Joost Gribnau - Visualization and characterization of regulators of X-inactivation

Background

In mammals, dosage compensation of X-linked genes is achieved by inactivation of one of the two X-chromosomes in female somatic cells. At the onset of random X-inactivation, around the implantation stage of the embryo, one X-chromosome is designated to remain active (Xa) and all other X-chromosomes will be inactivated (Xi). Genetic studies have revealed that silencing of the X-chromosome initiates from one location on the X-chromosome, termed the X-inactivation center (Xic). The Xist gene, which is located within the Xic, is required and sufficient for X-inactivation. Xist encodes a non-coding RNA that coats the inactive X-chromosome, thereby directly or indirectly attracting chromatin components and chromatin modifiers, which initiate and establish epigenetic changes rendering the Xi transcriptionally inactive. In most cells the Xi is visible as a dense stainable structure in the nucleus, called the Barr body. Once the X-chromosome is inactivated, this inactive state is inherited clonally through many rounds of cell division (for review see Plath 2002).

The first notable change in chromatin structure on the Xi, is global tri-methylation of the lysine 27 residue of histone H3 (H3-K27), which happens concomitantly with the initial *Xist* RNA spread along the chromosome. Accumulation of this histone modification is mediated by the Polycomb group proteins EED and Ezh2, which are transiently enriched on the Xi at the onset of X-inactivation (Silva 2003, Plath 2003). EED and Ezh2 act in a complex and are also directly or indirectly required for global di-methylation of histone H3 lysine 9 (H3-K9) of Xi chromatin, which happens after the accumulation of H3-K27 tri-methylation (for review see Cohen 2002). Other chromatin modifications found to occur in the early stages of X-inactivation are global hypo acetylation of H3-K9 and hyper acetylation and hypo methylation of histone H3 lysine 4. Several other epigenetic changes happen relatively late during the X-inactivation process including global hypo acetylation of histone H4, accumulation of the histone variant macrohistone H2A and CpG island methylation. These later changes may be responsible and sufficient for the maintenance of the silent state of the Xi in somatic cells. In addition, Brca1 has been found to colocalize with the Xi in somatic cells, but its role in the X-inactivation process is not well defined.

Despite the recent progress in determining different chromatin modifications and proteins involved in the initiation, establishment and maintenance of the inactive X, little is known about the initial stages of the X-inactivation process, especially how Xist RNA silences the Xi, how Xist RNA associates with and spreads along the X-chromosome, and how the choice which X-chromosome to inactivate occurs. The X-inactivation process starts with choosing one of two identical X-chromosomes to remain active and the other one to become inactivated. In humans and mice this choice process can be skewed to some extent in which more of either the paternal or the maternal inherited X chromosomes are inactivated. The skewing process is mediated by the Xce locus, which overlaps and extends downstream of the Xist gene. In a clinical setting, skewing of X inactivation has been linked to penetrance of X-linked diseases such as Rett syndrome in heterozygous women. If an X-linked disease locus is linked to a strong Xce (which means a higher chance to remain active) compared to the wild-type X-chromosome this will result in a higher likelihood for the disease to be manifested. To date it remains unclear how the choice process is regulated. Different models explaining the choice process have been described. The prevailing model predicts a blocking factor, which binds and prevents inactivation of one of the X chromosomes. The blocking factor has to be present in a sufficiently low concentration to

ensure that it will bind only one X chromosome per diploid chromosome set. A second model, the 'transvection' model predicts that the X chromosomes physically meet in space, cross communicate and decide which of the X chromosomes will be inactivated. The choice process may also be stochastic, in which the Xic with the highest concentration of Xist RNA will trigger silencing complex formation. Initiation of such a complex could in turn down regulate silencing on the second X chromosome. None of these models are mutually exclusive and may all to some extent contribute to the choice process.

Another intriguing and unexplained part of mammalian X-inactivation is spreading of Xist RNA over the Xi. It is unclear whether Xist RNA spreads along the chromosome in a linear fashion, using chromatin entry sites, or whether it jumps along chromatin loops. Finally, although many chromatin modifications have been detected on the Xi to date none of those have been implicated in the initial silencing of the X-chromosome. Many data suggest that Xist RNA functions in association with proteins, and identifying these proteins would help to elucidate the mechanism of spreading and silencing by Xist RNA. In order to increase our understanding of these different processes we have established a system in which Xist RNA is tagged. The tag allows live visualization of Xist RNA, purification of Xist binding proteins, and targeting of candidate proteins, implicated in the X-inactivation process, to Xist RNA in order to identify their role in X-inactivation.

Aims

The aim of my lab is to understand the role of the chromatin remodeling complexes and chromatin modifications in gene expression or silencing. We use the X-inactivation system as a model system to study these processes. We have generated Xist tagged ES cell lines. The tagging system is based on an insertion of 16 stem-loop RNA binding sites for bacteriophage MS2 coat protein into the Xist gene and simultaneous expression of a $MS2_{coat}$-fusion proteins which will bind the MS2 stemloop. Live imaging of X inactivation by tagging the Xist RNA with GFP allows temporal recording of Xist transcription, spreading and nuclear localization on a cellular level and could shed new light on our understanding of this process. In addition, the use of Xist tagged cell lines to target putative regulators of X inactivation or specific chromatin modifiers to the Xi provides a powerful tool to elucidate their individual role in X inactivation or chromatin remodeling in general. The robustness and uniformity of histone modifications on the Xi provides a convenient readout for detecting chromatin changes. MS2 tagged Xist ES cells are also a potent platform for protein purification and may help identify new players in the X inactivation process.

Publications of the last 5 years

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