

Class 26 Dosage Compensation

Learning Goals

- 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 and other known and postulated molecules involved in dosage compensation
- Interpret experiments about how members of the dosage compensation pathway might interact.
- Predict outcomes on sex and viability of organisms with defects in dosage compensation genes.

Drosophila sex determination: XX Female; XY Male

Based on the information in the table below, what determines whether a fly will be a normal male?

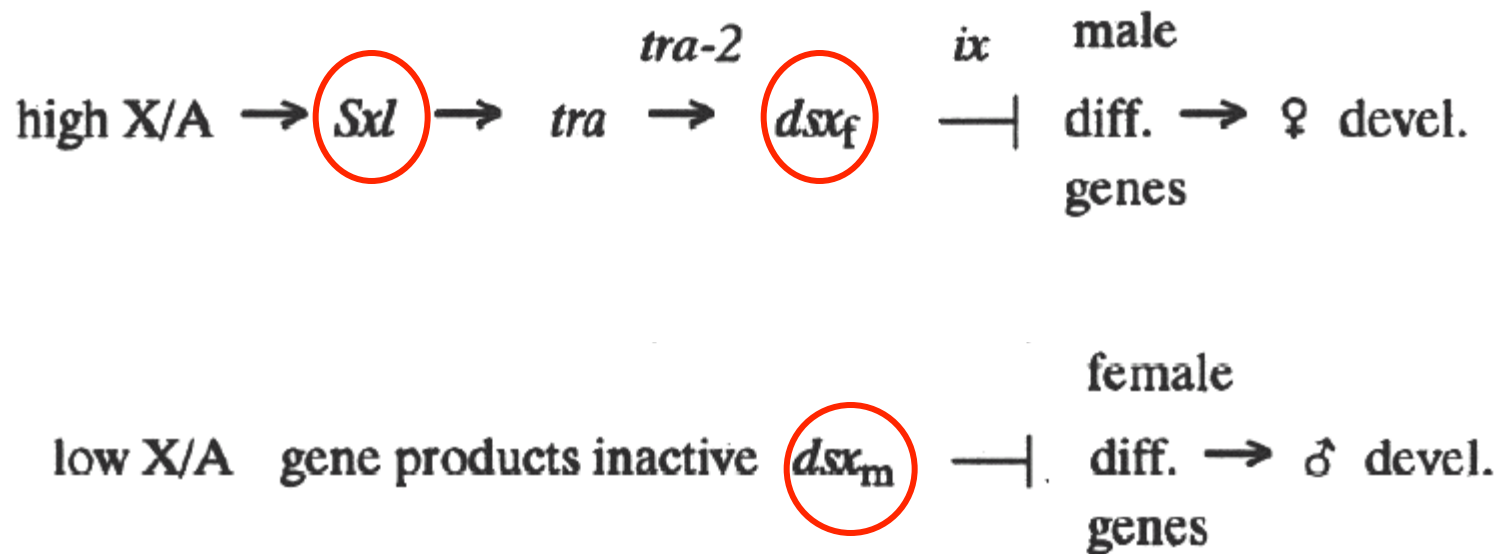
- a. Number of X chromosomes
- b. One Y chromosome
- c. An X:A ratio of 1
- d. An X:A ratio of less than 1
- e. An X:A ratio of .5

X chromosomes	Y chromosome	Autosome Sets	Sex
4	0	4	female
2	0	2	female
2	0	3	intersex
2	1	4	male
1	1	2	male
1	0	2	male

X:A ratio controls both *Drosophila* and *C. elegans* sex determination

In *Drosophila*, the result of a 1:1 “X:A” ratio is the transcription of certain genes (Sex Lethal).

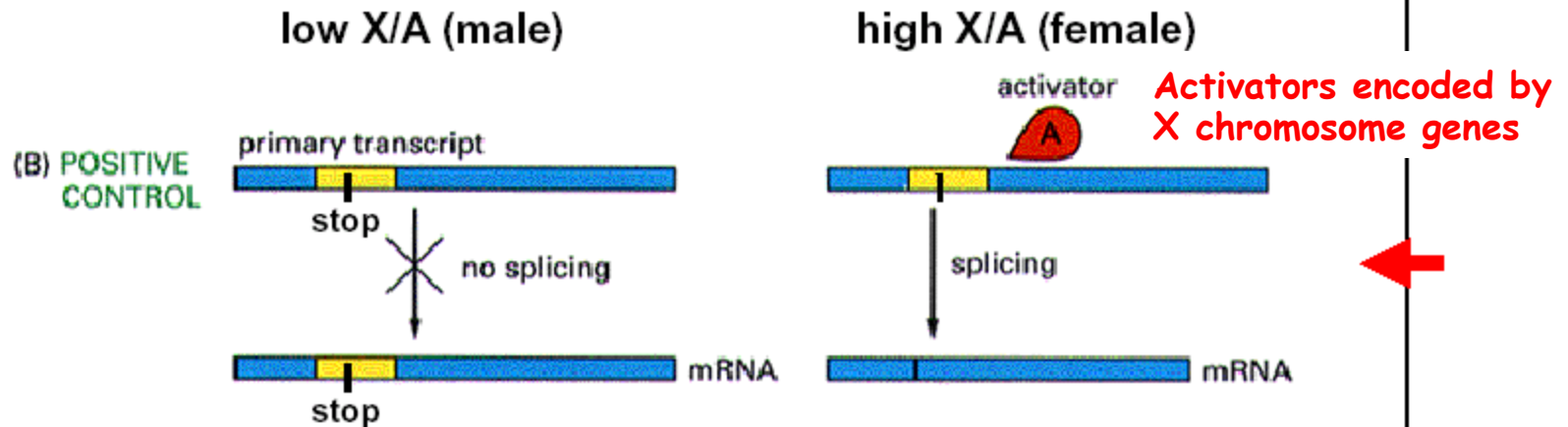
The genetic pathway controlling sex determination in *Drosophila*



In *Drosophila*, the activation of products requires splicing

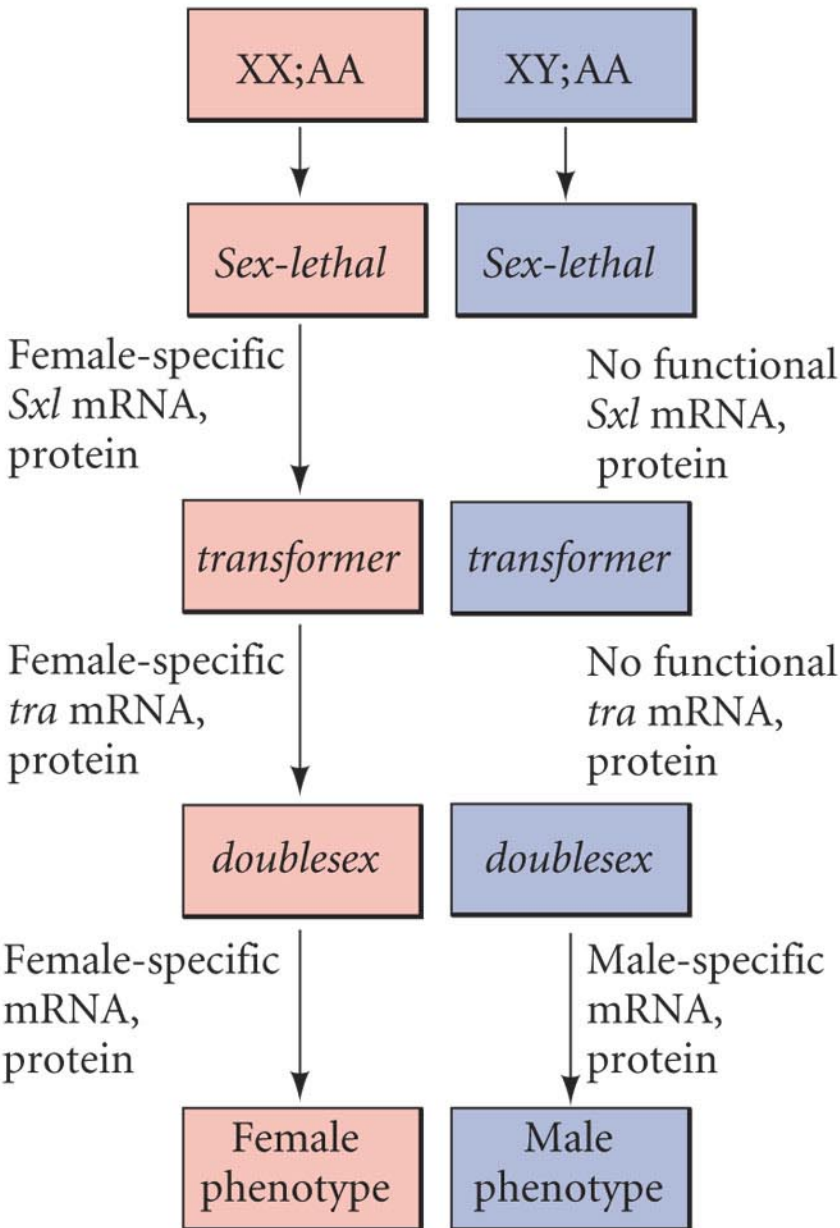
The splicing happens in females

Mechanism of switch (for Sxl gene)



From The Art of MBoC² © 1995 Garland Publishing, Inc.

Sxl protein then goes on to allow transformer transcript to be spliced (not spliced in males)



A *Drosophila* with a loss of function mutation in *transformer* will have what kind of gonad?

- Male if it is XY, female if it is XX
- Female if is XY, male if it is XX
- Male no matter what
- Female no matter what

In *C. elegans* and *Drosophila*,
sex determination and dosage
compensation are
interconnected...

Dosage compensation

Female vs. male

Most organisms have 2 copies of each autosome

2 copies of X

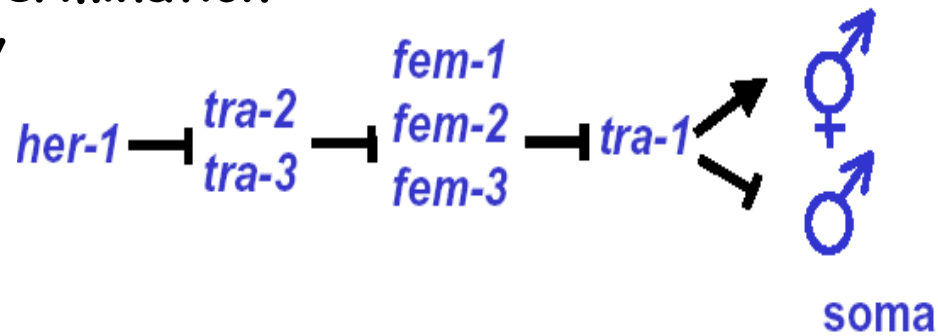
1 copy of X

If the amount of transcript produced from each chromosome is important, then there must be some compensation mechanism that allows both XX and X to be viable genotypes

Dosage compensation fits into the sex determination pathways for invertebrates

C. elegans

Sex determination pathway

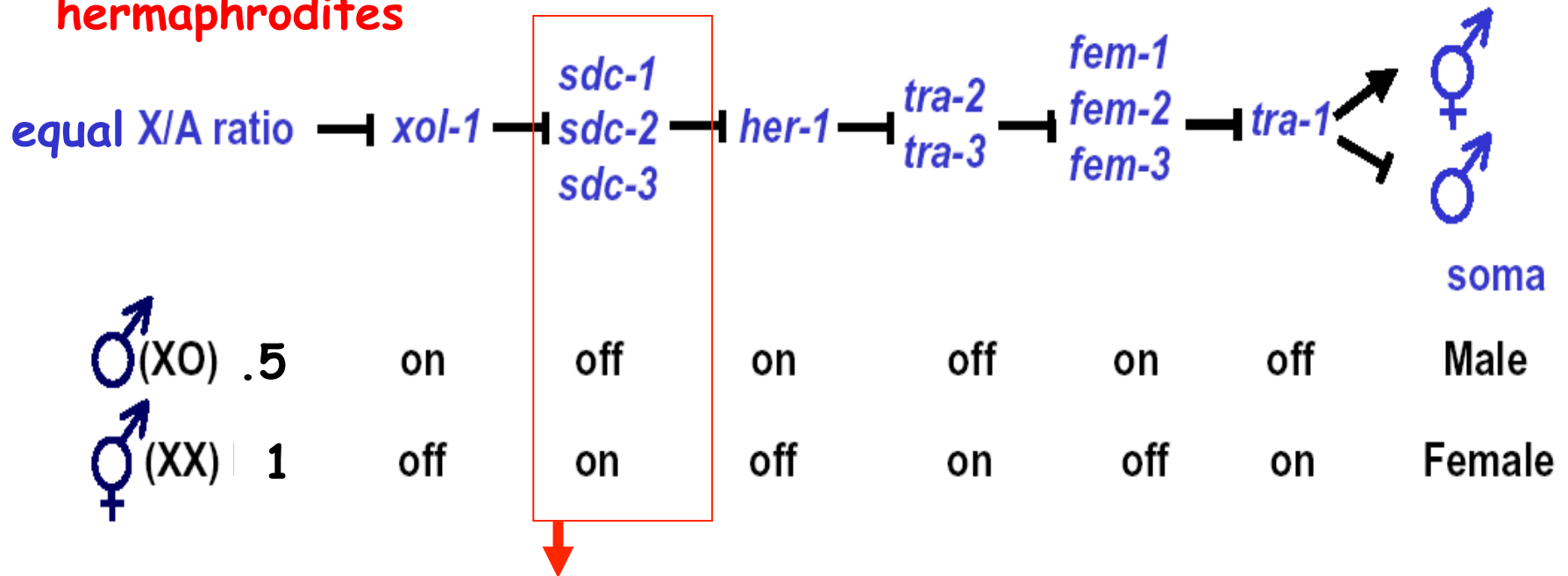


on	off	on	off	Male
off	on	off	on	Female

C. elegans

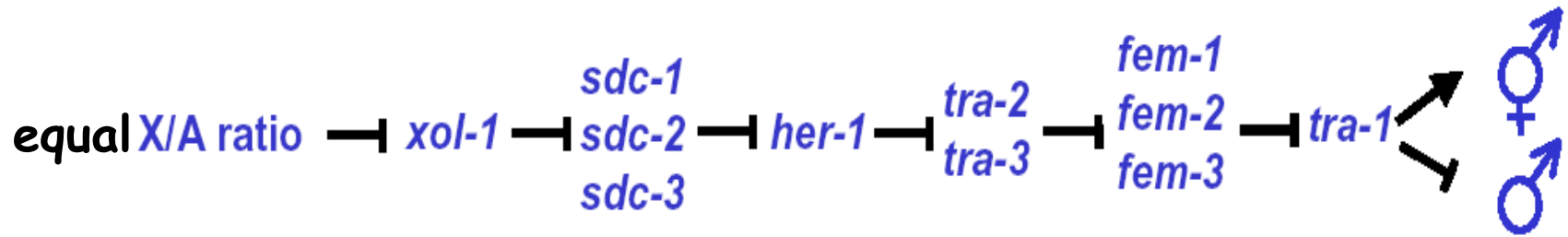
X:A ratio determines activation of “dosage compensation genes” that direct the level of expression from X

Transcription from each X chromosome is decreased by 1/2 in hermaphrodites



SDC proteins bind to both X chromosomes and downregulate expression of X transcripts

SDC proteins also bind to prevent expression of her-1 (her-1 is not on X chromosome)



Which of the following is true of a worm that is homozygous mutant for *xol-1* (lf)--one of the dosage compensation genes?

- a. XX embryos develop into male worms
- b. XO embryos develop into hermaphrodite worms
- c. XO embryos die due to lack of X chromosome gene products
- d. XX embryos die due to overproduction of X chromosome gene products

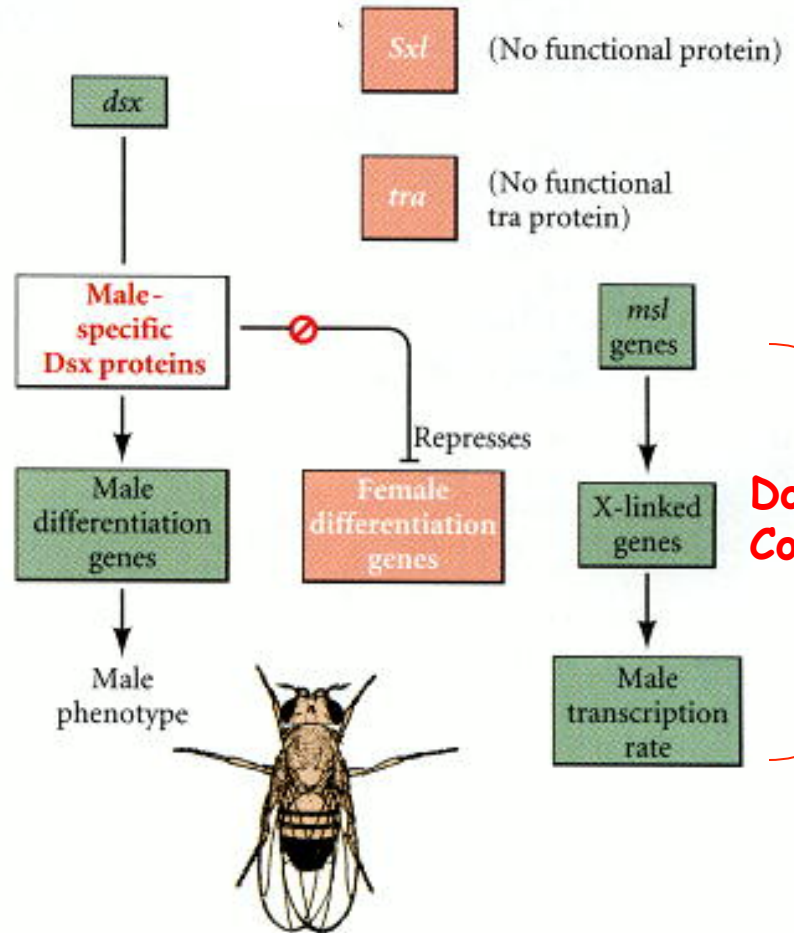
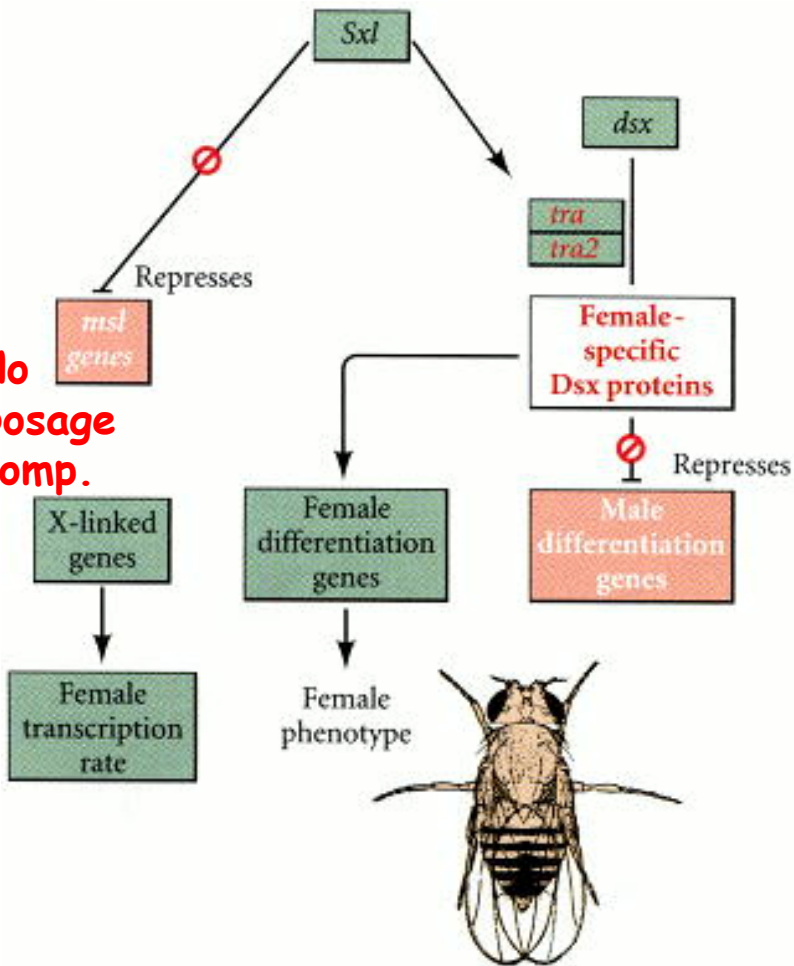
If they could survive, the *xol-1* mutant XO animals would have a:

- a. Male gonad
- b. Hermaphrodite gonad
- c. Something in between
- d. No gonad

Drosophila is the opposite of *C. elegans*:
transcription from the X is increased 2x in males

female

male

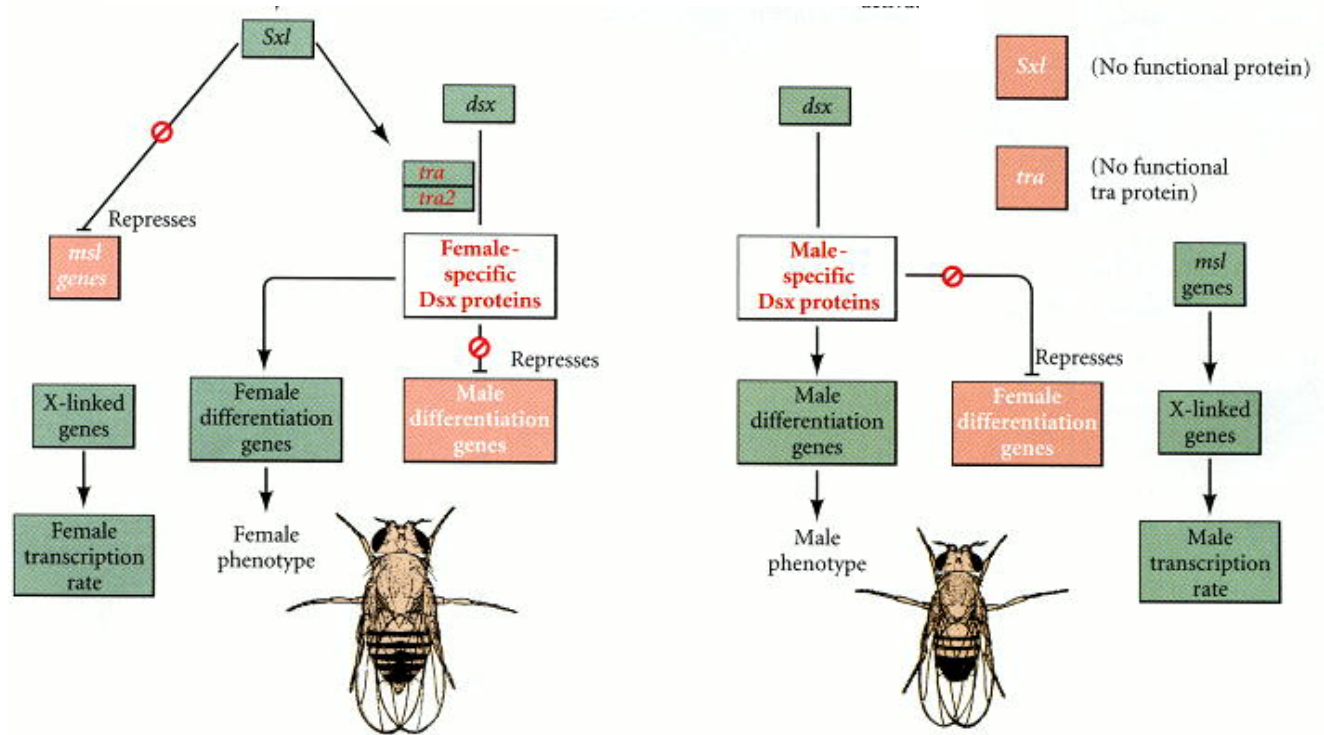


No
Dosage
Comp.

Dosage
Comp.

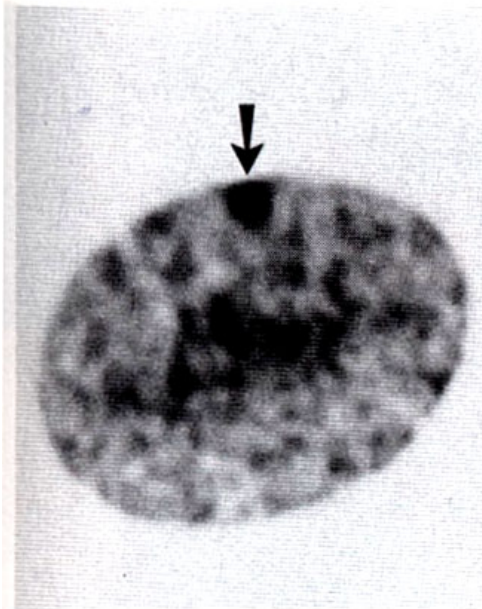
Now think about *Drosophila*. In which chromosomal combination would a loss of function mutation in the *Sxl* (sex lethal) gene be lethal?

- a. XX
- b. XY
- c. Neither
- d. Both

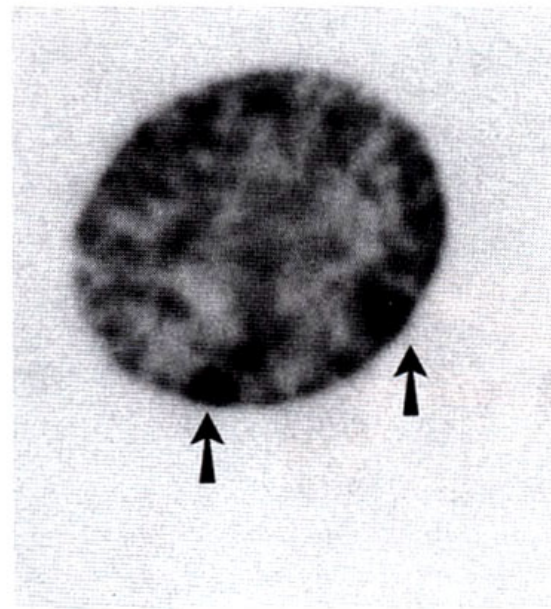


In mammals: dosage compensation is accomplished by inactivating most, but not all, of one X chromosome (rather than by changing transcription levels)

The “Barr body” represents the inactive X chromosome

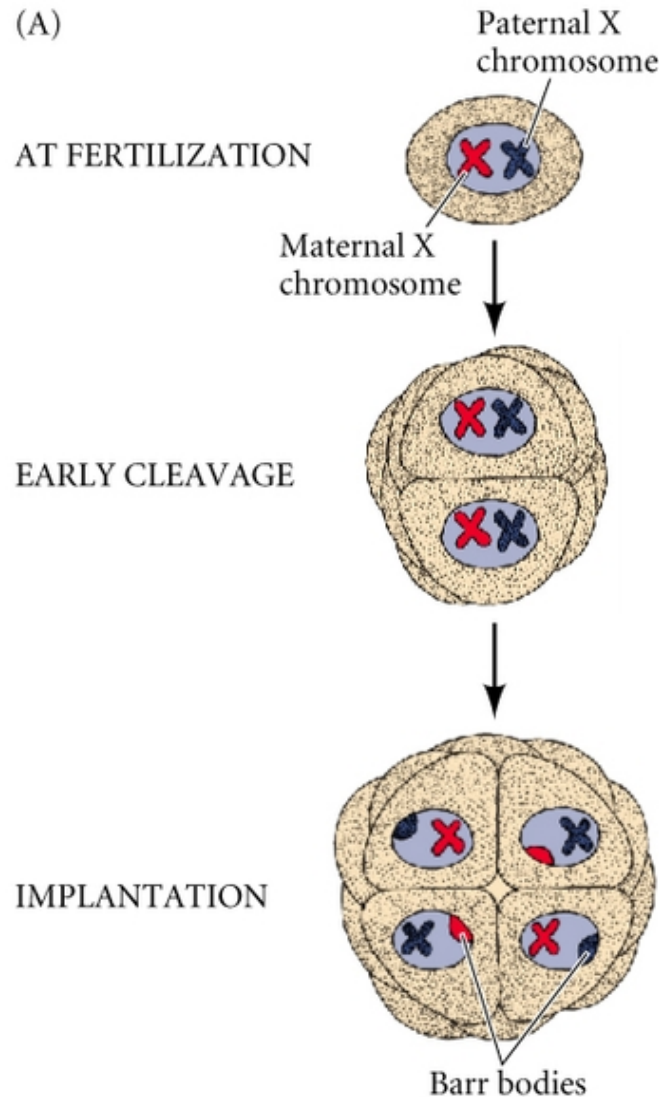


Cell from XX female



Cell from XXX female

Inactivation is random and takes place early in development, between 4-cell and 32-cell stage



X inactivation in action: Calico cats



a.

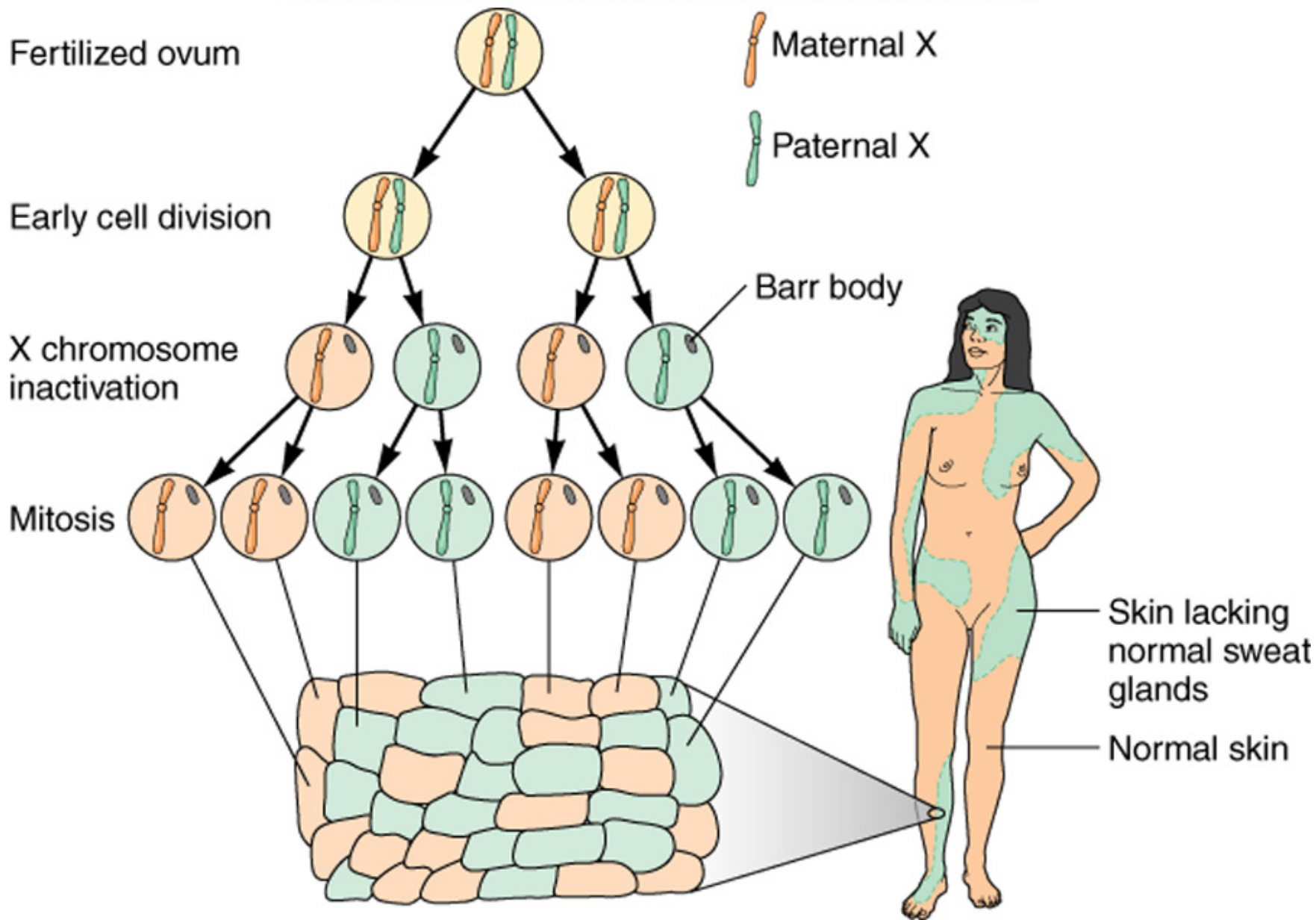


All female mammals are “mosaics” for genes on the X chromosome, illustrated here by coat color.

O (orange, dominant) and o (black, recessive) are two alleles on the X chromosome for coat color. White coat color is due to an autosomal gene.

What about in humans?

Anhidrotic Ectodermal Dysplasia



Important feature of X-chromosome inactivation:

- The entire X chromosome is not inactivated—there is a small section where transcription still takes place
- This has consequences for individuals with abnormal sex chromosome combinations:
 - **XXY: usually infertile**
 - **XO: usually infertile**

But XXX: normal

Worksheet question from last time:

You are doing chromosomal analysis of an individual with two X chromosomes who appears externally male. You find that one of the X chromosomes contains a translocation from the Y chromosome, containing the SRY gene.

If you were able to look at multiple tissue samples from this individual's gonad, you would expect to see:

- a. The gonadal cells have all differentiated into testis tissue
- b. The gonadal cells have all differentiated into ovarian tissue
- c. The gonadal cells have not differentiated into testis or ovarian tissue
- d. Some gonadal cells have differentiated into testis, some into ovarian tissue

Say there are approximately equal amounts of testicular and ovarian tissue in the gonad from the previous example. What characteristics would this person have?

- a. ductal system male, external male secondary sex characteristics
- b. ductal system male, external female secondary sex characteristics
- c. ductal system female, external female secondary sex characteristics
- d. both female and male ductal systems; both female and male external secondary sex characteristics
- e. no ductal system; male secondary sex characteristics

How does X inactivation happen in mammals?

A complex on the X chromosome, the **XIC** is responsible for X inactivation

The main gene of interest is: **Xist**

- does not get translated into a protein
- instead, makes a long RNA that “coats” the X chromosome
- once Xist mRNA is transcribed, additional methylation events take place on promoters of genes along the X chromosome; these methylations are preserved so that the same chromosome remains inactivated even after many rounds of cell division

How does Xist work?

Consider the following experiment. A transgenic XY mouse is generated which contains an additional copy of Xist integrated onto chromosome 3. When individual cells are studied, there is evidence of a "barr body" in each cell, but sometimes that inactivated chromosome is an X chromosome, and sometimes it is chromosome 3.

This suggests that:

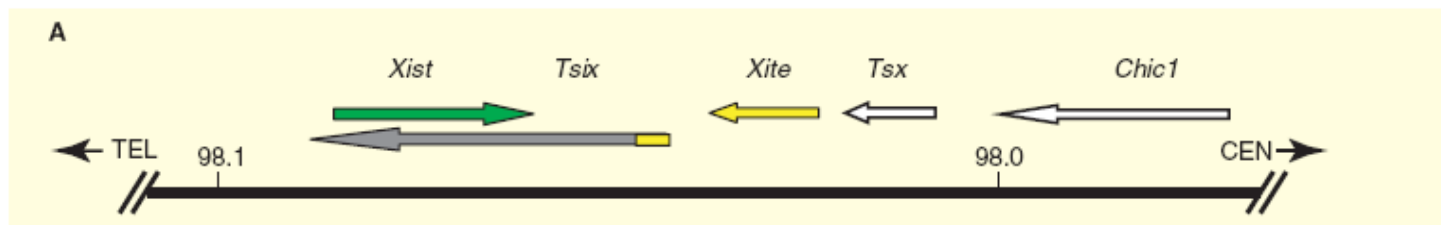
- a. Xist mRNA inactivates the chromosome it is transcribed from
- b. Xist mRNA inactivates only the X chromosome
- c. Xist mRNA randomly inactivates chromosomes.

The state of Xist is **opposite** to the rest of the X chromosome

When Xist promoter is **ACTIVE** (unmethylated),
Xist is made, and that X chromosome is inactivated

When Xist promoter is **INACTIVE** (methylated),
Xist is not made, and that X chromosome is active

XIC region of the X chromosome

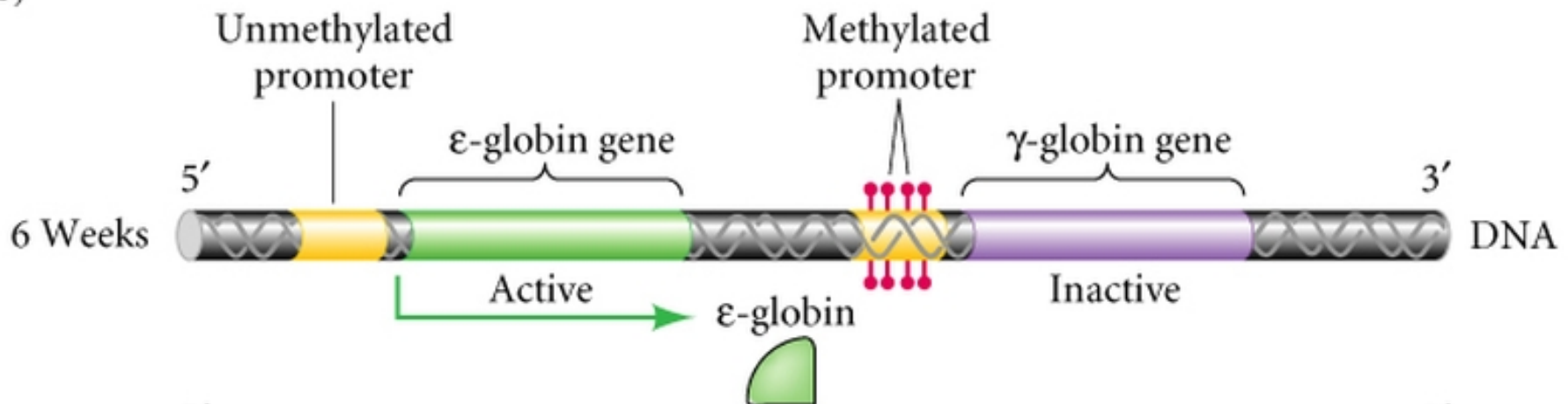


Maintenance: once Xist is activated on one of the X chromosomes:

- methylation events on THAT X chromosome take care of further inactivating the X chromosome genes
- these methylation states are preserved as cells divide

Differential methylation of promoters controls the transcriptional state of genes

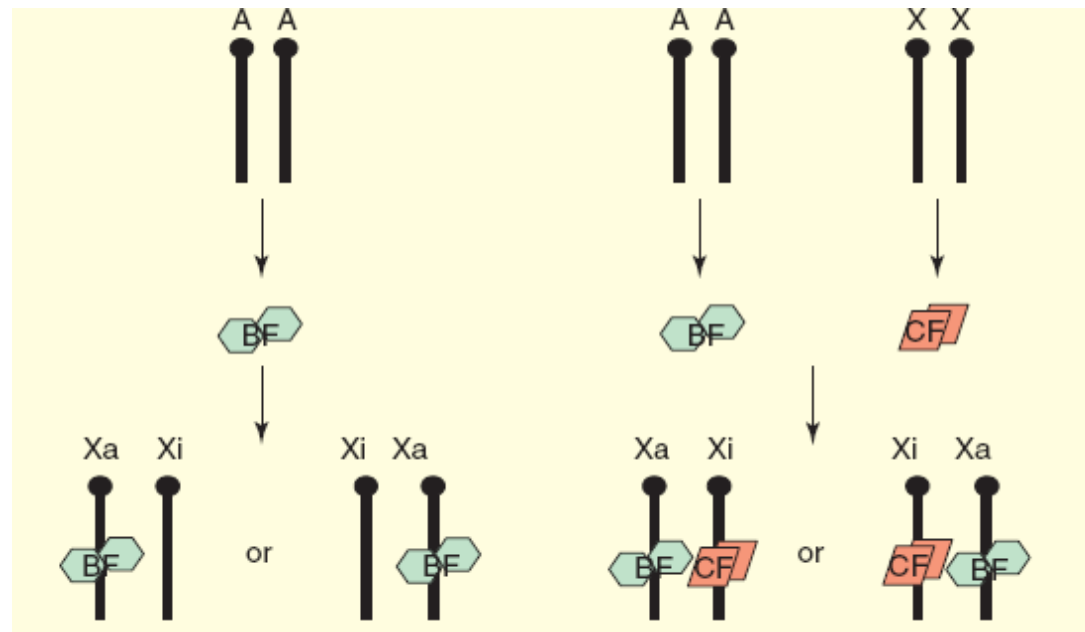
(B)



Regulation of Xist: how is the inactive X chosen? We don't have the answers yet, but rather a set of hypotheses

Positive regulation: a competence factor that helps turn on transcription of stable Xist RNA to initiate inactivation; this factor would act on the X chromosome that gets inactivated

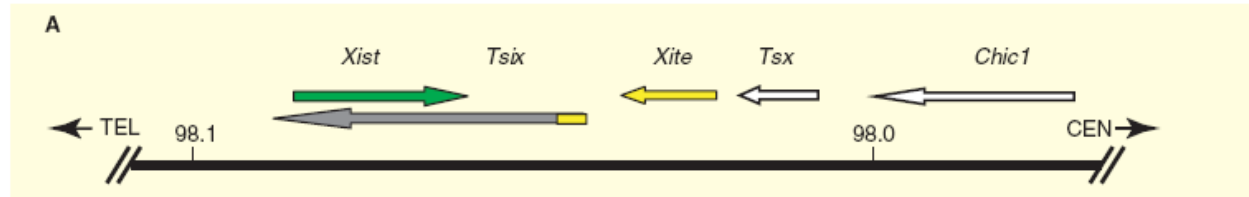
Negative regulation: blocking factors thought to prevent the inactivation of one X chromosome; these factors would act on the X chromosome that doesn't get inactivated



Negative regulators

1. **Tsix** : an antisense transcript exactly complementary to Xist, found on the opposite strand of DNA
 - Tsix is expressed at high levels from the active X chromosome and at low levels from the inactive (the opposite of Xist).
 - Tsix expression precedes inactivation

XIC region of the X chromosome



Alexander and Panning, 2006

If **Tsix** protects an X chromosome, what would you expect to see in mice that are heterozygous for a mutation deleting the promoter of **Tsix**?

- a. An x chromosome would be randomly inactivated
- b. The x chromosome with the **Tsix** mutation would always be inactivated
- c. The x chromosome without the **Tsix** mutation would always be inactivated
- d. No x chromosomes would be inactivated

2. Blocking Factor:

- transcribed from an autosome: same level of blocking factor will be present in genotype XX or XY.
- this amount of blocking factor could be enough to PROTECT just one X chromosome (Xist cannot be active).
- on the other X chromosomes, Xist will be active.

Thus “counting” of chromosomes is likely accomplished by protecting a single X.

