IX. Appendix B

Molecular mechanism and pattern of X chromosome inactivation

A. Molecular mechanism of X chromosome inactivation

Today, it is believed that two forms of XCI have evolved. Silencing of the father's X chromosome in marsupials and in the placenta of some eutherians depends on the imprinted form, whereas random inactivation, as proposed by Lyon, is found in somatic cells of all eutherians studied to date. In what follows, the molecular mechanisms known to play a role in the latter form of XCI are discussed.

A.1. Counting of X chromosomes

Cellular counting, sensing the number of X chromosomes, depends on the X-inactivation centre.

The observation in polyploid individuals[1330,1331] and in people with a supernumerary number of X chromosomes that one X chromosome remains active for every two sets of autosomes implies the existence of a cellular counting mechanism that senses the dosage effects of the X chromosomes. The molecular basis of such a mechanism remains unclear, but it has been shown that it depends on a 20 kb bipartite domain[1332] within the X-inactivation centre, a ~1 Mb locus located on Xq13.2[1333].

A.2. Onset, establishment and maintenance of X chromosome inactivation

The onset of XCI correlates with cellular differentiation and occurs in a stepwise fashion. XCI involves late replication, histone modifications and CpG island methylation.

In non-differentiated female cells, both X chromosomes are active. The onset of XCI correlates with cellular differentiation[1334], occurring in the mouse at E6.5. Several observations of cells undergoing differentiation in culture indicate that XCI occurs in a stepwise fashion, as indicated in Fig. IX-1. The first event observed in the initiation of XCI is the physical coating, *in cis*, of X_i with *XIST* RNA. *XIST*, located in the X-inactivation centre, is exclu-

sively expressed from X_i[1335-1337]. It is believed that the *XIST* transcript recruits proteins, which transform the chromatin into a closed and transcriptionally inactive form[1338]. Prior to X-inactivation, the *TSIX* transcript, transcribed from the *XIST* anti-sense strand, counteracts the *XIST* transcript, thereby effectively regulating the onset of XCI in the early embryo[1339]. Other hallmarks in the onset, establishment and maintenance of X_i include late replication[1340], H3-K9/K27 methylation[1341,1342], hypoacetylation on H4[1343], enrichment in histone macroH2A1[1344] and CpG island methylation[1345].

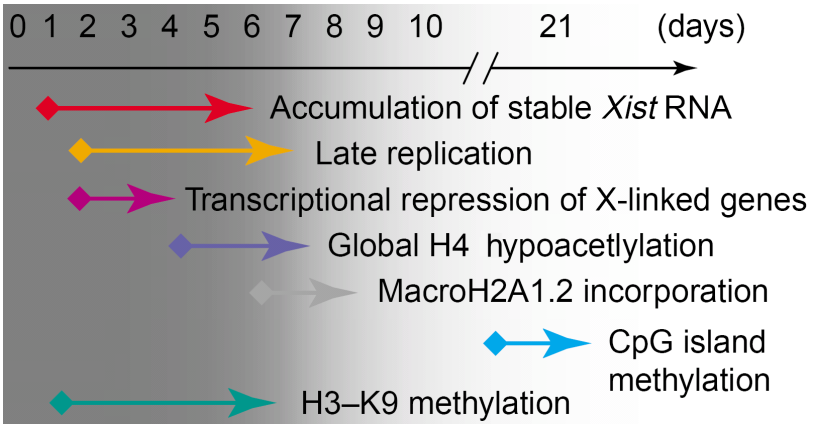

Fig. IX-1 | **X chromosome inactivation occurs in a stepwise fashion.**

From several studies investigating differentiating mouse XX embryonic stem cells, it became clear that X chromosome inactivation proceeds in a stepwise manner over a period of at least 21 days. The shaded area represents an estimate of the developmental window during which cells have the capacity to inactivate chromatin in response to *Xist* RNA expression.

Image adapted from *1338*.

B. X chromosome inactivation pattern

XCI is neither continuous nor static. Instead, it varies along the X chromosome, in the population and with age. It can be non-random and is tissue-specific.

B.1. Escape from X chromosome inactivation

Up to 15% of X-chromosomal genes escape XCI. Their distribution along the X chromosome reflects its evolution.

Although one of the most remarkable features of X_i chromatin is its stability and its clonal inheritance through many rounds of cell division, XCI is not an absolute feature; it has been observed that certain loci escape XCI. In females, genes situated in such domains are expressed from both X chromosomes. Apart from genes in the pseudoautosomal region, a region similar between the X and the Y chromosome, up to 15% of X-chromosomal

genes escape XCI. The proportion of genes escaping XCI in different regions of the X chromosome reflects the evolutionary history of the sex chromosomes[24].

B.2. Reactivation

Reactivation of previously inactivated loci has been reported.

Gradual reactivation of inactivated loci during adulthood, presumably due to wearing out of the inactivation effect, was reported by Cattanach. He noted a change in coat colour in mice carrying an X chromosome with an autosomal insertion containing the genes *albino*, *pink-eye* and *ruby-eye-2*. White patches of coat in four-month-old mice turned brown over time and were black at one year of age[1346]. A recent molecular study of Cattanach's rearrangement found that the autosomal insertion was hyperacetylated on H4 and that *XIST* RNA associated only very weakly with it, both indications for transcriptional activity[33]. Lingenfelter and co-workers showed that the escape from XCI of *mSmcx* occurs by reactivation of a previously entirely inactive state[1347].

B.3. Skewed X chromosome inactivation

Non-random XCI can arise (i) to maintain functional euploidy when an X chromosome expresses a deleterious mutation or (ii) through stochastic processes at the onset of XCI.

Studies on human balanced and unbalanced chromosomal rearrangements involving the X chromosome revealed that somatic XCI is not always a random process as was initially postulated. In addition, many X-linked syndromes are known in which carrier females exhibit skewed XCI[1348,1349]. As in X;A translocations, it is believed that there is a strong selection against cells losing their functional euploidy (see I.A.3.2.2.2). This results in preferential inactivation of the X chromosome expressing the deleterious mutation.

However, skewed XCI is not only associated with disease but is also present in the general population[1096]. It is thought to arise by stochastic processes in the small number of progenitor cells present at the onset of XCI[1350,1351].

B.4. Tissue specificity of the X chromosome inactivation pattern

Since XCI proceeds gradually when cells are no longer pluripotent, tissue specificity of the XCI pattern arises.

The pattern of XCI (e.g. escape from XCI and randomness of XCI) is tissue-specific because random XCI of the primitive ectoderm does not occur simultaneously, but proceeds gradually in the subpopulations of the embryonic ectoderm and mesoderm[1352], when cells are no longer pluripotent. For example, the degree of escape from XCI of *mSmcx* has been shown to be tissue-specific in embryonic and adult mice[1095]. In a study involving 270 phenotypically unremarkable women, Sharp *et al.* showed that XCI patterns varied widely between different tissues in several females[1096]. Gale and colleagues provided another example of tissue-specific XCI patterns in the human population[1097].

B.5. Variation of the X chromosome inactivation pattern within the population

A remarkable 10% of X-linked genes show variable levels and patterns of XCI.

Allelic expression of *mSmcx* is characterised by a high cell-to-cell variability[1347], and the variability of XCI patterns has also been noted in humans. Carrel and Willard, for example, reported on *REP1*, mutations in which are responsible for the X-linked eye disorder choroideremia (OMIM 303100), showing that its inactivation status varies among different females in the population[1353]. Performing a comprehensive X-inactivation profile, the same authors found a remarkable 10% of X-linked genes showing variable levels and patterns of XCI, indicating an unsuspected degree of expression heterogeneity among females[24].

B.6. Variability of the X chromosome inactivation pattern with age

Age dependency of the XCI pattern is likely due to secondary selection processes.

Apart from tissue specificity and population variation of its XCI pattern, the study of *mSmcx* revealed that the degree of escape from XCI differed throughout embryonic development[1347]. The study of Sharp and coworkers, already mentioned under IX.B.4, showed that skewing ratios ≥ 90:10 occur in 7% of women under 25 and in 16% of women over 60, indicating an age dependency of the phenomenon. The authors also showed that the variation of XCI patterns with respect to tissue specificity increases with age[1096]. These ob-

servations are in line with the belief that the major factors in the aetiology of skewed XCI are secondary selection processes; a particular X chromosome is non-randomly inactivated if a selective advantage is thus conferred (see IX.B.3).