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LINE-1 Activity in Facultative Heterochromatin Formation during X Chromosome Inactivation

Jennifer C. Chow,[1,2,3] Constance Ciaudo,[1,2,3,4] Melissa J. Fazzari,[5] Nathan Mise,[6,7] Nicolas Servant,[1,8,9] Jacob L. Glass,[5] Matthew Attreed,[5] Philip Avner,[6] Anton Wutz,[10] Emmanuel Barillot,[1,8,9] John M. Greally,[5] Olivier Voinnet,[4] and Edith Heard[1,2,3,*]

[1]Institut Curie, Paris F-75248, France
[2]CNRS UMR3215
[3]INSERM U934
[4]Institut de Biologie Moléculaire des Plantes du CNRS UPR2357, Université de Strasbourg, Strasbourg 67084 Cedex, France
[5]Department of Genetics and Center for Epigenomics, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[6]URA1968 Institut Pasteur, Paris 75015, France
[7]Department of Pharmacology, Jichi Medical University, Tochigi 329-0431, Japan
[8]INSERM U900
[9]Mines ParisTech, Fontainebleau F-77300, France
[10]The Wellcome Trust Centre for Stem Cell Research, University of Cambridge, Cambridge CB2 1QN, UK
*Correspondence: edith.heard@curie.fr
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SUMMARY

During X chromosome inactivation (XCI), Xist RNA coats and silences one of the two X chromosomes in female cells. Little is known about how XCI spreads across the chromosome, although LINE-1 elements have been proposed to play a role. Here we show that LINEs participate in creating a silent nuclear compartment into which genes become recruited. A subset of young LINE-1 elements, however, is expressed during XCI, rather than being silenced. We demonstrate that such LINE expression requires the specific heterochromatic state induced by Xist. These LINEs often lie within escape-prone regions of the X chromosome, but close to genes that are subject to XCI, and are associated with putative endo-siRNAs. LINEs may thus facilitate XCI at different levels, with silent LINEs participating in assembly of a heterochromatic nuclear compartment induced by Xist, and active LINEs participating in local propagation of XCI into regions that would otherwise be prone to escape.

INTRODUCTION

In mammals, one of the two X chromosomes is transcriptionally inactivated in females (see Chow and Heard, 2009 for a recent review). In placental mammals, X chromosome inactivation (XCI) involves the noncoding Xist transcript, which coats the chromosome from which it is produced and triggers inactivation. Although Xist is essential for the XCI process (Marahrens et al., 1997; Penny et al., 1996), the molecular mechanisms involved in silencing over 1000 genes across the chromosome remain unclear. With fewer than 2000 molecules of Xist RNA per nucleus (Buzin et al., 1994), silencing the 150 Mb X chromosome must require some kind of spreading process. Moreover, X:A translocations show different degrees of XCI spread from the X chromosome segment into autosomal DNA, suggesting that specific sequences might facilitate spreading. Gartler and Riggs (Gartler and Riggs, 1983) hypothesized that specific "way stations," which would be particularly enriched on the X, could ensure this spreading function. Lyon proposed that long interspersed elements (LINE-1 or L1) might represent way stations, because they are enriched on the X, compared with autosomes (Boyle et al., 1990), and because L1 density correlates with the efficiency of XCI spread into autosomal regions in both X;autosome translocations and Xic transgene contexts (Lyon, 1998). LINEs are autonomous, non-LTR retrotransposons, measuring up to ~6–7 kb. Although the majority of the L1 copies in the mammalian genome are truncated at their 5′ ends and are transcriptionally and retrotranspositionally incompetent, a number of young, full-length LINEs exist in both mouse and human genomes. The activity of these intact elements can lead to de novo somatic and germ line L1 retrotransposition events. Given the potential danger that extensive mobility represents to genome integrity, LINEs are believed to be stringently controlled, particularly in the germ line, by DNA methylation and RNAi mechanisms (Goodier and Kazazian, 2008). Nevertheless, some mobility of young, active LINEs can be observed during early development (Kano et al., 2009), in human ES cells (Garcia-Perez et al., 2007) and in cancer cells (Belancio et al., 2008). Interestingly, young active L1 elements appear to be particularly enriched on the X chromosome, on the basis of bioinformatics analyses, suggesting that their retention may be advantageous for XCI,

although this has never been tested experimentally (Abrusan et al., 2008; Bailey et al., 2000).

Further support for a role for LINEs in XCI comes from the observation that, on the human X, regions containing genes that escape inactivation are relatively L1 poor (Bailey et al., 2000). Indeed, although the majority of genes are silenced on the inactive X, some genes ("escapees") are transcribed from both X chromosomes (Yang et al., 2010). On the human X, up to 15% of X-linked genes escape XCI to some extent (Carrel and Willard, 2005). Bioinformatics analyses comparing the genome environments of escapees versus genes that are subject to XCI suggests that the XCI potential of specific regions/genes on the inactive X may be mediated by a variety of sequence elements, with some facilitating XCI and others promoting escape (Carrel et al., 2006; Wang et al., 2006). For example, the escapee *Jarid1c* has clearly evolved sequences enabling it to resist inactivation whatever its X-chromosomal location (Li and Carrel, 2008). Some cases of escape could also be due to inadequate inactivation-facilitating sequences and/or the influence of nearby escapees (the bystander effect). CTCF-binding sites have been identified between escapees and inactivated regions and have been hypothesized to play a role in facilitating escape by blocking the spread of inactivation (Filippova et al., 2005). However, the existence of sequences facilitating spread, as opposed to maintenance, of XCI, particularly in escapee neighborhoods, has not been addressed.

A potential role for repeats in the onset of XCI was recently proposed (Chaumeil et al., 2006). One of the earliest events in XCI is the creation, by Xist RNA, of a silent nuclear compartment, depleted of RNA Polymerase II (Pol II) and transcription factors. This inner compartment of the Xi was predicted to consist mainly of silent repeats both in the mouse (Chaumeil et al., 2006) and in humans (Clemson et al., 2006). In differentiating mouse ES cells, genes were found to be located outside the Xist RNA compartment initially, but to move into it as XCI proceeded, whereas genes that escaped from XCI remained external. This demonstrated that escapees and inactivated genes show different nuclear organization and suggested that repeats might play a role in such spatial segregation.

In this study, we set out to investigate the implication of repeats, particularly LINEs, in XCI. We show that silent LINEs participate in formation of the silent nuclear compartment induced by Xist RNA. We also make the surprising discovery that a subset of young, full-length LINEs are transiently expressed from the X chromosome undergoing inactivation and provide evidence that the expression of young LINEs may be exploited, in some regions of the X, to facilitate the local spread of silencing into active regions of the otherwise inactive X chromosome.

RESULTS

Transcription of Repetitive Elements on the X Chromosome during ES Cell Differentiation

Previously, we showed that during early stages of XCI, signals for repetitive transcripts were rapidly lost within the Xist RNA compartment, following Xist RNA coating (Chaumeil et al., 2006; Okamoto et al., 2005). This analysis involved RNA FISH using a Cot1 DNA probe that contains a mixture of different types of repeats. In the present study, we examined the transcriptional activity of various repetitive elements during XCI by RNA FISH using a combination of probes for Xist and specific repeats, such as SINEs and LINEs, on undifferentiated and differentiating (days 2, 4, and 8–10) female ES cells. SINEs consist of 150–200 bp B1 and B2 repeats, which are enriched in GC-rich, gene-dense regions of the genome. LINEs are up to 6–7 kb in length and are most abundant in gene-poor, AT-rich regions. In undifferentiated ES cells, both SINE- and LINE-derived RNA could be detected from the two active X chromosomes (surrounding the Xist primary transcript signals; Figures 1A and 1B). Upon differentiation, rapid loss of SINE transcripts (B1 or B2) was detected within the Xist RNA domain (Figure 1A), with kinetics similar to those previously found for Cot1 RNA depletion (Chaumeil et al., 2006). Transcription of LINEs, investigated with a full-length L1 probe (TNC7), was also lost within the Xist RNA compartment during early (days 2–4) differentiation, with slightly slower kinetics, compared with the SINE transcripts (Figure 1B, upper and middle panels). DNA FISH with an X chromosome paint, combined with either a SINE or a LINE probe, showed that these sequences are present throughout the X chromosome detected by the paint (Figure 1C), but their transcripts are silenced within the Xist RNA-coated portion of the X chromosome early during XCI. However, in addition to this depletion of LINE RNA within the Xist compartment, L1 transcripts were enriched just adjacent to the Xist RNA domain, at day 4 of differentiation (Figure 1B). L1 RNA was also detected at the site of the active X chromosome at this stage, in both female (Figure 1B) and male (Figure S1A available online) cells. This apparent L1 RNA accumulation on both X chromosomes at day 4 of differentiation might be simply due to the higher density of LINEs on the X compared to the rest of the genome (Figure 1C) (Mikkelsen et al., 2007; Waterston et al., 2002). However, this hypothesis alone is unlikely to account for the phenomenon, because by day 10 of differentiation, when L1 transcript signals showed a global reduction in the nucleus and on the active X chromosome, LINE expression on the inactive X persisted (Figure 1B). Furthermore, at this stage of differentiation, L1 RNA accumulation overlapped with the Xist RNA domain, in contrast to the situation at day 4 where it was adjacent to Xist (Figure 1B; middle panel). This unusual profile of L1 RNA on the Xi was no longer present in fully differentiated mouse embryonic fibroblasts (MEFs) (Figures 1B and 1D). Collectively, these results show that the bulk of X-chromosomal SINEs and LINEs are rapidly silenced within the Xist RNA compartment early on in XCI. However, a subpopulation of LINEs is specifically expressed from the inactive X chromosome during later stages of XCI.

Young, Full-Length LINEs Are Transcribed from the Inactive X Chromosome during Late Differentiation Stages in Female ES Cells

We next assessed whether the L1 RNA associated with the X chromosome undergoing inactivation was derived from specific full-length LINEs, driven by their own promoters, or from truncated L1s (which usually lack 5′ promoter regions; Ostertag and Kazazian, 2001) lying within other transcription units. Promoter activity of mouse L1 elements lies in tandemly