORF1p. An open arrowhead indicates the PCR product corresponding to an insert containing the spliced version of the Alu expression vector. The Alu expression plasmid (P lane) was used as the control for unspliced products (small arrow). DNA from untransfected cells were used as the negative control (−). M is DNA marker. **C.** ORF1p enhances SINE retrotransposition in chicken cells. Cells were transfected with the tagged Alu plus the human ORF2p ($L1_{RP}$) supplemented with different amounts of the ORF1p expression vector (0, 1, 2 or 6 μg). Columns represent the mean number of G418 resistant colonies for each time point ($n=5$) and S.D. shown as error bars. Results significantly different from the no ORF1 reference transfection with *p*-values of $p \leq 0.05$ (Student paired *t*-test) are indicated by an asterisk (*).

Comparisons between different subtypes of human and rodent L1 elements show great sequence variation in this coiled-coil region (Furano, 2000; Mears and Hutchison, 2001; Boissinot and Furano, 2001; Goodier et al., 2001). Because both mouse and human ORF1p enhanced Alu

retrotransposition, the conserved C-terminus could have a direct role in this effect. However, the N-termini of the rodent and human ORF1ps, though very different in DNA sequence, share a similar structural coiled-coil domain, making it possible that both or either of the domains contribute to the observed increase in Alu amplification.

L1 ORF1 protein expression has been detected in several normal human tissues, such as vascular endothelium, testis, epididymus, placenta (Ergun et al., 2004), and the mammary gland (Asch et al., 1996). This expression is elevated in many cancerous tissues such as ductal carcinomas (Miki et al., 1996), breast carcinomas (Asch et al., 1996), and testicular tumors (Bratthauer and Fanning, 1992). Additionally, some transformed cell lines express the L1 ORF1 protein (Hohjoh and Singer, 1996). Due to the ability of ORF1p to aid Alu retrotransposition, it is possible that genetic instability due to Alu retrotransposition would be enhanced in these L1 ORF1p expressing tissues. Indeed, this increase in Alu activity and subsequent genomic instability would likely be further enhanced in cancerous tissues, where ORF1p expression is higher. Furthermore, our observation suggests that the level of Alu activity in a cell could be correlated with the cell's endogenous ORF1p production.

4.1. Model of potential of ORF1p role in SINE retrotransposition

Several roles have been suggested for ORF1p in the L1 retrotransposition cycle. These include the regulation of ORF2p expression, the recruitment of ORF2p to the L1RNP, or the targeting of the L1 complex to the nucleus and possibly facilitation of strand exchange at the insertion step (Martin, 2006). ORF1p may enhance Alu retrotransposition in two different ways: through direct binding to the RNA or indirectly by enhancing the function of ORF2p or other cellular factors involved in the process (Fig. 5). Because of the chaperone nature of ORF1p, it is reasonable to consider a direct interaction of ORF1p with the SINE RNA. In this case, ORF1p may play a role in protecting the transcript from degradation, or aiding the SINE RNP complex in reaching the nucleus. Interestingly, in contrast to SINEs, retropseudogenes require ORF1p for amplification (Esnault et al., 2000). A previously proposed explanation for this difference, suggests that the SRP9/14 proteins that bind Alu transcripts could potentially "replace" the need for ORF1p in Alu amplification (Dewannieux et al., 2003; Martin, 2006). However, other SINEs, like the rodent B2 elements, are not known to bind SRP9/14 and do not require additional supplementation of ORF1p in HeLa cells for amplification (Dewannieux and Heidmann, 2005). Our own observations suggest that the ORF1p requirement may be dictated by the type of RNA polymerase generating the transcript (pol II vs. pol III) (Kroutter et al., unpublished).

Alternatively, ORF1p may have an indirect role by interacting with components such as ORF2p or cellular factors to facilitate retrotransposition (Fig. 5). It is possible that ORF1p may have a role in increasing the half life of ORF2p or aiding its targeting to the nucleus. In addition, ORF1p may help to target or recruit other cellular factors to the nucleus. As mentioned above, ORF1p may have a potential role in facilitating strand exchange during L1 insertion. It is possible that ORF1p helps Alu insertion in a similar manner. There are observed differences between SINE and LINE amplification and regulation (Hulme et al., 2007). Further analysis of the differential role that ORF1p plays in SINE vs. LINE mobilization could be instrumental in demonstrating how their amplification pathways deviate.

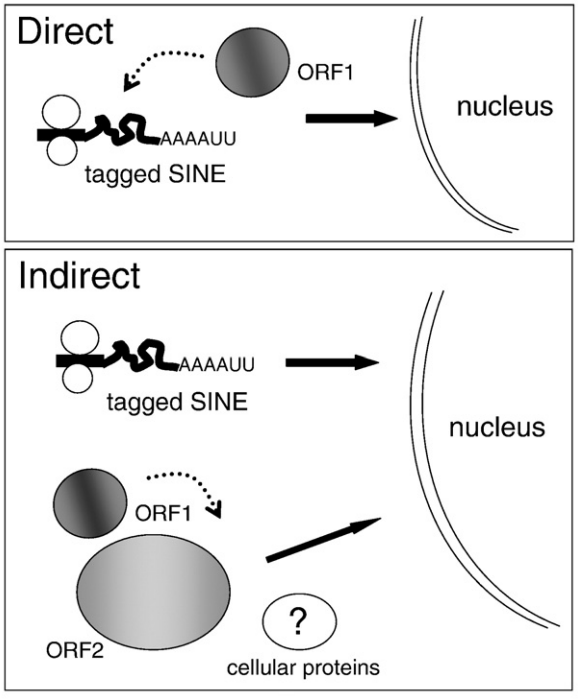

Fig. 5. Model of potential roles of ORF1p in SINE retrotransposition. Although only ORF2p is strictly required for SINE retrotransposition, supplementation with ORF1p enhances their mobilization. There are two potential scenarios of how ORF1p increases Alu retrotransposition. First, because of the chaperone nature of ORF1p, the direct interaction of ORF1p with the SINE RNA may play a role in protecting the transcript from degradation or aiding the RNP complex to reach the nucleus. Alternatively, in the second scenario, ORF1p may have an indirect role by interacting with other components such as ORF2p or cellular factors to facilitate retrotransposition. In addition, ORF1p could have a role in increasing the half life of ORF2p or targeting other cellular factors and ORF2p to the nucleus.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gene.2008.04.007.

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