Seminars in Cell & Developmental Biology 56 (2016) 3–8

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Activators and repressors: A balancing act for X-inactivation

Leeanne Goodrich, Barbara Panning*, Karen Nicole Leung

Department of Biochemistry and Biophysics, University of California, San Francisco, 600 16th Street, San Francisco, CA 94158, United States

ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form 6 May 2016
Accepted 6 May 2016
Available online 17 May 2016

Keywords:
Random X-chromosome inactivation
Non-coding RNA
Xist
Tsix
X-inactivation center
Pluripotency
Embryonic stem cell
Epiblast stem cell
Early post-implantation development
Rlim/Rnf12

ABSTRACT

In early female embryos X-chromosome inactivation occurs concomitant with up regulation of the non-coding RNA, Xist, on the future inactive X-chromosome. Up regulation of Xist and coating of the future inactive X is sufficient to induce silencing. Therefore unlocking the mechanisms of X-chromosome inactivation requires thorough understanding of the transcriptional regulators, both activators and repressors, which control Xist. Mouse pluripotent embryonic stem cells, which have two active X chromosomes, provide a tractable ex vivo model system for studying X-chromosome inactivation, since this process is triggered by differentiation signals in these cultured cells. Yet there are significant discrepancies found between ex vivo analyses in mouse embryonic stem cells and in vivo studies of early embryos. In this review we elaborate on potential models of how Xist is up regulated on a single X chromosome in female cells and how ex vivo and in vivo analyses enlighten our understanding of the activators and repressors that control this non-coding RNA gene.

Contents

The central question in X chromosome inactivation (XCI) is: how do cells with two X chromosomes (Xs) silence only one of their Xs in the correct developmental fashion? In rodent extraembryonic tissues, only the paternally inherited X is silenced [1,2], a process termed imprinted XCI. In the embryo proper of rodents and of other placental mammals XCI is random, and the X inherited from either parent may be silenced [3]. Because XCI is random in embryonic tissues, there is an additional level of complexity-machinery that allows cells to randomly select one X for silencing is integrated into this system. In this review we will describe general models for how XX cells can achieve random silencing of one X, and discuss factors implicated in this process, focusing findings from the mouse system.

X chromosome silencing can be divided into two stages [4]. Establishment of silencing occurs first, when one X transitions from the active to the inactive state. Once silencing is established, the silenced X is stably maintained throughout all subsequent cell divisions. A key player in XCI is the X-linked gene *Xist*, which encodes a non-coding RNA that coats the inactive X (Xi) *in cis* [5]. *Xist* is up regulated on the X that will be silenced concomitant with the initial establishment of silencing and continues to coat the Xi during

* Corresponding author.
E-mail address: barbara.panning@gmail.com (B. Panning).

http://dx.doi.org/10.1016/j.semcdb.2016.05.005
1084-9521/

the maintenance of silencing. In addition, *Xist* is sufficient to silence a *cis*-linked chromosome (X or autosome) during a brief developmental window [6], consistent with a key role in establishment of silencing. Thus the developmental cues that trigger silencing and the mechanisms that allow random choice of one X for silencing all converge on *Xist*. Identifying the factors that promote *Xist* up regulation on the future inactive X is pivotal to understanding the control of XCI.

1. Developmental control of XCI

Xist up regulation during random XCI is developmentally controlled [7,8]. In the mouse model, activation of *Xist* expression occurs shortly after implantation [9,10]. Prior to implantation, the inner cell mass (ICM) of the XX blastocyst, which will give rise to the embryo proper, is composed of cells with two active Xs (Xas). Implantation, which begins at approximately embryonic day (E) 4.5, triggers a dramatic reorganization of the ICM. Within 24 h, by E5.5, the ICM cells convert to the epiblast, a change to a pseudostratified epithelium accompanied by *Xist* up regulation and establishment of silencing [11].

A host of dramatic changes occur during implantation [12]. The exit from the glycoprotein shell of the zona pellucida and establishment of contact with the uterine membrane is characterized by changes in signaling molecules, extracellular matrix contacts, mechanical forces, and oxygen and nutrient availability [11]. It remains to be determined whether *Xist* up regulation is a consequence of one or more of these changes. Understanding how developmental signaling pathways connect with the transcriptional regulators that control *Xist* is a key question in XCI.

While the peri-implantation developmental window is difficult to access *in vivo*, pluripotent stem cells isolated from the ICM provide a model system for the study of XCI *ex vivo*. Pluripotent stem cells resembling cells in the ICM, embryonic stem cells (ESCs), can be isolated and propagated *ex vivo* under appropriate signaling milieus [13]. XX ESCs maintain two Xas, mirroring the cells of the ICM and when differentiated in culture *Xist* is up regulated on the prospective Xi [9]. *In vivo* a complex combination of inputs such as extracellular matrix contacts and nutrient availability may intersect with growth factor signaling to direct *Xist* up regulation. *Ex vivo* analyses may not fully recapitulate the events that control *Xist* expression. Depending on the differentiation method used, *Xist* up regulation can take several days and is generally highly asynchronous in the ESC system. In contrast, the process appears to be synchronous and complete within a 12–24 h window *in vivo* [14] [10]. These differences suggest that critical players may be over looked *ex vivo*. Factors that are not normally important *in vivo* may play a role under non-physiological *ex vivo* signaling conditions. These concerns highlight the importance of testing the effects of candidate regulator mutations *in vivo* as well as *ex vivo*. Therefore, whenever possible, we will compare and contrast the mutational analysis of potential *Xist* activators and repressors in culture and in embryos.

1.1. *Xist* cis-regulatory elements

In other examples of developmentally controlled up regulation, crucial *cis*-regulatory elements exhibit a poised chromatin state before they receive the signal that triggers the developmental transition [15,16]. This poised state is thought to facilitate rapid changes in gene expression in response to developmental signaling molecules. *In vivo*, *Xist* exhibits rapid up regulation in concert with a key developmental transition, suggesting that *Xist cis*-regulatory elements in ICM cells may be in a poised state.

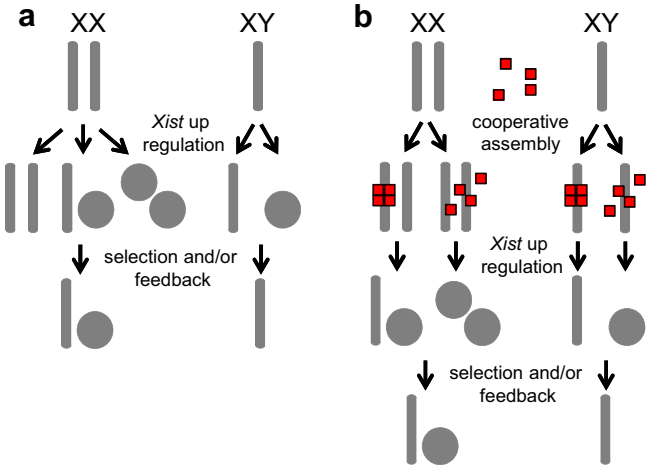

Fig. 1. Models in which there is no input from the number of Xs. (a) All Xas (gray bars) in XX or XY ESCs/ICM cells (top row) have a small probability of *Xist* up regulation. As a result the epiblast initially consists of cells (middle row) in which neither, one, or both Xs are silenced in XX individuals and in which the X in XY individuals in active or inactive (Xi indicated by gray circle). Selection or feedback ensures that the final population of cells in the embryo (bottom row) consist of one Xa and one Xi in XX animals and one Xa in XY animals. (b) An autosomal gene product (red squares) is used to designate the Xa. Binding of this product is highly cooperative, such that it can assemble on only one X in XX or XY cells (middle row, left Xs). In some cells cooperative binding may not occur on any Xs within the appropriate time window (second row, right Xs). *Xist* is up regulated on the unbound Xs and silencing occurs (third row). As in (a), feedback or selection ensures that all somatic cells contain one Xa and one Xi in XX animals and one Xa in XY animals.

Consistent with models for poised expression, *Xist* is transcribed at low levels from all Xas in XY or XX ESCs or ICM cells [10,17,18]. This expression is two to three orders of magnitude lower than the level seen after *Xist* up regulation, when Xist RNA coats the Xi. Crucial to understanding *Xist* expression is identification of its *cis*-regulatory elements. In principle a different set of elements may direct the low level expression from Xas in ESCs/ICM, the rapid up regulation of *Xist* expression during establishment of silencing in differentiating cells, and the abundant expression from the Xi during maintenance of silencing in differentiated cells. Chromatin capture methods suggest that *Xist cis*-elements in ESCs may lie in an approximately 500 kb topologically associated domain (TAD) [19]. It remains to be determined whether elements in this TAD play a role in low-level *Xist* expression in ESCs/ICM. In addition this TAD may contain elements that poise *Xist* for up regulation upon differentiation. While poising is an attractive model for precise developmental control of *Xist*, other models may also explain the rapid and synchronous up regulation of *Xist* on the future Xi upon implantation.

1.2. Classes of models

The observation that individuals with supernumerary Xs silence all but one X, lead to the 'n-1′ rule [20]. This rule postulates that one X per diploid genome remains active, and all additional Xs are silenced [21]. On the basis of the n-1 rule, models postulating that there is a robust system for ensuring only one X remains active have been proposed. Models vary on (i) whether the mechanism functions before or after *Xist* up regulation and (ii) whether there is an input from the number of Xs.

1.3. Models without input from the number of Xs

One class of model posits that all Xs are competent for *Xist* up regulation regardless of the number of Xs in the cell [22] (Fig. 1a). In this class of model there are two ways that could ensure that *Xist*