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An overview of X inactivation based on species differences

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ABSTRACT

X inactivation, a developmental process that takes place in early stages of mammalian embryogenesis, balances the sex difference in dosage of X-linked genes. Although all mammals use this form of dosage compensation, the details differ from one species to another because of variations in the staging of embryogenesis and evolutionary tinkering with the DNA blueprint for development. Such differences provide a broader view of the process than that afforded by a single species. My overview of X inactivation is based on these species variations.

Contents

1. Introduction: a parable

Thinking about my contribution to this special issue, I remembered the ancient Chinese parable of the elephant and the blind men [1]. As you may recall, John Godfrey Saxe's poem about it [2], begins:

"It was six men of Indostan

To learning much inclined,

Who went to see the elephant

(Though all of them were blind),

That each by observation

Might satisfy his mind."

The first, feeling his broad side, thought the elephant was a wall; the second feeling the tusk, thought he was a spear; the third with the squirming trunk within his hands, thought he was a snake; the fourth, feeling his knee thought he was a tree; the fifth who touched his ear, thought he was a fan, and the sixth seizing his swinging tail, thought he was a rope. Saxe concludes his poem:

"And so these men of Indostan

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Disputed loud and long . . .. . .

Though each was partly in the right.

And all were in the wrong."

When I read the current literature about X inactivation, I often think of the question posed by this parable: "How can anyone describe the whole until he has learned its parts?"

Because the mouse has proved to be such an excellent model for studies of the early embryonic events responsible for X inactivation, most of our conclusions are based on findings from mice. Yet, studies of other mammals are broadening our understanding of this developmental process, so that our view of this elephant is becoming more accurate. We can no longer ignore the fact that no single mammal can inform us about the molecular mechanisms of X inactivation. In addition, studies of several mammalian species reveal biological truths, not otherwise apparent from the study of a single species. I want to tell you about lessons learned from even the little we know about species differences in X inactivation. For more details about these variations on a common theme I refer readers to the abundant literature on the subject [3–7], including papers in this special issue.

2. Overview based on non-mammalian species

First, a brief review of what we have learned about the mammalian version of sex dosage compensation from studies of non-mammals. We know that most organisms with an XO/XX or XY/XX system of sex determination not only need to compensate for the sex difference in dosage of X-linked genes (one allele *versus* two alleles), but also expression of these genes must be balanced with that of autosomes, where in most cases two alleles are expressed [8]. Compensation takes place predominantly in only one of the sexes, either male as in flies or females as in worms. This balancing act is usually accomplished by amplifying the expression of the X-linked genes [9]; such a compensatory measure is enough when upregulation of X-linked genes occurs on the single X chromosome of males (see Fig. 1: fly). But when it increases the expression of all X chromosomes, there needs to be a second step: the excessive expression in females, who have two X chromosomes, must be reduced (Fig. 1: human). We also know that although dosage compensation may be required during some stages of gamete differentiation, it is maintained only in somatic cells [10], and its silence in somatic cells is highly stable and usually irreversible [11].

Other similarities in the compensatory process include an intimate association with sex determination. One cannot be a male fly or a female worm without undergoing dosage compensation. In each case, the process takes place very early in embryonic development, coordinated with the time of sex differentiation. In addition, not all X-linked genes need to be dosage compensated at the level of a chromosomal mechanism; rather it is required only in tissues where the gene is expressed [8]. In some cases balanced expression between X-linked and autosomal genes could be mediated in a piecemeal fashion by repressing interacting autosomal genes—or by altering rates of translation, or protein degradation. Instead, X dosage compensation is most often carried out by modifying the transcription of a bloc of X-linked genes all in one sweep. In addition, non-coding RNAs play an important role in effecting changes in transcription, at least in flies [12] and mammals [13–15]. The transcriptional changes are mediated by remodeling the chromatin of the relevant X chromosome [16].

But then, the variations begin. Each species seems to have its own unique way of accomplishing X dosage compensation, (Fig. 2). Flies boost the expression of genes on the single X in males [17], worms reduce the expression of both X chromosomes in females [18], and mammals inactivate all but the one X chromosome that remains active in either sex [19]. And birds and monotremes seem to have evolved other mechanisms for sex dosage compensation [20] (see Withorth and Pask's review in this issue page). One cannot help being impressed by what seems to be an infinite number of possible variations. In addition we have learned that the role players are not the same [15], even in related species [21,22], nor is the role that they play [23]. Clearly, as more species are studied, we see that Jacob was right, when he suggested that evolution acts like a tinkerer, using materials at hand, not at all concerned about maintaining the identical model in all species [24,25].

3. X inactivation: the mammalian means of X inactivation

One expects that X inactivation, the mammalian method of X dosage compensation, might be more homogeneous among the various mammals. All eutherian and marsupial mammals do it. Here, the compensation is achieved by maintaining a single active X in somatic cells of both males and females, as all other X's in the cell are inactivated early in embryonic development. In this case, the major theme is chromosome inactivation by means of an important non-coding X-linked gene (encoding Xist RNA in all the eutherian mammals examined). This intranuclear RNA binds to its chromosome of origin, and modifies it to inhibit transcription of any additional sets of X linked genes in both sexes. The inactivating event commences during a limited window in early development and is maintained by a series of reinforcing events that follow [26].

However, here too, there are numerous variations on the theme. The major differences that we know about, have to do with the time of onset of the silencing event [4], whether the paternal X is imprinted or not [27], the nature of the long non coding RNAs that have a role in the process [6,26], and the stability of the inactivation, once it occurs [28].

Although such differences in the details of X inactivation were surprising to some, they might have been anticipated because of evolutionary tinkering with the DNA blueprint for development, with the staging of embryogenesis, with the epigenetic machinery among species, as well as the eclectic effects of selective pressures that influence the underlying mechanisms.

3.1. Time of onset depends on the staging of embryogenesis

We should expect that the time when X inactivation is initiated would vary among species because they differ in the staging of embryogenesis. X inactivation is not seen in the mouse embryonic stem (ES) cells [29,30], until pluripotency factors (Oct3/4, NANOG and SOX2 that inhibit the chromatin remodeling RNAs [29,30] are less abundant. Therefore, it seems that silencing cannot occur until the time a tissue begins to differentiate. It is first seen, in the earliest differentiating tissues [31], which are the placental tissues. It seems that the time of tissue differentiation, which varies widely among mammals [Table 6.1 in 26], determines the onset of X inactivation.

3.2. Parental imprinting results from the paternal imprint acquired by many mammals during spermatogenesis

From the study of several mammalian species, we know that when the interval between fertilization and the earliest events in the dosage compensation process is short, then there is likely to be imprinted paternal X inactivation, at least in the earliest differentiating tissues [26,32]. (Also see Withorth and Pask' review in this issue). It is likely that the species differences in the time interval between fertilization and tissue differentiation determine whether or not imprinting occurs; the shorter the interval, the less likely that the sperm imprint on *Xist* will be erased.