

Dosage compensation

Reading: these notes (not covered well in Gilbert); 8th ed: Chp 5: 119-124; 550 ; 9th ed: 50-51, 63, 628

Learning Goals Be able to:

Explain and compare the basic mechanisms of dosage compensation in *Drosophila* and *C. elegans*.

Compare the mechanism of dosage compensation in mammals to invertebrates.

Explain the role of Xist in dosage compensation: how it is involved in initiation of compensation, maintenance, choice of which X is inactivated and counting the number of X chromosomes.

Design experiments that demonstrate how members of the dosage compensation pathway might interact.

Predict outcomes on sex and viability of organisms with defects in dosage compensation genes.

Males and females have different numbers of sex determining chromosomes in most species. In the organisms we have discussed, males routinely have one X chromosome, while females have two. In order for the expression of X-linked genes to be equalized between the sexes, there must be a mechanism for regulating the level of expression of these genes. This is called dosage compensation, and it can be achieved in two different ways: either the activity of X-linked genes can be doubled in males to match that of females (*Drosophila*), or the activity of X-linked genes can be halved in females to match that of males (*C. elegans*, mammals). In mammals, dosage compensation is accomplished by inactivating most of one X chromosome in each cell of females.

Mechanisms used in *Drosophila* and *C. elegans*.

Drosophila and *C. elegans* use a similar method for dosage compensation, but they do it in opposite ways. *Drosophila* increase the rate of transcription in males; *C. elegans* decrease the rate of transcription in hermaphrodites.

Drosophila: The dosage compensation genes controlling X chromosome expression were identified by sex-specific lethal mutations that lower X expression and kill males but do not affect females (hence their names, male specific lethal, *msl*). These genes (*msl* and *mle*) normally function to upregulate X expression in XY animals. **In XX animals these genes are turned off by Sxl activity.** The *msl* and *mle* gene products bind to the male X chromosome at multiple sites and are presumed to act by altering the chromatin conformation.

C. elegans: In *C. elegans*, the activity of X-linked genes on both X chromosomes is decreased by half in hermaphrodites to match that of males. This happens through the binding of a complex of proteins to both X chromosomes in hermaphrodites. Overall gene expression from the X chromosome is thus decreased, probably through chromosome condensation, or general transcription inhibition. As in *Drosophila*, the genes controlling dosage compensation were identified in screens for mutations that cause lethality in one sex but not the other. Mutations that lower X expression generally kill males but not hermaphrodites; mutations that raise X expression generally kill females, but not males. X-linked transcription factors, transcribed very soon after fertilization, are present in double the amount in an XX embryo compared to an XO embryo. High levels of these transcription factors actually inhibit a gene called *xol-1*, so that it is made only in XO animals (where the levels of those transcription factors is lower). The *sdc* genes, only active in hermaphrodites (negatively regulated by *xol-1*), play two roles: they activate the dosage compensation machinery in XX animals (*dcd* genes) so that transcription from the X is turned down, and they negatively regulate *her-1* transcription so that HER-1 protein is synthesized only in males (remember, it's linked back to the sex determination pathway!).

Summary of *Drosophila* and *C. elegans* dosage compensation: In *Drosophila*, gene products only made in males form a multiprotein complex that, in association with RNAs, binds to many sites along the male X chromosome. They upregulate the transcription of genes on the X. In *C. elegans*, a multiprotein complex is made specifically in hermaphrodites, and binds to both X chromosomes to downregulate transcription from the X.

X chromosome inactivation in mammals

The inactive X chromosome in mammals is visible as a dense heterochromatic Barr body. Inactivation does not occur immediately upon fertilization, but rather happens after several rounds of division. One X chromosome is inactivated in each cell of the embryo; the selection of which X chromosome is inactivated is random (sometimes the maternal X, sometimes the paternal X). Since the inactivation happens at about the 32 cell stage, females are mosaic for expression of X-linked genes. A highly used example of the results of X inactivation is the calico cat (Fig 5.22). Orange fur color and black fur color are caused by two different alleles of the same gene on the X chromosome. Calico cats are heterozygous for the alleles. A patch of orange fur is derived from a cell in which the X chromosome carrying the black allele was inactivated, while black fur is derived from a cell in which the opposite X chromosome has been inactivated. A male can have only one of the two alleles, and is thus black or orange but not both (white fur color, by the way, is conferred by an autosomal gene, so both males and females can have white). There is one exception to the random inactivation of the X chromosome: in the extraembryonic trophoblast, the paternal X chromosome is preferentially inactivated.

Interestingly, if there are more than two X chromosomes present, all but one are inactivated. Thus, an XXX individual would have 2 Barr bodies. This indicates that the cell can "count" the number of X chromosomes that are present.

How does the cell count X chromosomes, choose an X chromosome for inactivation, and then initiate and maintain this inactivation?

In mouse, a region of the X chromosome was found that, when missing, prevented inactivation of that chromosome. This region was termed the X inactivation center (XIC). Within the XIC, a gene was then found, called Xist (X inactive specific transcript), which seems to be responsible for the ability of the X chromosome to become inactivated. Evidence for Xist being the critical factor is:

1. Xist is expressed at high levels only from the inactive X. In other words, Xist is opposite in terms of its expression to the rest of the X chromosome. It is active on the inactive X and inactive on the active X!
2. The Xist RNA localizes to the inactive X chromosome
3. Xist expression precedes overt X inactivation
4. In differentiated ES cells with a targeted mutation in the Xist gene, the targeted chromosome does not undergo inactivation
5. If Xist is placed onto an autosome (and transcribed), that autosome gets inactivated.

Interestingly, Xist RNA does not appear to encode a protein, but rather exerts its effects as an RNA. This is similar to *Drosophila* in that the untranslated roX RNAs also associate with the compensated X chromosome.

A model for X chromosome inactivation in mammals:

A) Initiation. Xist is transcribed from both X chromosomes at an early stage, but this Xist RNA is unstable, and degrades rapidly. The X that will become inactivated subsequently begins to use an alternative promoter for the Xist transcript. The new Xist RNA is more stable and accumulates and spreads along the X chromosome in an RNA-protein complex. This X chromosome is thus inactivated.

B) Maintenance (how is transcription repressed only on one X?)

Histones regulate chromatin structures and gene expression. The inactive X is associated with decreased acetylation of histone H4 and the presence of a novel histone, histone macroH2A.

Sequences that have decreased acetylation have increased methylation, and the methylation prevents the transcription of these sequences. Methylases and methyl transferases add a methyl group to cytosines. The methylation state of DNA is propagated during replication-- newly replicated strands are re-methylated by methyl transferases.

If a promoter region is methylated, transcription of that transcript is decreased. Thus, a combination of decreased acetylation and increased methylation allows an X chromosome to be inactivated. The Xist promoter is methylated on the active X (Xist is off) and not methylated on the inactive X (Xist is on).

C) Choice: How does a cell choose X chromosome for inactivation?

Xist itself may be involved in the choice. Evidence: deletions within the Xist gene can lead to non-random inactivation. Regulation of Xist transcription (as described above) may be involved in choice of X chromosomes for inactivation. There are two speculations on the action of blocking:

- 1) There may be a positively acting "competence factor" that is needed to turn on transcription of the stable Xist RNA and/or initiate inactivation in other ways.
- 2) There may be a negatively acting "blocking factor" that can prevent X inactivation on one X per cell. Support for a negatively acting factor has been found in an antisense transcript that is found on the opposite strand from Xist and is exactly complementary to Xist. This transcript is called Tsix (Xist spelled backwards). Tsix appears to negatively regulate Xist on the active X chromosome (ie, it is expressed at high levels from the active X chromosome, while Xist is expressed at high levels from the inactive X). It may act by blocking Xist RNA accumulation. If Tsix is mutated, the mutant X is preferentially inactivated.

D) Counting. How does a cell know when more than one chromosome has to be inactivated? The above postulated "blocking factor" could conceivably account for counting. If the blocking factor is present in a limited amount (located on an autosome, transcribed in the same amount no matter how many X chromosomes are present), then each cell might only have enough blocking factor to "protect" a single X chromosome from inactivation. In cases where more than two X chromosomes are present, more X chromosomes would be inactivated by Xist. Thus the cell does not count X chromosomes per se, but rather only protects a single X.

Implications of dosage compensation for humans

As we discussed in the last class, sometimes an individual's genetic sex does not match their phenotypic sex. Dosage compensation plays into secondary sexual phenotype because of one important feature of X chromosome inactivation: there is a region of the X chromosome that is NOT inactivated. This region contains several important genes whose protein products are involved in female fertility. For a human female to have normal fertility, they must have two copies of these genes, so that they transcribe enough of the products required. This has repercussions for individuals with missing or extra X chromosomes. Two examples:

XO Female. Gonad is an ovary (no SRY gene); ductal system is female (estrogen is produced, but neither testosterone nor AMH is produced). Fertility is impacted. XO individuals have a range of other phenotypes including occasional mental disabilities, but the most notable phenotype is fertility. Because these individuals lack the second X chromosome, they do not have two copies of those never inactivated genes on the X chromosome. This can affect fertility in two ways: the level of estrogen produced is apparently decreased (which can affect the ductal structures), and the germ cells themselves do not completely mature.

XXY Male. Often completely normal externally, but with fertility problems. These individuals usually have decreased levels of testosterone (presumably due to the higher levels of estrogen), although this is easily treatable. Sometimes (rare), individuals develop female secondary sex characteristics due to extra levels of estrogen. The fertility issues are the main problem: the extra protein products from the non-inactivated X chromosome genes inhibit differentiation of sperm. XXY males produce sperm, but their sperm are abnormal.

Interestingly, extra X chromosomes in females don't seem to affect fertility—perhaps the presence of even more proteins required for fertility doesn't negatively impact the differentiation of the oocytes and their support cells.

Review Questions

1. Describe the general mechanisms of dosage compensation in *Drosophila* and *C. elegans* and how they are different.
2. Why are human females considered "mosaic"? How does this relate to dosage compensation?
3. What lines of evidence demonstrated that Xist was responsible for X inactivation?
4. How does dosage compensation work in mammals (proposed and known mechanisms)? Are there any similarities to invertebrates?
5. How is methylation related to X inactivation?
6. What are the hypotheses for how a cell "chooses" which X chromosome to inactivate?