CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2017-2018 :

"Le chromosome X -
paradigme de la génétique et l'épigénetique"

5 février, 2018

Cours II

Régulation génétique et épigénétique du chromosome X inactif

Genetic and Epigenetic Regulation of the Inactive X chromosome

COLLÈGE DE FRANCE 1530

SUMMARY OF LAST WEEK

Advantages and costs of Sexual Reproduction versus Asexual Reproduction

Sex Determination: *Diverse mechanisms are used to determine sex*

- **Sex determination is a rapidly evolving trait in many lineages**
- **Non-genetic:** (ie no DNA sequence difference between the sexes) environmental, temperature-dependent, epigenetic…
- **Genetic:** Sex determining locus or loci –leads to evolution of sex chromosomes
- **Mixed (genetic and non-genetic):** Sex chromosomes and environmental sex determination eg many Fish, plants…

Sex Determination in mammals:

Testis Determining Factor (TDF) = Sry transcription factor that determines male sex
Evolved from SOX3 (X-linked in eutherians; autosomal in other organisms)
Female sex determination – eg DAX1 encoding a nuclear hormone receptor.
Delicate balance between male + female signaling pathways for sex determination in embryogenesis and adulthood

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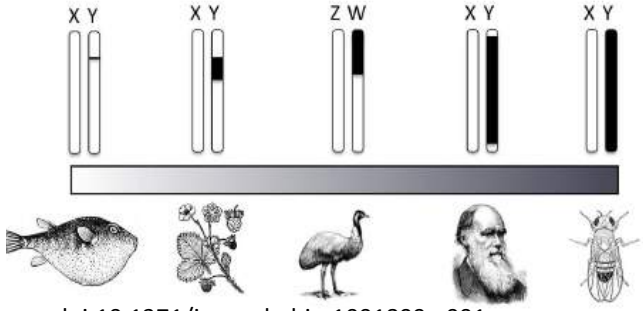
Sex Chromosomes:

- **Different systems** – XX/XY; ZW/ZZ; UV
- **Evolution from pair of Autosomes:** appearance of proto-sex chromosomes (eg proto X/proto Y) – recombination suppression and degeneration of Y
- **Fate of the Y:** Some mammals have lost their Y; others have stabilised it or even added new genes to it
- **Sex chromosomes differences:** The degree of sex chromosome differentiation ranges widely across species

Ranging from a single sex-determining locus (pufferfish), to a small differentiated region (strawberry and emu), to most of the sex chromosomes except for short recombining regions (humans), and to the entire sex chromosome pair (Drosophila).



doi:10.1371/journal.pbio.1001899.g001

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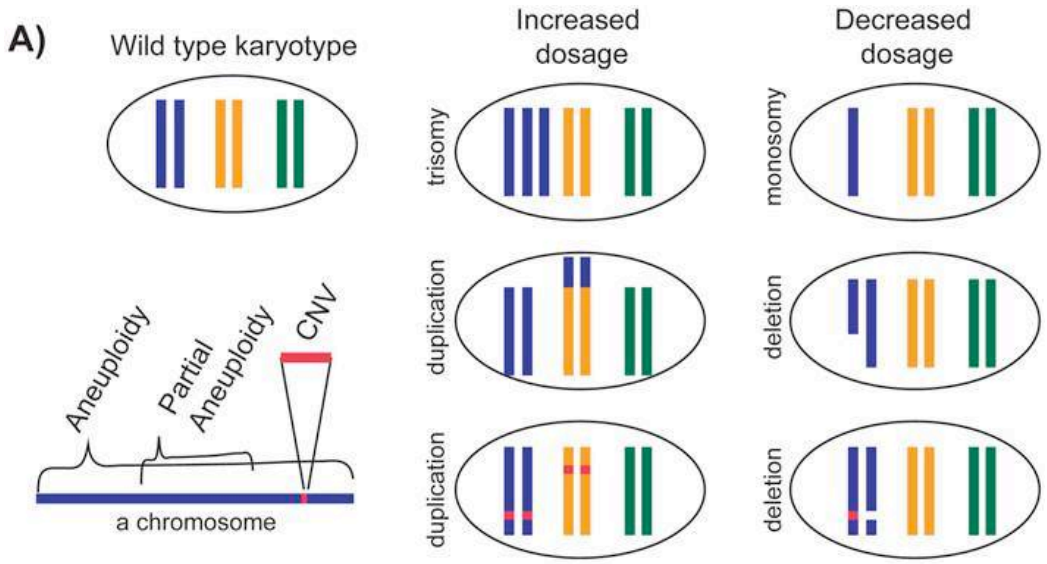
Need for sex chromosome dosage compensation :

- Evolution of very different strategies in different species

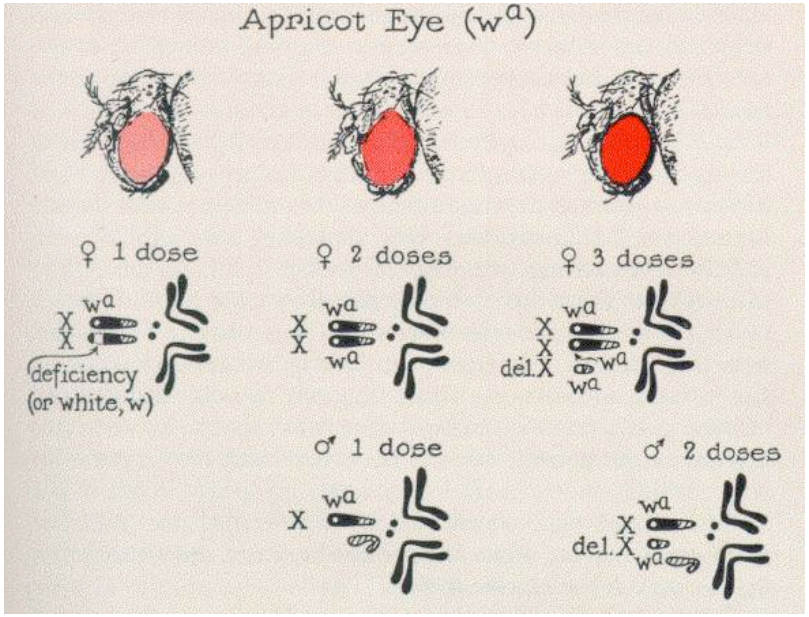
Does Dosage Matter?



- Chromosome monosomy or trisomy <u>are almost always lethal</u> for normal organisms
- Copy number changes of even a single gene can cause problems (e.g. haploinsufficiency)
- Thus maintenance of correct gene dosage at multiple scales (single genes to whole chromosomes) is important for an organism's fitness.
- However, dosage compensation responses to any type of aneuploidy are known to exist
- Even in trisomy 21, not ALL genes show expected 1.5-fold increase in expression suggesting that *dampening* must occur.
- Also, genes / regions in other parts of the genome are affected..
  => while some gene expression varies according to dose, other genes become compensated (presumably to reduce deleterious dosage imbalance)

Sex Chromosome Dosage Compensation

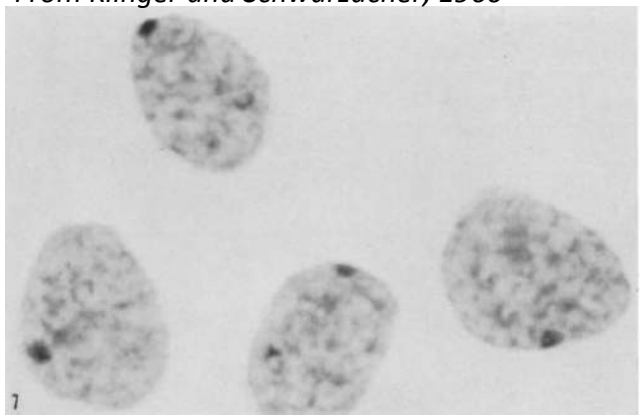
"Effects of dosage changes of sex-linked genes, and the compensatory effects of the gene differences between male and female"
(Muller et al. 1932)
=> "Dosage Compensation" (Muller, 1947)



Observing *apricot* mutation of the *white* gene:
2 X chromosomes in a female produce same eye color as one X chromosome in a male

Sex-chromatin in female mammals is seen in the mouse, rat, opossum, and man.
Ohno et al, 1959, 1960, 1961.

From Klinger and Schwarzacher, 1960



Barr and Bertram, 1949, *Nature*.
Ohno et al, 1959, *Exp Cell Res*.

Allocycly of the X-Chromosome in Tumors and Normal Tissues*

S. Ohno and T. S. Hauschka

(City of Hope Medical Center, Duarte, California; and Roswell Park Memorial Institute, Buffalo, New York)

The apparent functional difference between the two essentially isogenic homologs (at least in inbred strains) of the sex-chromosome pair within the same female nucleus poses an intriguing problem. Among the as yet untested explanations

Gene Action in the X chromosome of the Mouse

M.F. Lyon (1961)

Mary F. Lyon

(1925–2014)

"Grande dame of mouse genetics"

From Obituary by S. Rastan
Nature, 2015

of the year gave the same symptoms; (6) on *L. esculentum* × *L. pimpinellifolium* the symptoms were identical both in the inoculation from the vine and from diseased *I. holstani*.

From *I. holstani* the isolate has so far been transmitted to tobacco (varieties White Burley and Samsun) and to *Petunia*, by sap and by *Myzodes persicae*; to *Nicotiana glutinosa*, *Datura stramonium*, *Vigna sinensis* and *I. holstani* by sap. The percentage infection in the transmission from these species to the same species or to the other species that gave positive results in the inoculation from *I. holstani*, is higher than in the transmission from *I. holstani*.

We are trying to transmit the isolates from the herbaceous plants to grape vine. For this work we use symptomless grape vines, selected during three years and belonging to varieties that appeared to be very receptive to the 'infectious degeneration' in previous experiments on transmission by grafting from vine to vine.

Other work in progress is the identification of the isolates.

No rod-shaped virus particles were seen in a series of observations, using the electron microscope, with exudates obtained by Johnson's method and with drops prepared with Brandes's dipping method both with diseased grape vines (leaves, shoots and roots) and with infected herbaceous plants.

E. Baldacci
A. Amici
P. Bonola
E. Betto
G. Fogliani
E. Refatti

Istituto di Patologia vegetale,
Università di Milano.

[1] Amici, A., Baldacci, E., and Refatti, E., *Ann. Facoltà Agraria Milano (N.S.)*, **7**, 41 (1958).

GENETICS

Gene Action in the X-chromosome of the Mouse (*Mus musculus* L.)

Ohno and Hauschka[1] showed that in female mice one chromosome of mammary carcinoma cells and of normal diploid cells of the ovary, mammary gland and liver was heteropyknotic. They interpreted this chromosome as an *X*-chromosome and suggested that the so-called sex chromatin was composed of one heteropyknotic *X*-chromosome. They left open the question whether the heteropyknosis was shown by the paternal *X*-chromosome only, or the chromosome from either parent indifferently.

The present communication suggests that the evidence of mouse genetics indicates: (1) that the heteropyknotic *X*-chromosome can be either paternal or maternal in origin, in different cells of the same animal; (2) that it is genetically inactivated.

The evidence has two main parts. First, the normal phenotype of *XO* females in the mouse[2] shows that only one active *X*-chromosome is necessary for normal development, including sexual development. The second piece of evidence concerns the mosaic phenotype of female mice heterozygous for some sex-linked mutants. All sex-linked mutants so far known affecting coat colour cause a 'mottled' or 'dappled' phenotype, with patches of normal and mutant colour, in females heterozygous for them. At least six mutations to genes of this type have been reported, under the names mottled[3-5], brindled[3], tortoiseshell[3], dappled[6], and 26*K*[7]. They have been thought to be allelic with one another, but since no fertile males can be obtained from any except, in rare cases, brindled, direct tests of allelism have usually not been possible. In addition, a similar phenotype, described as 'variegated', is seen in females heterozygous for coat colour mutants translocated on to the *X*-chromosome[7,8].

It is here suggested that this mosaic phenotype is due to the inactivation of one or other *X*-chromosome early in embryonic development. If this is true, pigment cells descended from cells in which the chromosome carrying the mutant gene was inactivated will give rise to a normal-coloured patch and those in which the chromosome carrying the normal gene was inactivated will give rise to a mutant-coloured patch. There may be patches of intermediate colour due to cell-mingling in development. The stripes of the coat of female mice heterozygous for the gene tabby, *Ta*, which affects hair structure, would have a similar type of origin. Falconer[9] reported that the black regions of the coat of heterozygotes had a hair structure resembling that of the *Ta* hemizygotes and homozygotes, while the agouti regions had a normal structure.

Thus this hypothesis predicts that for all sex-linked genes of the mouse in which the phenotype is due to localized gene action the heterozygote will have a mosaic appearance, and that there will be a similar effect when autosomal genes are translocated to the *X*-chromosome. When the phenotype is not due to localized gene action various types of result are possible. Unless the gene action is restricted to the descendants of a very small number of cells at the time of inactivation, these original cells will, except in very rare instances, include both types. Therefore, the phenotype may be intermediate between the normal and hemizygote types, or the presence of any normal cells may be enough to ensure a normal phenotype, or the observed expression may vary as the proportion of normal and mutant cells varies, leading to incomplete penetrance in heterozygotes. The gene bent-tail, *Bt*[10], may fit into this category, having 95 per cent penetrance and variable expression in heterozygotes. Jimpy, *jp*, is recessive, suggesting that the presence of some normal cells is enough to ensure a normal phenotype, but Phillips[11] reported one anomalous female which showed the jimpy phenotype. Since it showed the heterozygous phenotype for *Ta* this animal cannot be interpreted as an *XO* female; it is possible that it represents an example of the rare instance when by chance all the cells responsible for the jimpy phenotype had the normal gene inactivated.

The genetic evidence does not indicate at what stage of embryonic development the inactivation of one *X*-chromosome occurs. In embryos of the cat, monkey and man sex-chromatin is first found in nuclei of the late blastocyst stage[12,13]. Inactivation of one *X* at a similar stage of the mouse embryo would be compatible with the observations. Since an *XO* female is normally fertile it is not necessary to postulate that both *X*-chromosomes remain functional until the formation of the gonads.

The sex-chromatin is thought to be formed from one *X*-chromosome also in the rat, *Rattus norvegicus*[14], and in the opossum, *Didelphis virginiana*[15]. If this should prove to be the case in all mammals, then all female mammals heterozygous for sex-linked mutant genes would be expected to show the same phenomena as those in the mouse. The coat of the tortoiseshell cat, being a mosaic of the black and yellow colours of the two homozygous types, fulfils this expectation.

Mary F. Lyon

Medical Research Council
Radiobiological Research Unit,
Harwell, Didcot.

[1] Ohno, S., and Hauschka, T. S., *Cancer Res.*, **20**, 541 (1960).
[2] Welshons, W. J., and Russell, L. B., *Proc. U.S. Nat. Acad. Sci.*, **45**, 560 (1959).
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[4] Lyon, M. F., *J. Hered.*, **51**, 116 (1960).
[5] Dickie, M. M., *J. Hered.*, **45**, 158 (1954).
[6] Phillips, R. J. S., *Genet. Res.* (in the press).
[7] Russell, L. B., and Bangham, J. W., *Genetics*, **44**, 532 (1959).
[8] Russell, L. B., and Bangham, J. W., *Genetics*, **45**, 1008 (1960).
[9] Falconer, D. S., *Z. indukt. Abstamm. u. Vererblehre*, **85**, 240 (1953).
[10] Garber, E. D., *Proc. U.S. Nat. Acad. Sci.*, **38**, 876 (1952).
[11] Phillips, R. J. S., *Z. indukt. Abstamm. u. Vererblehre*, **86**, 322 (1954).
[12] Austin, C. R., and Amoroso, E. C., *Exp. Cell Res.*, **12**, 419 (1957).
[13] Paris, W. W., *J. Anat.*, **91**, 360 (1957).
[14] Ohno, S., Kaplan, W. D., and Kinosita, R., *Exp. Cell Res.*, **18**, 415 (1959).
[15] Ohno, S., Kaplan, W. D., and Kinosita, R., *Exp. Cell Res.*, **18**, 417 (1960).

Genetic Basis for Graft-against-Host Immunological Reactions between Two Inbred Lines of Chickens

It has been established that the enlargement of the embryonic spleen which follows the injection of adult chicken blood into chick embryos is due, at least in part, to a proliferation of cells derived from the injected blood[1,2]. Cock and Simonsen[2] have shown that virtually no splenic enlargement occurs when the blood-donor and embryonic recipients are members of the same inbred line of chickens. The phenomenon of splenic enlargement seems to be fundamentally immunological in nature, and due to donor cells proliferating in response to those host antigens which differ from any in the donor.

It should be possible, by injecting blood from adult birds from one parental line into F_2-generation and back-cross embryos between two inbred lines, to analyse the antigenic difference of the other parental line. Assuming that the antigens of the parental lines are dominantly determined and that they segregate in crosses between the lines in a Mendelian fashion, then a proportion of F_2-generation embryos, and of embryos of the back-cross to the parent of the blood-donating line, will be expected to lack those genes which determine antigens occurring exclusively in the non-blood-donating line. The proportion of embryos which lack these genes will be $(\frac{1}{2})^n$ in the F_2-generation and $(\frac{1}{4})^n$ in the back-cross, where n is the number of pairs of genes involved. Since splenomegaly will occur only when the recipient embryo possesses antigens foreign to the donor cells, these are also the proportions of embryos in the respective crosses which will show no splenic enlargement. All F_1 hybrids and embryos of the back-cross to the parent of the non-blood-donating line will receive the genes which determine antigens peculiar to the non-blood-donating line and all these embryos will therefore show splenic enlargement. Thus, an estimate of the value of n can be obtained by observing the proportion of F_2 and back-cross embryos which show no splenic enlargement. The genetic basis for this method is essentially similar to that used in analysing histo-compatibility differences between inbred strains of mice using tumour transplantation[3], and skin transplantation[4].

Lyon, M. F. (1961), Gene Action in the X-chromosome of the Mouse (Mus musculus L.) Nature. 190 (4773): 372-3.

E. Heard, February 5th, 2018

COLLÈGE DE FRANCE 1530

The Discovery of X-Chromosome Inactivation "Lyonisation"

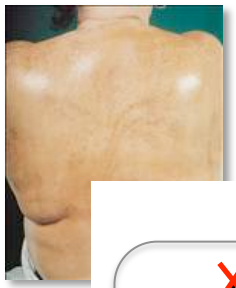
Mary Lyon

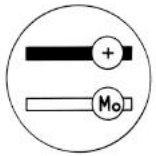
The hypothesis formulated by Mary Lyon in 1961 was that:

(1) the heteropyknotic X chromosome was genetically inactivated
(2) that it could be either paternal or maternal in origin in different cells of the same animal,
(3) that the inactivation occurred <u>early in embryonic development,</u> and once established was stably maintained

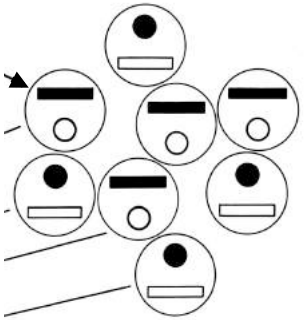
XO mice are normal fertile females => female mice needs only one X chromosome to develop normally (Welshons and Russell, 1959

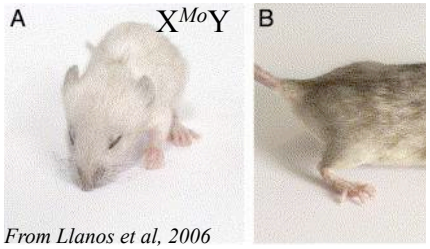
In 1962, Lyon put forward the hypothesis that X inactivation in females is the basis for **dosage compensation** in mammals (Lyon 1962).



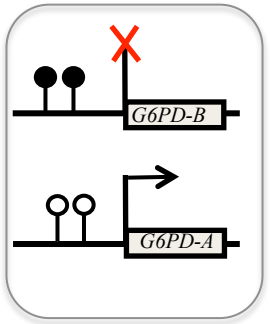


Adapted from Mary Lyon, Henry Stewart Talks

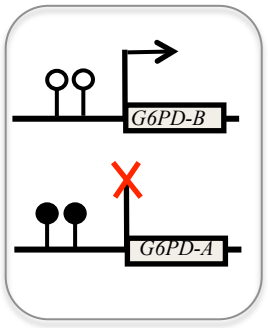




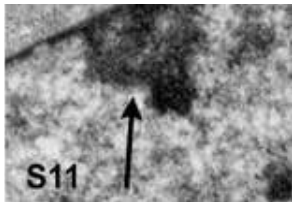
From Llanos et al, 2006



G6PD-A isozyme



G6PD-B isozyme



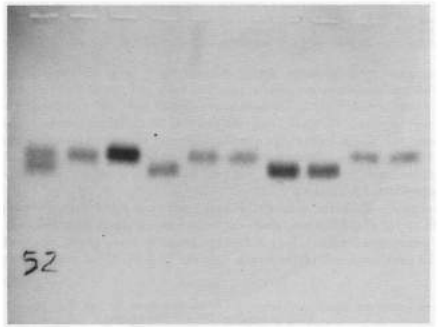


FIG. 2.—Electrophoretic pattern of G-6-PD from sonicates of cultured cells. Samples were run singly, starting from the origin at the top of the figure. From left to right are the AB phenotype of the cell culture from Mrs. De. prior to cloning, and the single bands of nine clones derived from the original cell lines. Variation in intensity of staining is due to inequality of enzyme concentration applied to the starch gel.

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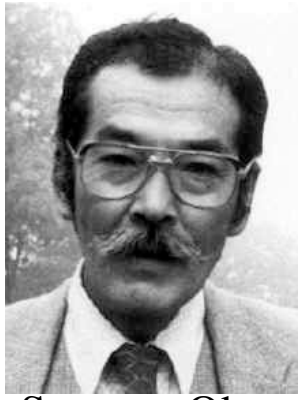
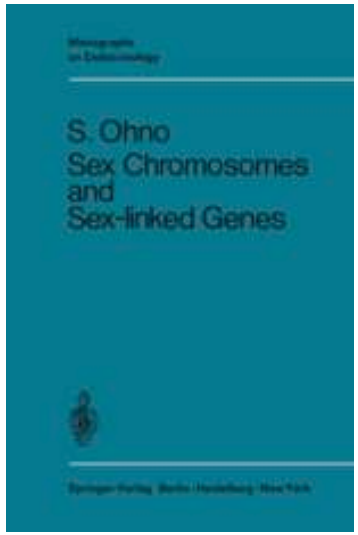
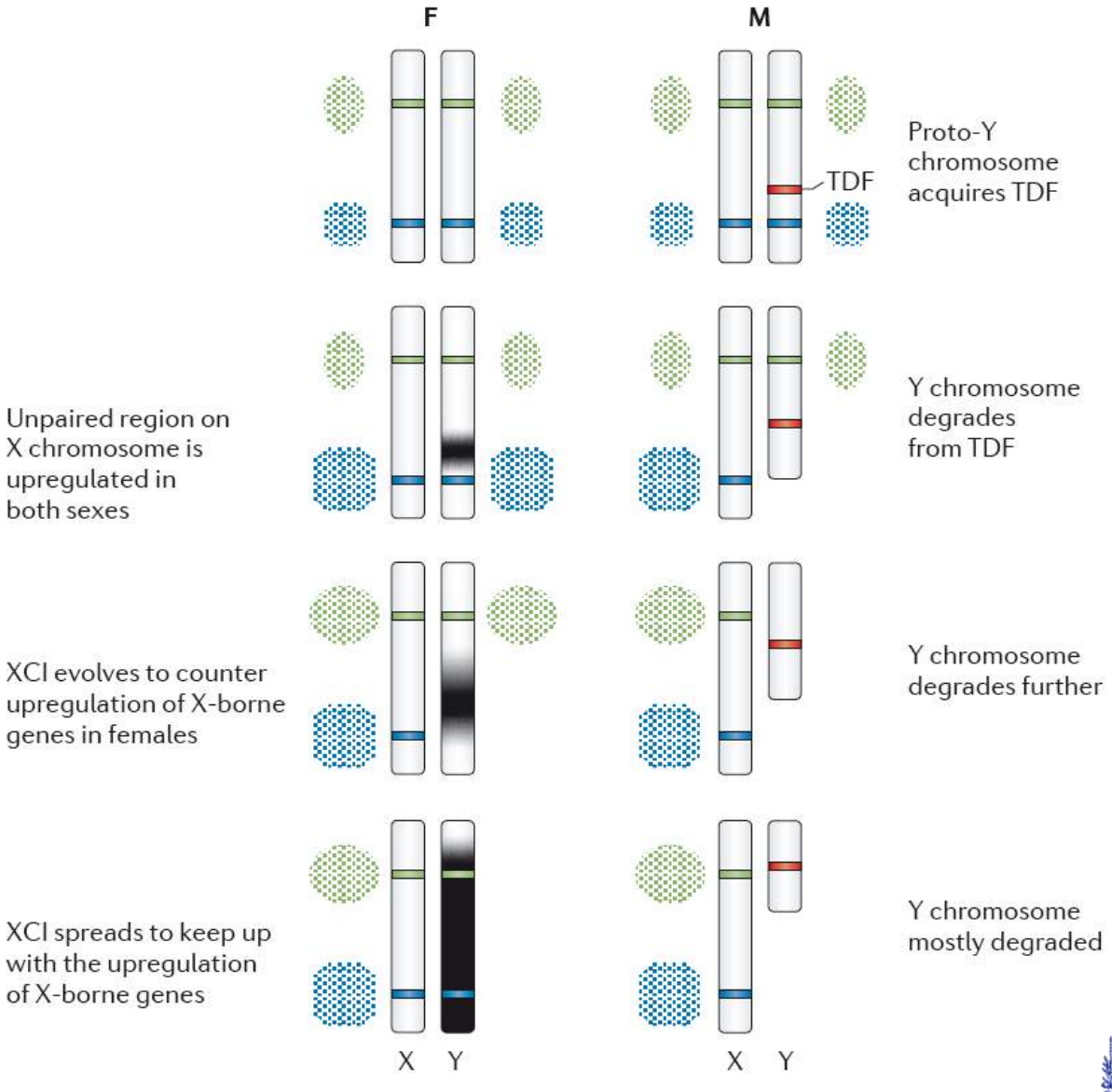
Ohno's Hypothesis (1967)



Susumu Ohno
(1928-2000)



Ohno S (1967) Sex Chromosomes and Sex-Linked Genes.



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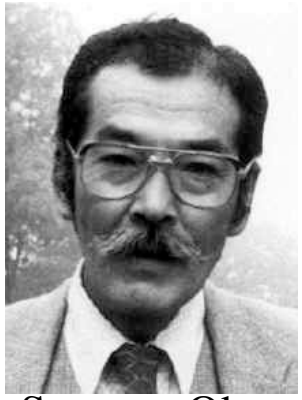

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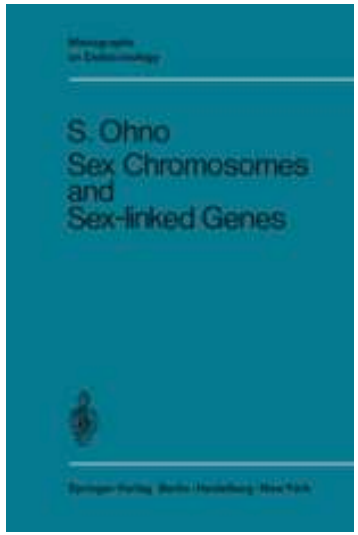

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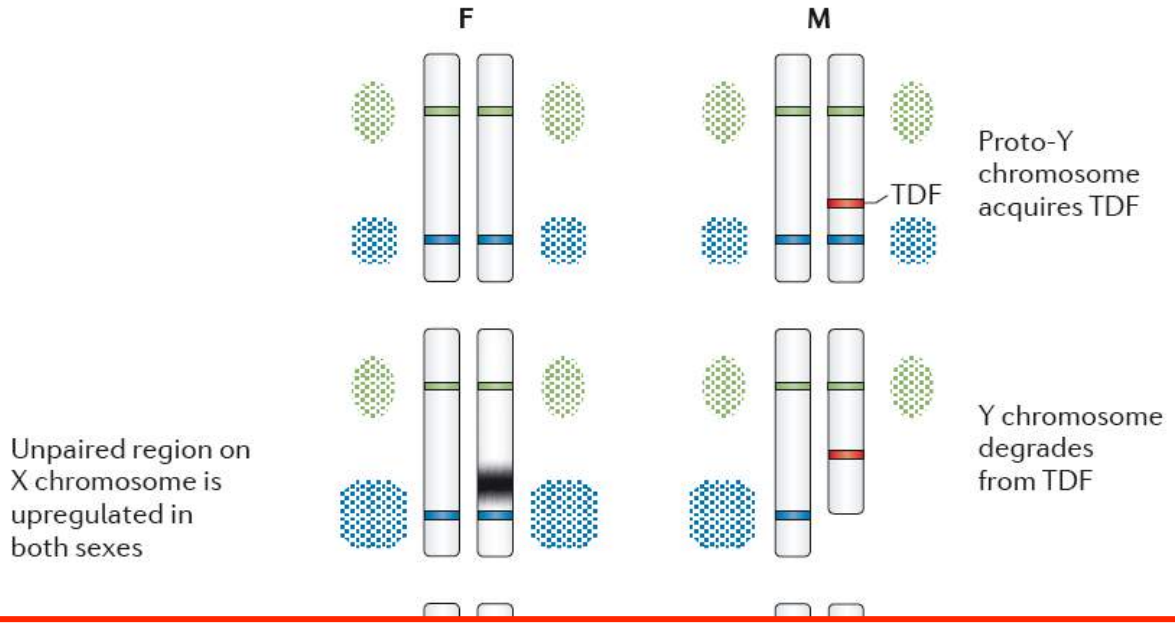
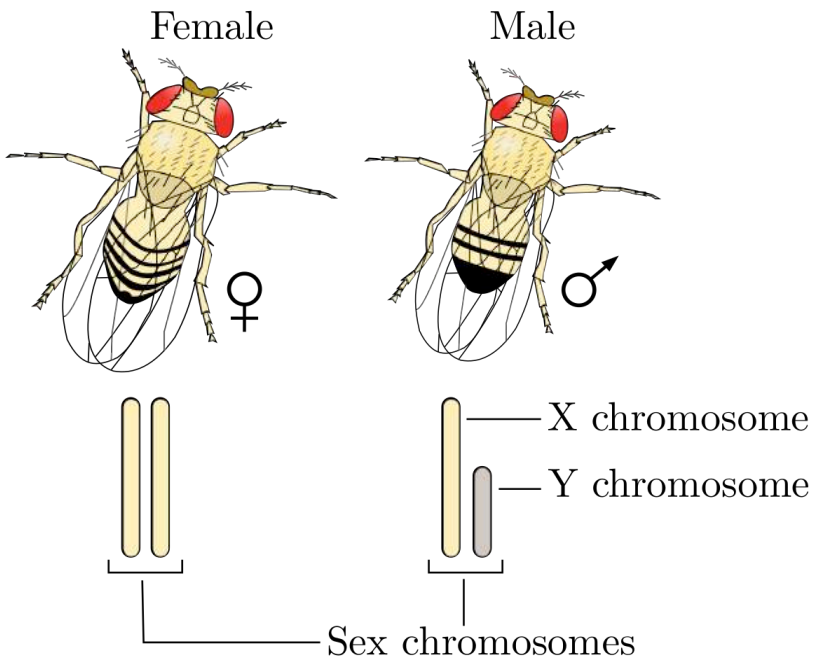


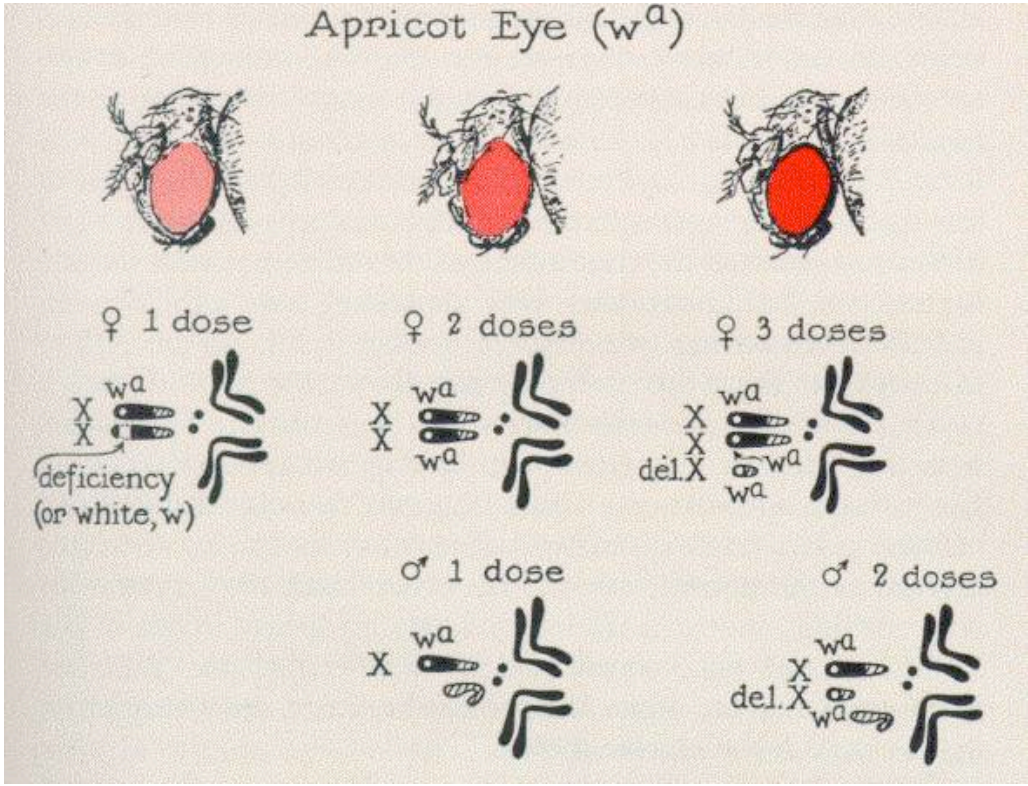
Ohno proposed that Y chromosome degradation might drive upregulation of the unpaired region of the X chromosome in males (M) and females (F) and this is then countered by X chromosome inactivation in females.

Do other organisms with sex chromosomes undergo dosage compensation via X inactivation and up-regulation of the X (compared to autosomes)?

What about *Drosophila*?

The term "Dosage Compensation" first coined by Muller in his 1947 Harvey Lecture





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Dosage Compensation in *Drosophila*

- Levels of nascent transcripts on the male X chromosome and on the paired X chromosomes of females were similar and the mechanism of compensation, responsible for correcting the difference in gene dosage between the sexes, operated at the level of transcription (Mukherjee & Beermann 1965)
- Mutants affecting males only showed that transcription up-regulation was likely the mechanism (Belote & Lucchesi, 1980).
- Epigenetic marking of the "hyper-active" X in males compared to the two Xs in females (Turner et al, 1992).

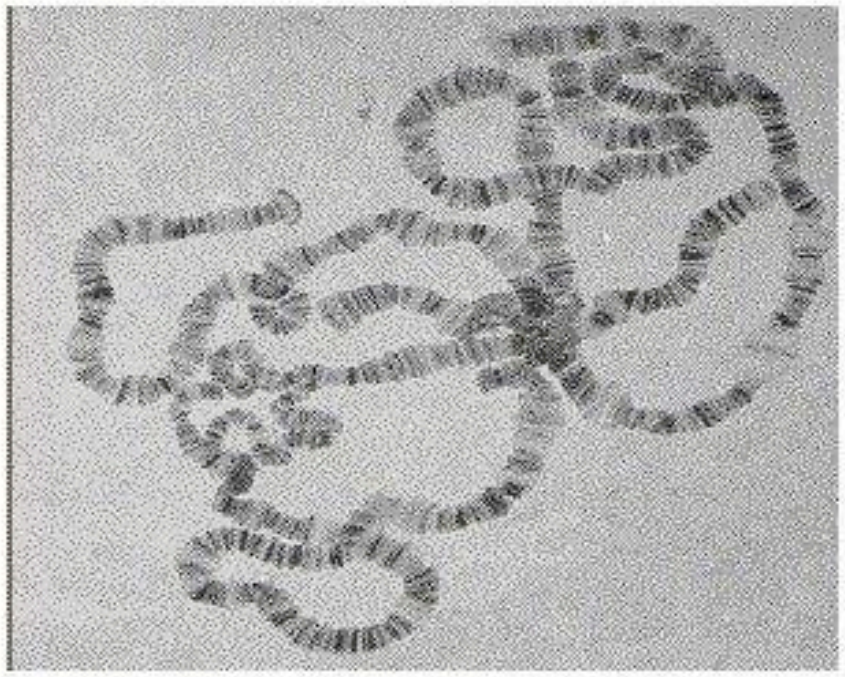
GENETICS

Mukherjee and Beermann, Nature, 1965

Synthesis of Ribonucleic Acid by the X-Chromosomes of *Drosophila melanogaster* and the Problem of Dosage Compensation

INVESTIGATIONS of the expression of various sex-linked genes in *Drosophila* have shown that for most of them their phenotypic manifestation is identical in male and female. Stern[1], Muller[2] and later Muller *et al.*[3] by use of mutations such as bobbed and apricot in various doses showed that the identity and equality of genic expression between the two sexes are not associated, *a priori*, with the development of sex, but rather involves a system of genes, plus and minus modifiers, which regardless of sex tends to repress the action of extra doses of sex-linked genes. Muller proposed the name 'dosage compensation' for this effect and concluded that for each sex-linked gene there is a set of 'compensator genes'[4].

Dosage compensation has been a subject of modern genetic research in *Drosophila*[5] and also in mammals[6–8], but the cytochemical examination of the mechanism of dosage compensation has been initiated only recently by Rudkin *et al.*[9].



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Mukherjee and Beermann, Nature, 1965

Synthesis of Ribonucleic Acid by the X-Chromosomes of *Drosophila melanogaster* and the Problem of Dosage Compensation

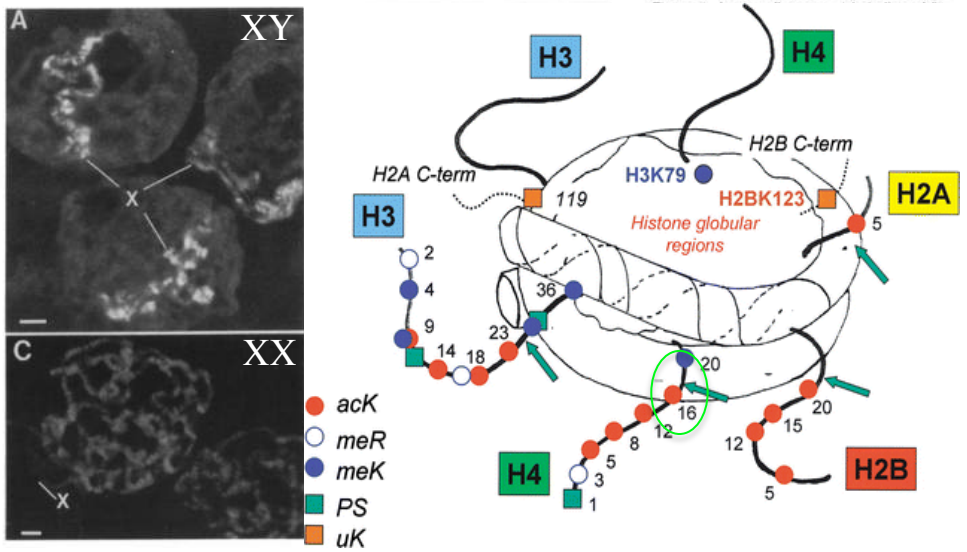
INVESTIGATIONS of the expression of various sex-linked genes in *Drosophila* have shown that for most of them their phenotypic manifestation is identical in male and female. Stern[1], Muller[2] and later Muller *et al.*[3] by use of mutations such as bobbed and apricot in various doses showed that the identity and equality of genic expression between the two sexes are not associated, *a priori*, with the development of sex, but rather involves a system of genes, plus and minus modifiers, which regardless of sex tends to repress the action of extra doses of sex-linked genes. Muller proposed the name 'dosage compensation' for this effect and concluded that for each sex-linked gene there is a set of 'compensator genes'[4].

Dosage compensation has been a subject of modern genetic research in *Drosophila*[5] and also in mammals[6–8], but the cytochemical examination of the mechanism of dosage compensation has been initiated only recently by Rudkin *et al.*[9].

Cell, Vol. 69, 375–384, April 17, 1992, Copyright © 1992 by Cell Press

Histone H4 Isoforms Acetylated at Specific Lysine Residues Define Individual Chromosomes and Chromatin Domains in Drosophila Polytene Nuclei

Bryan M. Turner,* Andrew J. Birley,† and Jayne Lavender*



E. Heard, February 5th, 2018

Sex Determination and Dosage Compensation in *Drosophila*

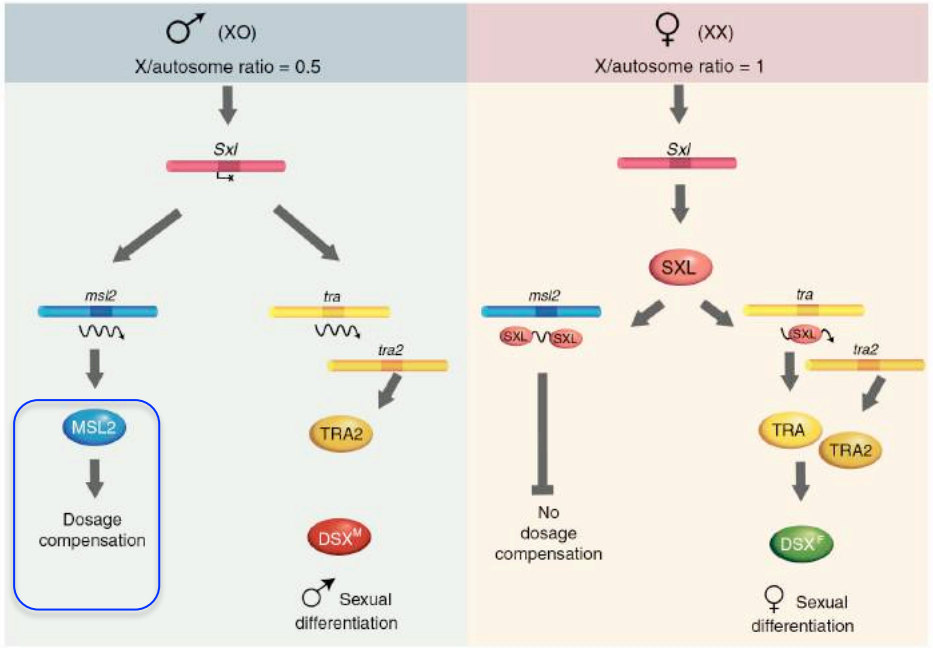
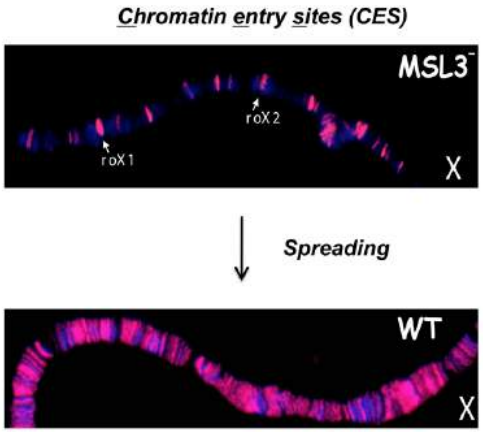
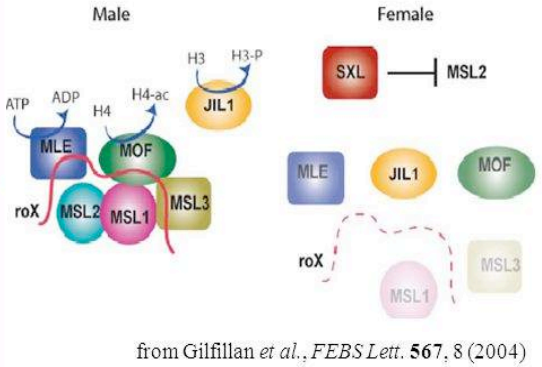


Figure 2. Diagram of the control of sex determination and dosage compensation. If the X/A ratio is equal to regulatory cascade leads to female sexual development. In females, the presence of the *Sxl* gene product prevents the translation of the *msl2* message and the assembly of the MSL complex. If the X/A ratio is only 0.5, absence of the cascade leads by default to male sexual development and to the formation of the MSL complex.

Epigenetics, Second Edition © 2015 Cold Spring



- MSL complex in males only
- Binds to the X only



from Gilfillan *et al.*, *FEBS Lett.* **567**, 8 (2004)

SXL in females prevents MSL2 translation

MSL2 in males stabilizes roX, MSL1, and MSL3

DCC binds to high affinity sites on X chromosome

DCC spreads to nearby sites on active chromatin

H4K16 acetylation impedes formation of condensed chromatin structure

• Up-regulation of the male X chromosome is achieved by recruitment of the MSL complex to increase levels of H4K16 acetylation and open chromatin, which results in increased transcription initiation and elongation.

• Recruitment of the MSL complex to the male X is mediated by a 2–4 fold enrichment in specific binding motifs.

•Heterochromatin elements counteract chromatin unfolding to achieve a precise doubling in gene expression.

Model for X-linked gene up-regulation in male *Drosophila*

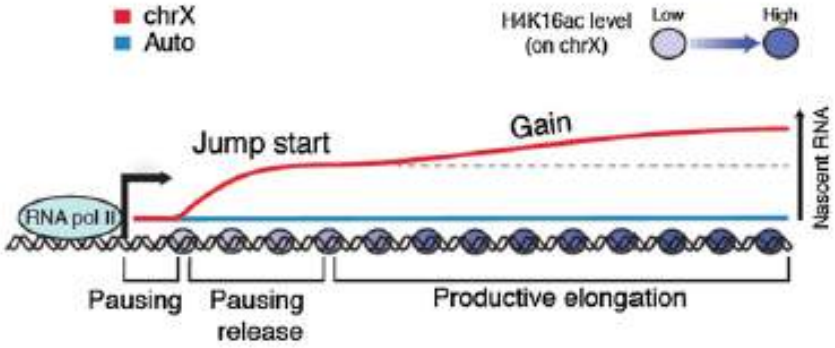
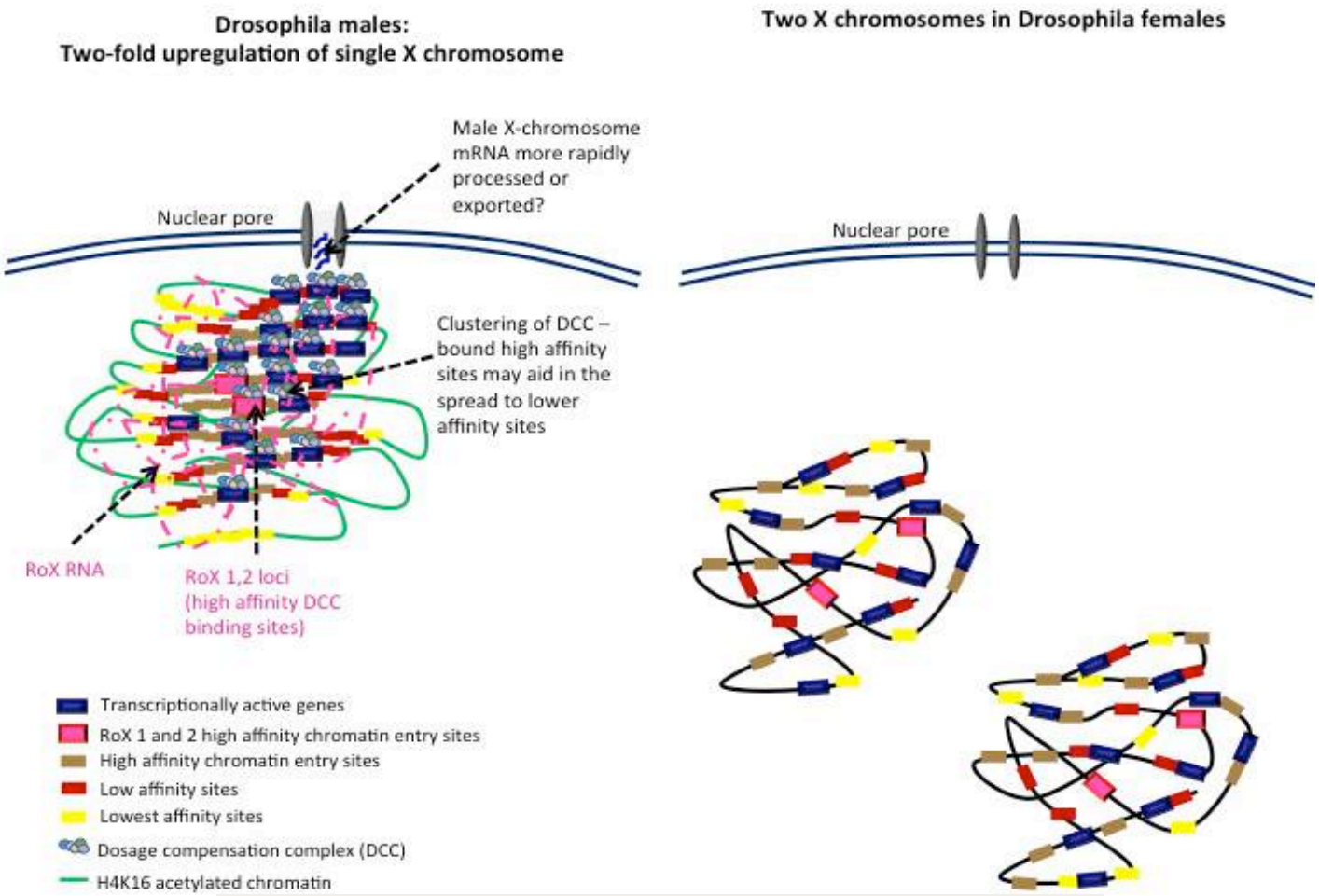


Figure 10 Jumpstart and gain model for dosage compensation. The transcription profile of genes on autosomes (Auto, blue line) compared to dosage compensated genes on *X* (chrX, red line). Both attract abundant Pol II, producing short transcripts while paused. An increase of mRNA production occurs on *X*-linked genes due to H4K16ac enriched on gene bodies. The facilitated steps include pause release (jumpstart), and a measureable gain during elongation. From Ferrari *et al.* 2013b; reprinted with permission from Elsevier (Amsterdam).

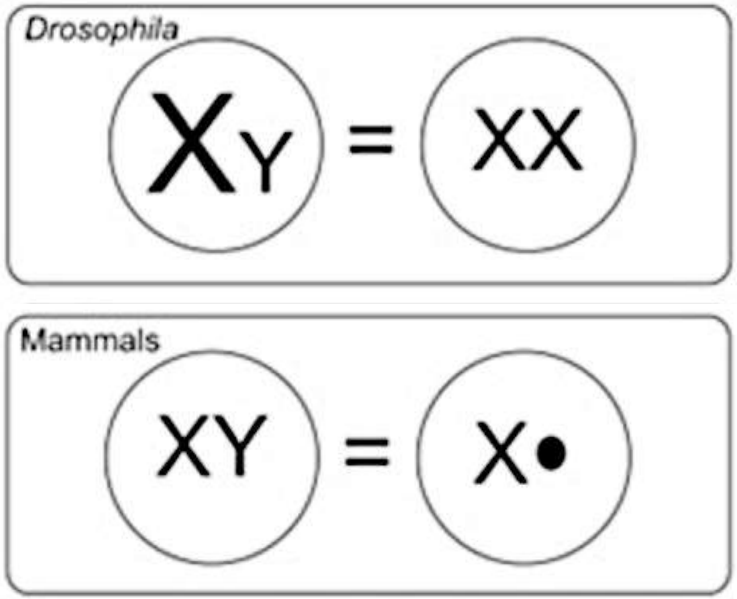
Dosage Compensation in *Drosophila*

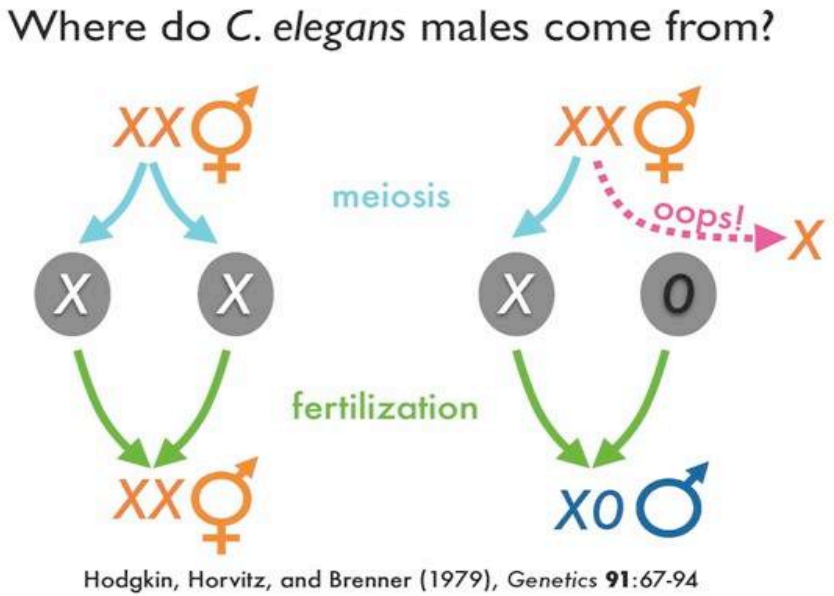


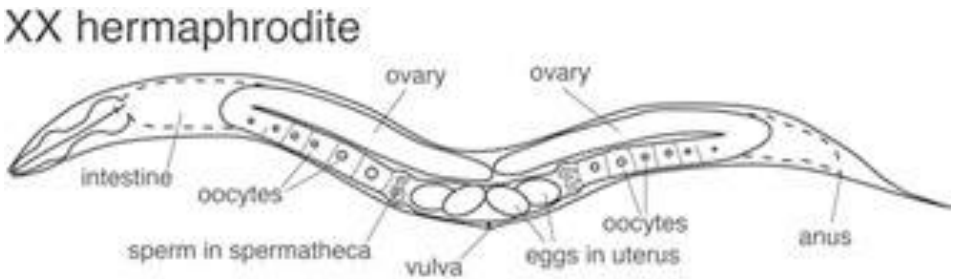
Organisation of the dosage compensated X?
(NEXT WEEK)

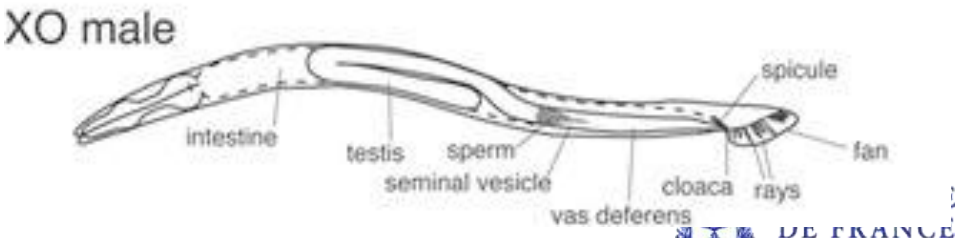
From Chow and Heard, 2010

Sex Determination and Dosage Compensation in *Caenorhabditis elegans*



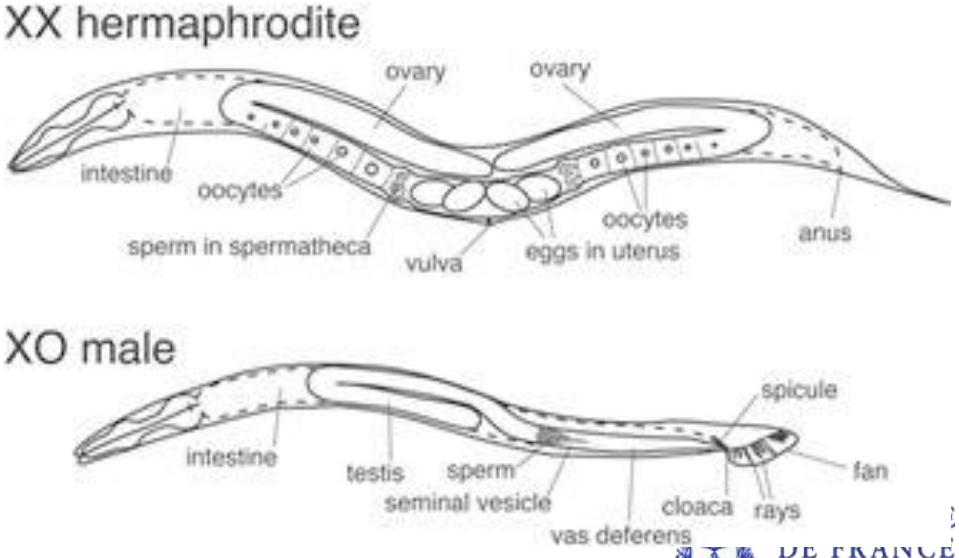






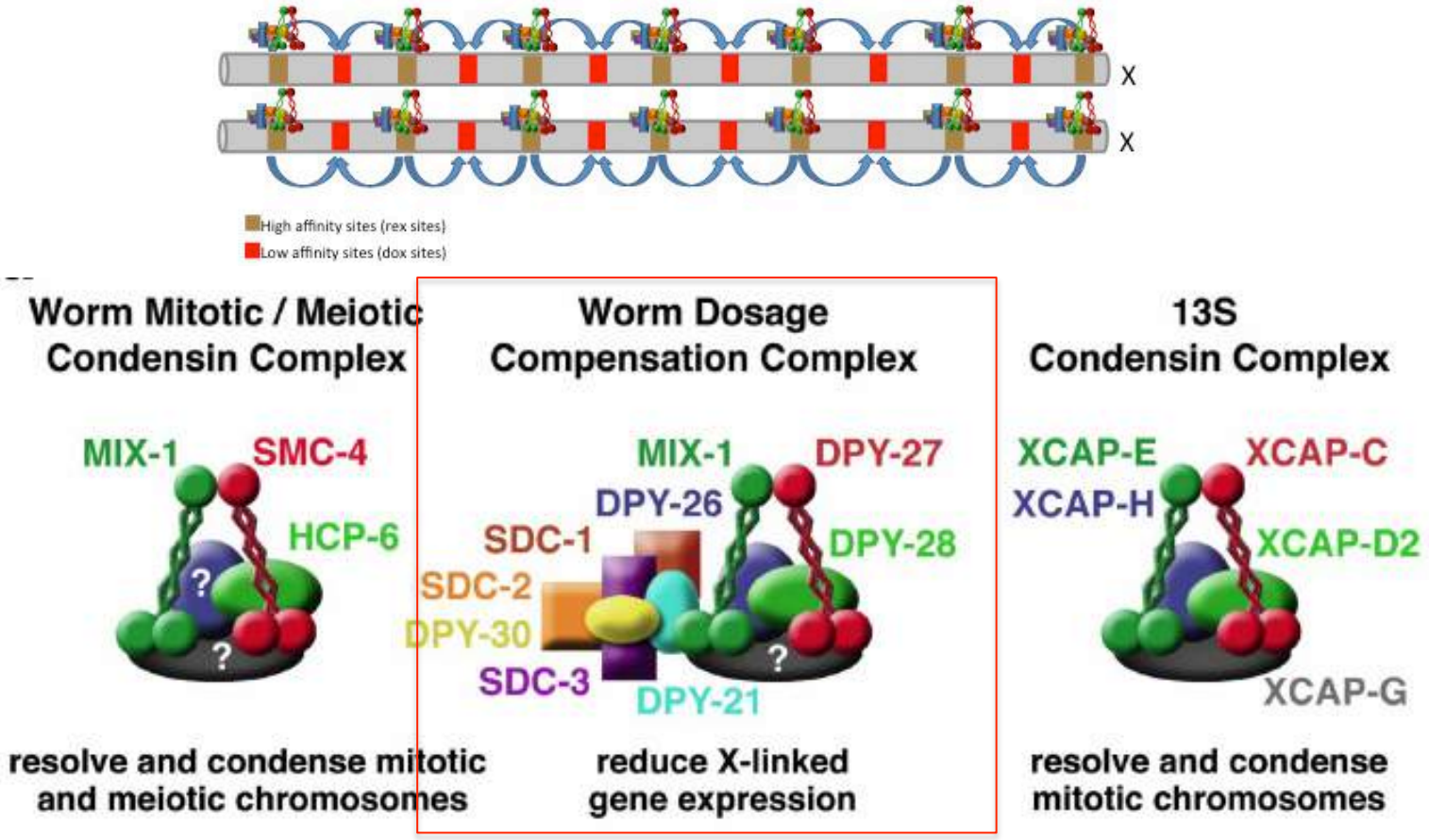
Sex Determination and Dosage Compensation in *Caenorhabditis elegans*

- Caenorhabditis elegans (or C. elegans), sex is determined by the X:A ratio (X chromosomes relative to sets of autosomes):
- XX worms develop into hermaphrodites; worms with only 1X (XO) develop as males
- There is no male specific chromosome (ie no Y)
- The same developmental X:A signal triggers sex determination and dosage compensation
- Following this common step of regulation, sex determination and dosage compensation are then controlled by distinct pathways
- Discovery of sex-specific lethal mutations that preferentially killed XX hermaphrodites in 1980s – disrupted dosage compensation & led to 2-fold increase in X-linked transcripts in XX but not XO animals
- Animals surviving have Dumpy phenotype due to overexpression of X-linked genes



Dosage Compensation in *Caenorhabditis elegans*

- Dosage compensation is achieved by a protein complex that binds both X chromosomes of hermaphrodites to reduce transcript levels by one-half.

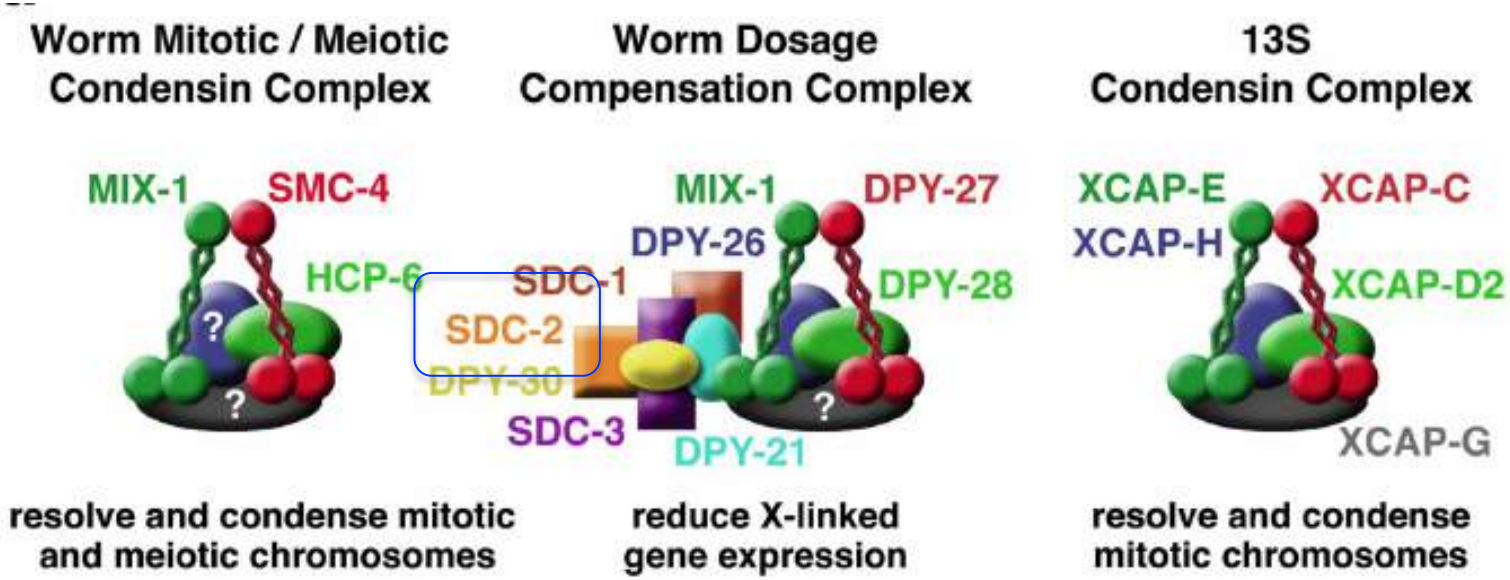


Meyer BJ, Casson LP: *Caenorhabditis elegans* compensates for the difference in X chromosome dosage between the sexes by regulating transcript levels. *Cell* 1986, 47:871-881.

Barbara J. Meyer (WormBook, 2005)

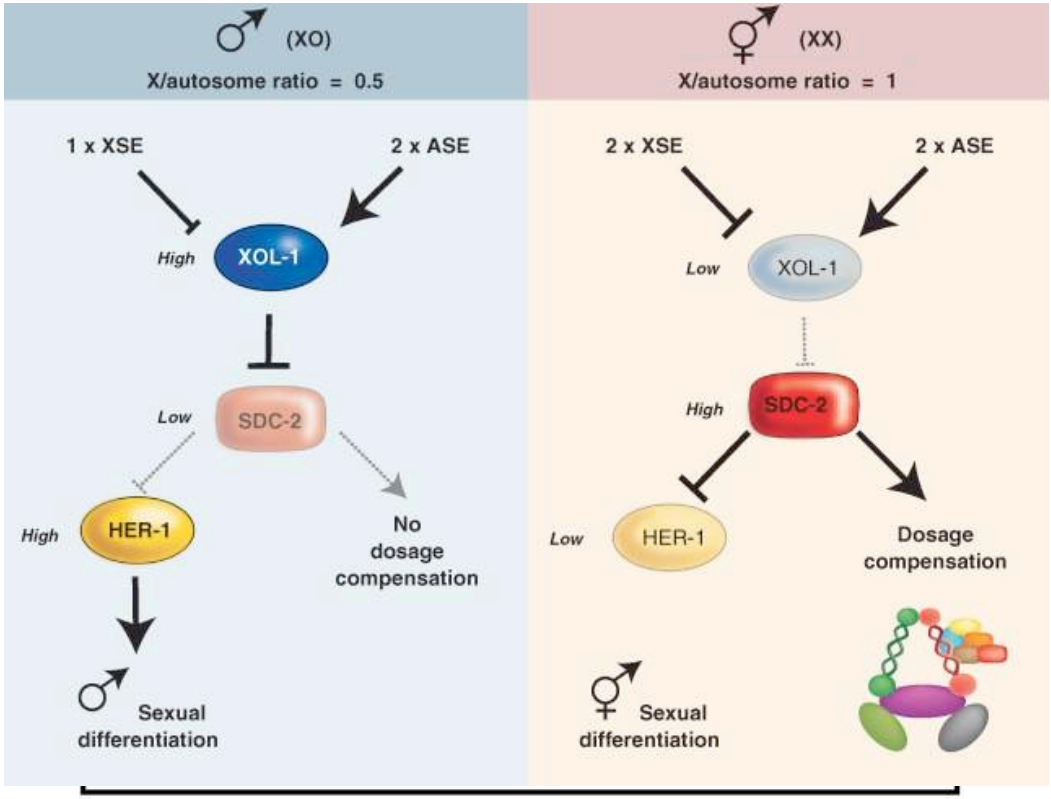
Dosage Compensation in *Caenorhabditis elegans*

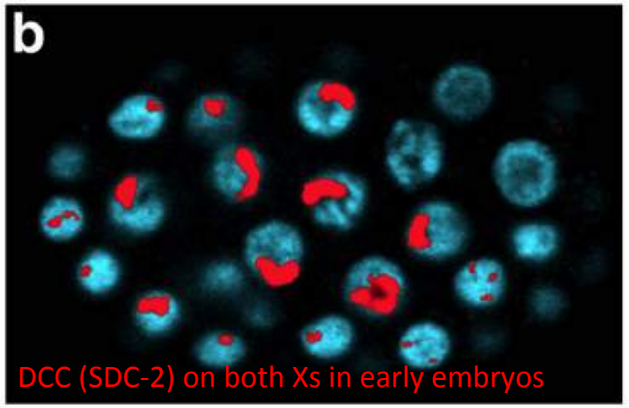
- Dosage compensation is achieved by a protein complex that binds both X chromosomes of hermaphrodites to reduce transcript levels by one-half.
- The dosage compensation complex resembles the conserved 13S condensin complex required for both mitotic and meiotic chromosome resolution and condensation
- => recruitment of ancient proteins to the new task of regulating gene expression.
- Targeting of DCC to the X is via small DNA elements that act as entry sites to recruit the DCC and to nucleate spreading of the complex to X regions that lack recruitment sites

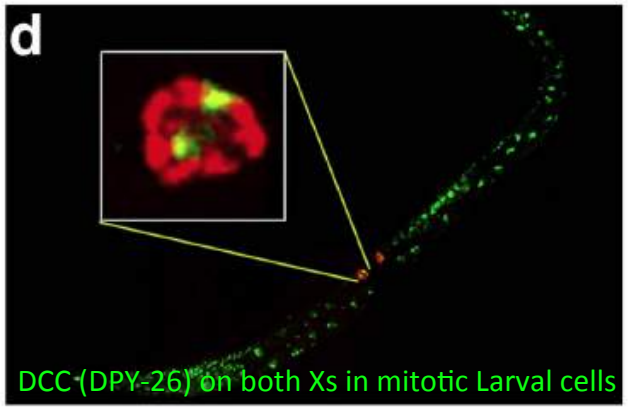


Barbara J. Meyer (WormBook, 2005)

Dosage Compensation in *Caenorhabditis elegans*



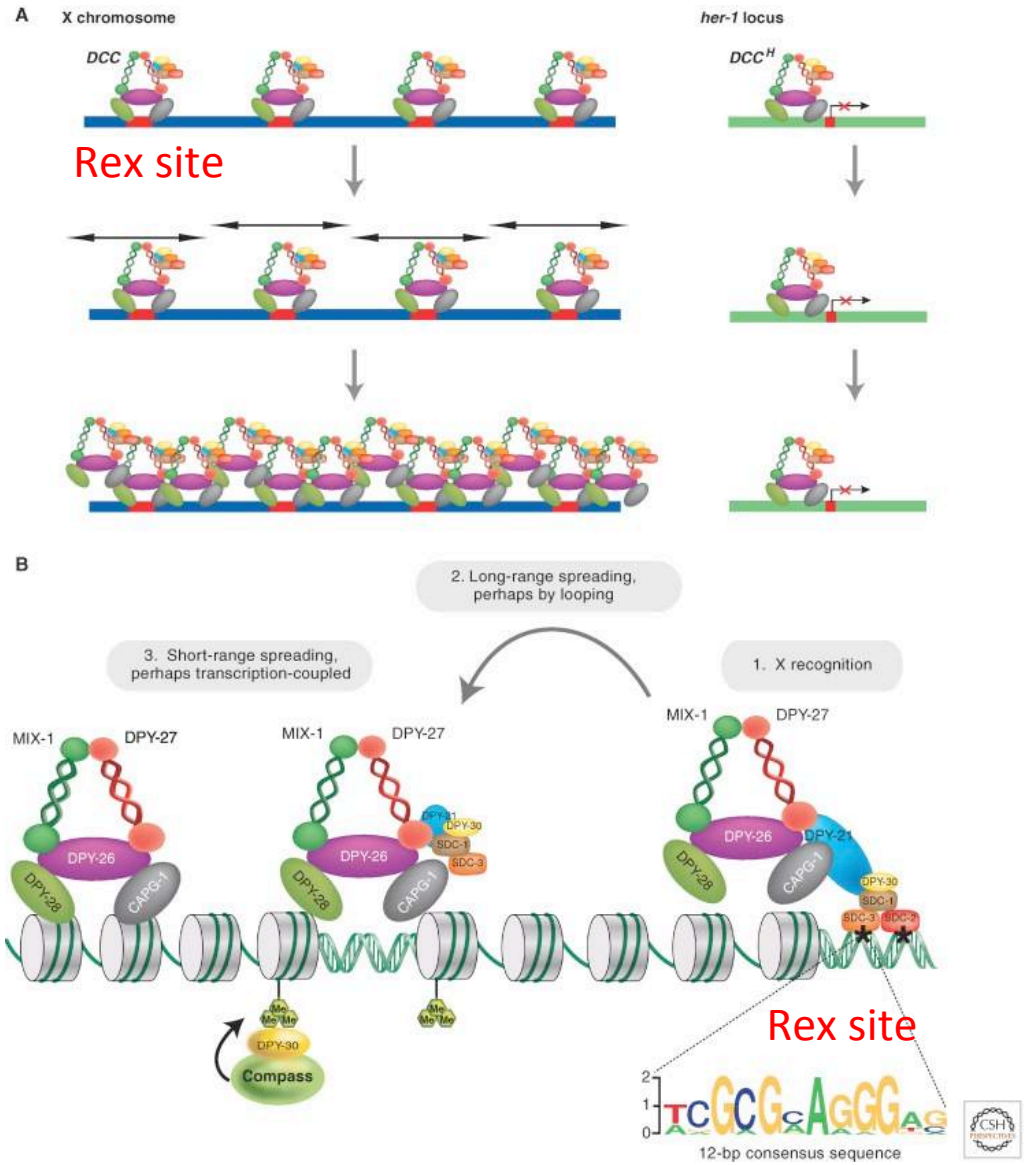


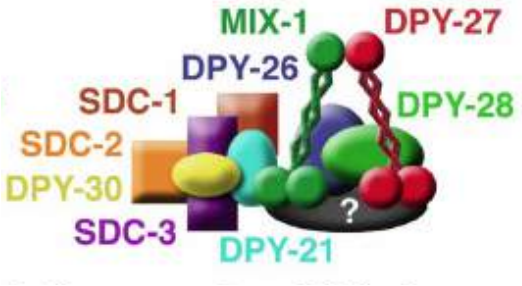


- **SDC-2** is expressed at around 40-cell stage
- It confers chromosome-specificity to dosage compensation and also hermaphrodite-specificity (by repressing the *her1* male sex-determining gene).
- *sdc-2* is repressed in males by the male-specific gene *xol-1*, the master sex-determination switch gene and direct target of the primary sex-determination signal

Barbara J. Meyer (WormBook, 2005)

Dosage Compensation in *Caenorhabditis elegans*

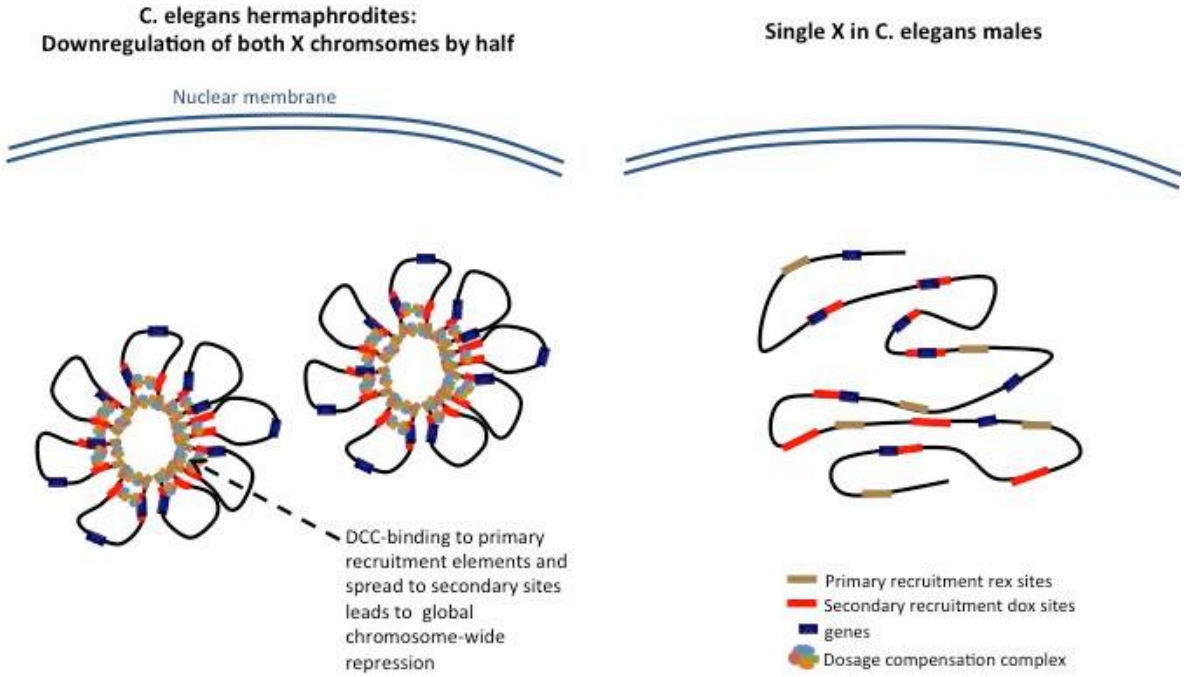


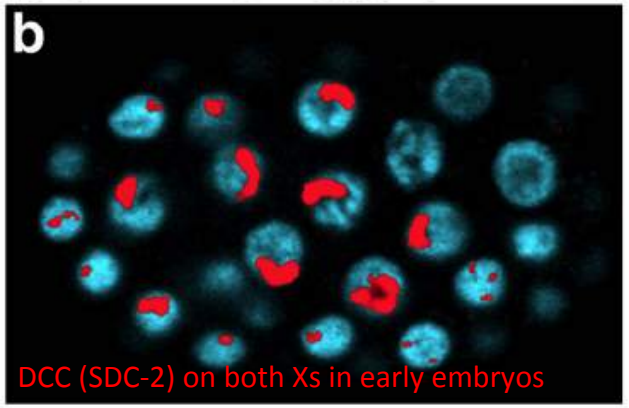


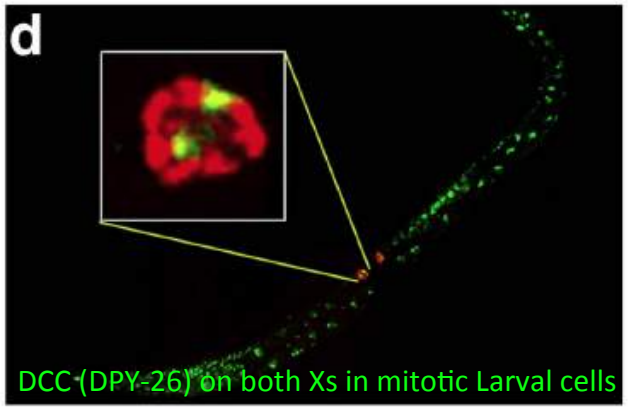
The X chromosome harbors multiple X-recognition elements (rex sites) that distinguish it from autosomes to recruit the DCC. The complex binds to rex elements on both hermaphrodite X chromosomes and then spreads in cis to neighboring X regions that lack recruitment sites, thus establishing X-chromosome-wide repression that is maintained throughout the lifetime of the animal.

Strome et al, CSH, 2014

Dosage Compensation in *Caenorhabditis elegans*





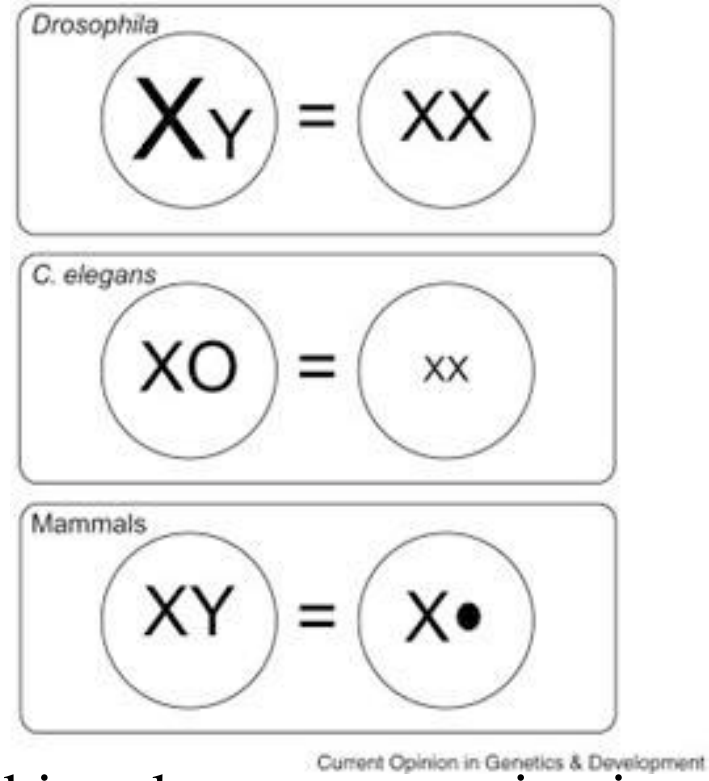


Organisation of the dosage compensated X?
(NEXT WEEK)

Barbara J. Meyer (WormBook, 2005)

Sex Chromosome Dosage Compensation

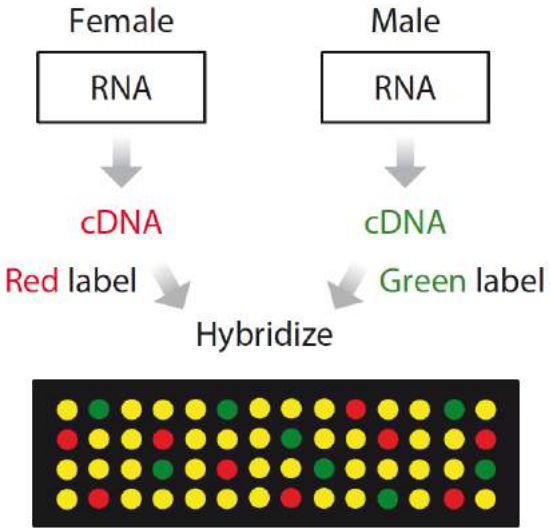
Globally dosage is compensated between the sexes
in three best studies model organisms
(although by very different strategies)



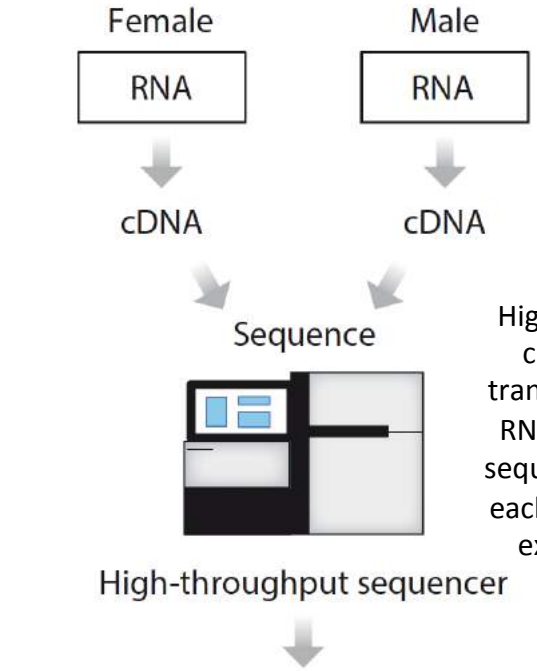
Sex-biased gene expression is present
(as expected in sex tissues– but also in other tissues)
(More in coming Weeks!)

Molecular Evidence for Dosage Compensation?

Comparative hybridization of labeled female (*red*) and male (*green*) cDNA generated by reverse transcription of RNA. The microarray contains thousands of spots of DNA corresponding to each gene in the genome. The intensity of the hybridization signal for each color is used to estimate the expression level of a gene in each sex. Spots with equal expression in the two sexes appear yellow.



Gene	Red signal	Green signal	Bias
A	4,561	102	Female
B	76	2,399	Male
C	5,796	5,788	None



High-throughput sequencing of cDNA generated by reverse transcription of female and male RNA (RNA-seq). The number of sequence reads corresponding to each gene is used to estimate its expression level in each sex.

Grath and Parsch, Ann. Reviews Genetics, 2016

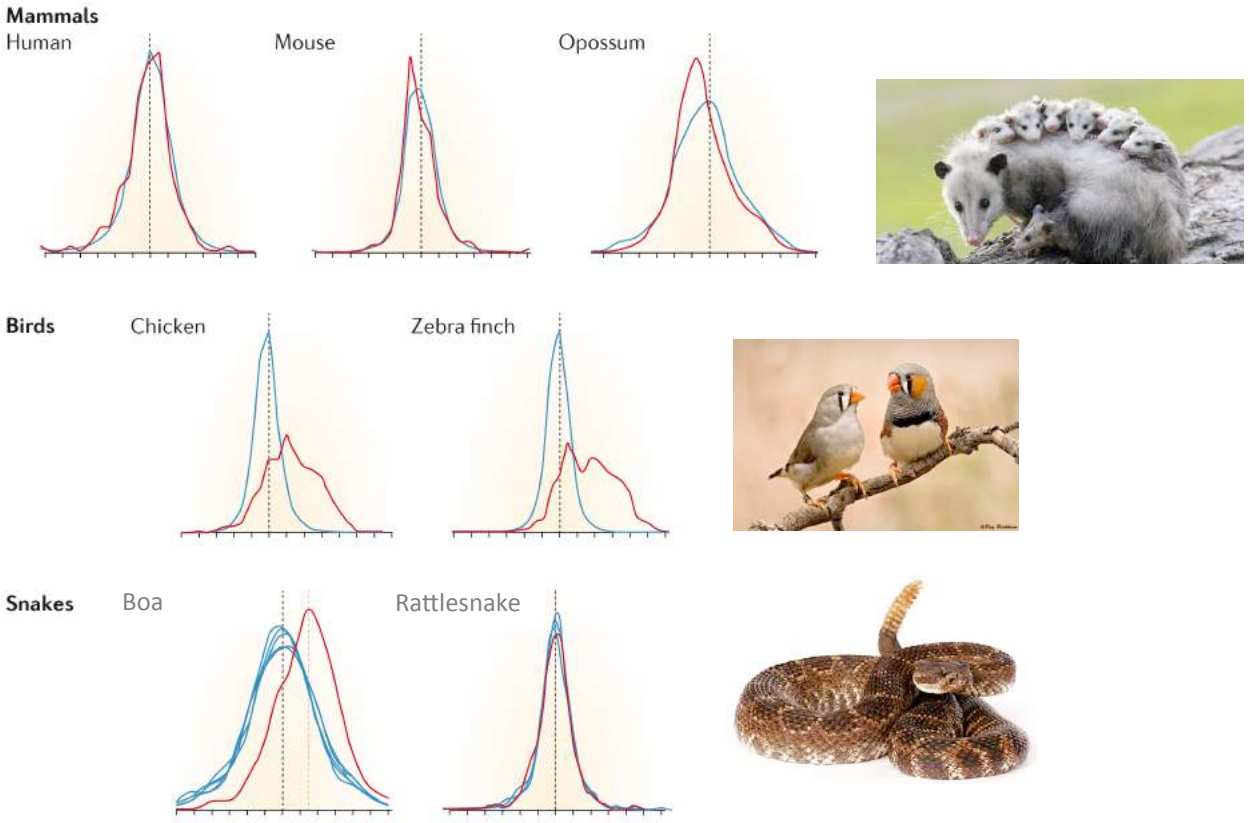
How widespread is Dosage Compensation between the Sexes?

Blue lines denote the male/female ratio for genes on **autosomes**

Red lines denote the male/female ratio for **sex chromosomes** (X or Z)

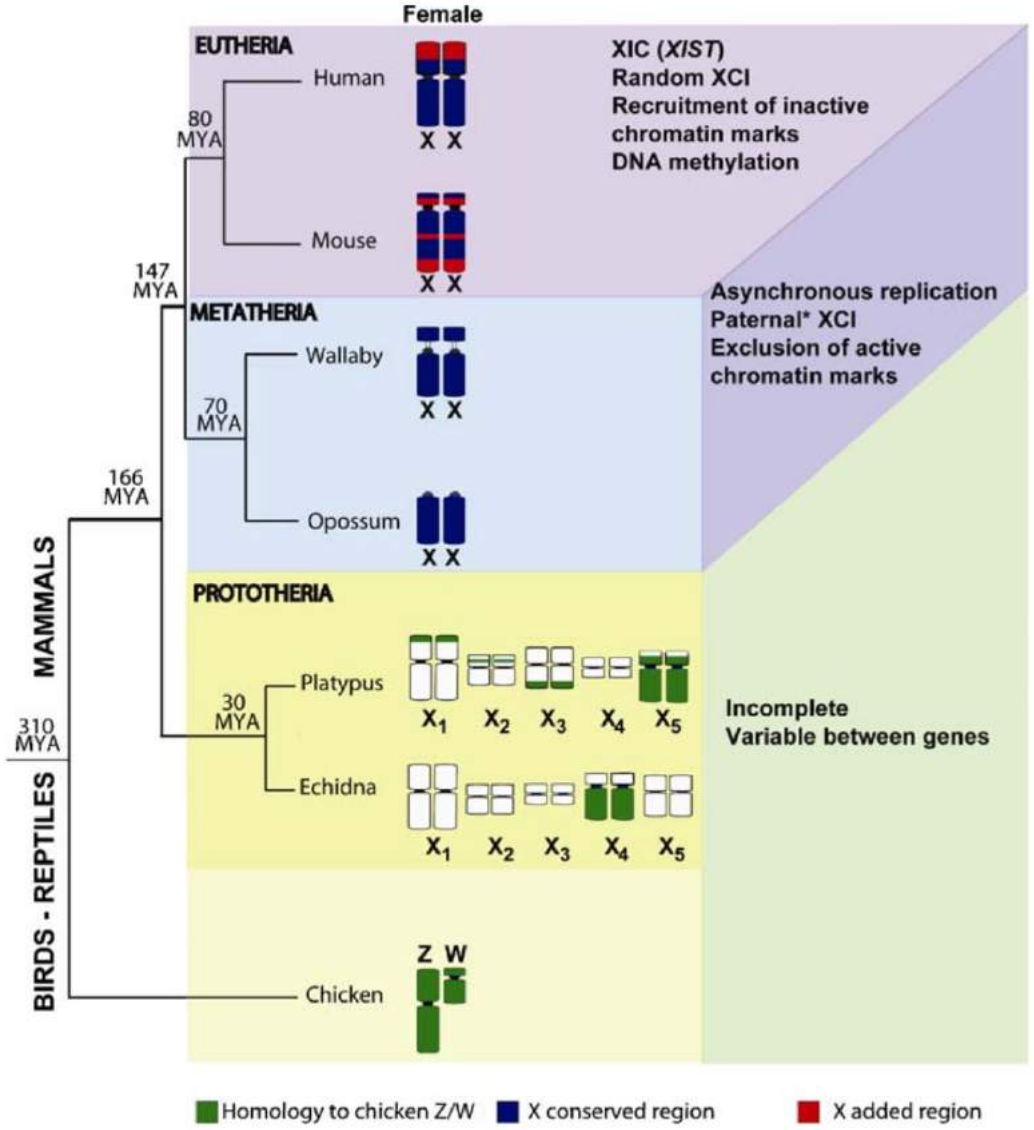
The frequency of genes with varying male (m)/female (f) ratios of expression is shown logarithmically

Zero ratio, denotes equal expression, represented by a dashed vertical line



Dosage compensation between the sexes is almost complete for the mammalian X chromosome, partial for the bird, rattlesnake and sole Z chromosome, and absent for the undifferentiated boa Z chromosome.

Chromosome-wide Dosage Compensation may be the exception rather than the rule…



(Deakin, Chaumeil, Hore, Graves, Chrom Res 2009)

Evolution of dosage compensation **revisited**

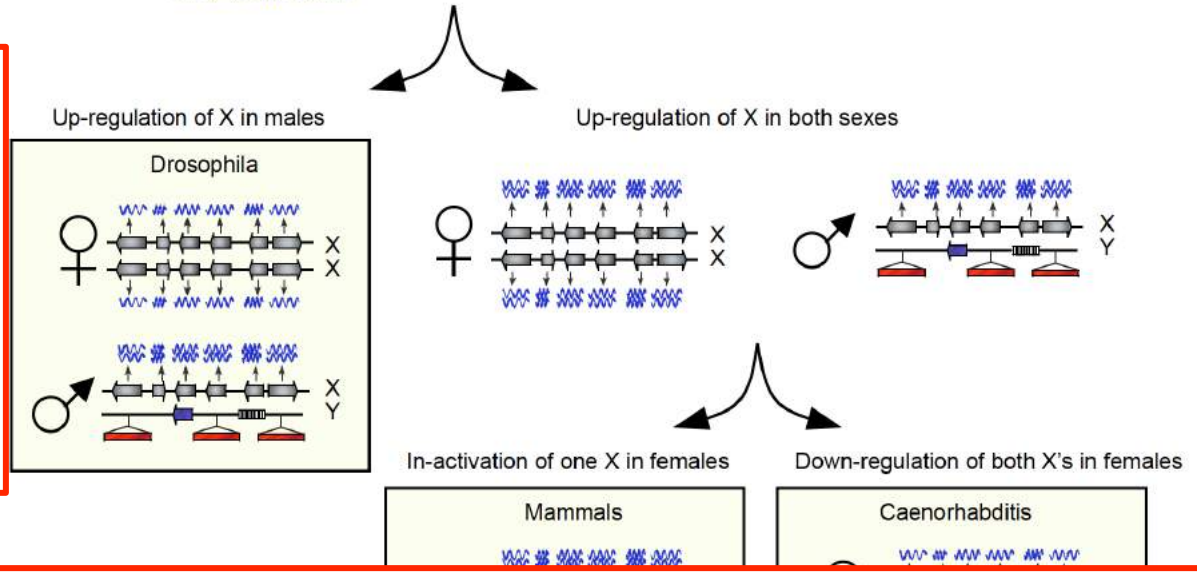
Two types of **sex chromosome dosage compensation** (often confused with each other):

- (1) one to balance gene expression genome-wide via changes in relative expression of X- or Z-linked genes versus autosomal genes => critical for fitness. X/Z up-regulation??
- (2) another, to equalise sex-linked gene expression between the sexes (XX/XY, or ZW/ZZ).

Also, in some cases, evolution of adaptive changes in expression of ancestral autosomal genes that evolved into sex-linked genes …

Support for Ohno's hypothesis?
As measured by X:autosome expression ratios, X chromosome up-regulation is still controversial…

Up-regulation may have evolved at the gene by gene level – and by different molecular mechanisms?



Although equality of X chromosome expression between the sexes has been demonstrated in flies, mammals and worms, tests for the doubling of expression levels globally along the X chromosome have returned contradictory results...

Dosage Compensation in *Drosophila:* an alternative view

- “Inverse Hypothesis” has been proposed
- s with multiple X chromosomes
- omal genes in triple X metafemales which

Review Article

Cytogenetic and Genome Research

Cytogenet Genome Res 2016;148:52–67
DOI: 10.1159/000445924

Accepted: January 27, 2016
by M. Schmid
Published online: May 12, 2016

Parallel Universes for Models of X Chromosome Dosage Compensation in *Drosophila*: A Review

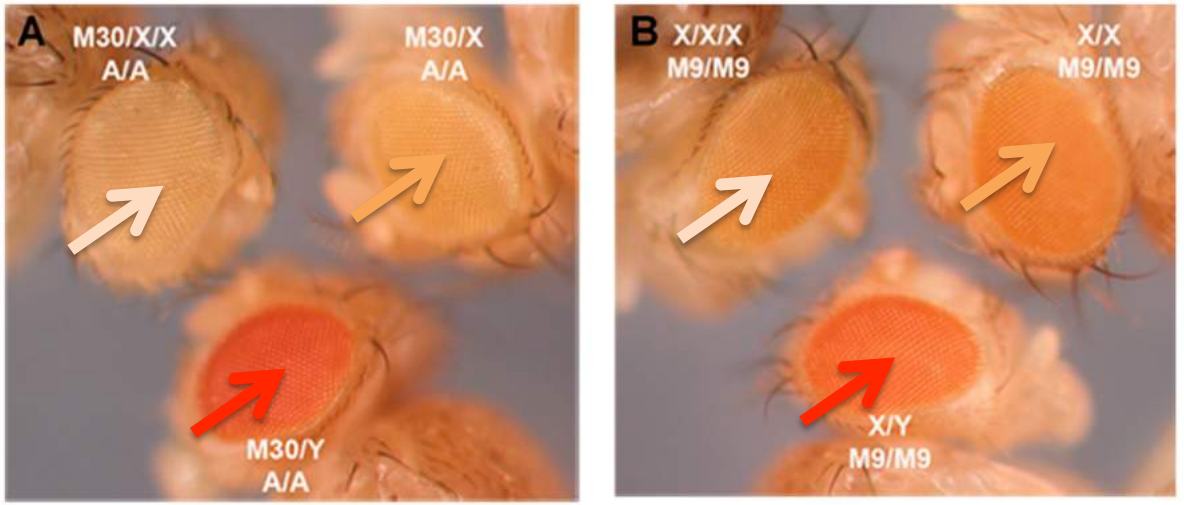
James A. Birchler

Division of Biological Sciences, University of Missouri, Columbia, Mo., USA

- osophila depends on **genome-wide**
- ather than just the X.

ye color comparisons of an
somal mini white reporter, M9

The MSL complex might have evolved to localize to the X chromosome so as to sequester the histone modifiers MOF and JIL1 kinase away from the autosomes to mute the inverse dosage effect that would otherwise occur there.



Normalization of global expression patterns should not be corrected to autosomal levels, given that one model to be distinguished suggests they are modulated, but rather to the DNA or via “per cell” methods.

Ohno's Hypothesis Refuted?

Mammalian X chromosome inactivation evolved as a dosage-compensation mechanism for dosage-sensitive genes on the X chromosome

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[a]Laboratoire de Biométrie et Biologie évolutive, Université Lyon 1, Centre National de la Recherche Scientifique, Villeurbanne F-69622 cedex, France; [b]Department of Ecology and Evolutionary Biology, Graduate School of Life Sciences, Tohoku University, Aoba-ku, Sendai 980-8578, Japan; [c]Smurfit Institute of Genetics, University of Dublin, Trinity College, Dublin 2, Ireland; and [d]Instituto Gulbenkian de Ciência, P-2780-156 Oeiras, Portugal

Edited* by Michael Freeling, University of California, Berkeley, CA, and approved February 3, 2012 (received for review October 13, 2011)

Expression reduction in mammalian X chromosome evolution refutes Ohno's hypothesis of dosage compensation

Fangqin Lin[a], Ke Xing[a], Jianzhi Zhang[b,1], and Xionglei He[a,1]

[a]Key Laboratory of Gene Engineering of Ministry of Education, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, 510275, China; and [b]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109

Edited by Masatoshi Nei, Pennsylvania State University, University Park, PA, and approved June 08, 2012 (received for review January 31, 2012)

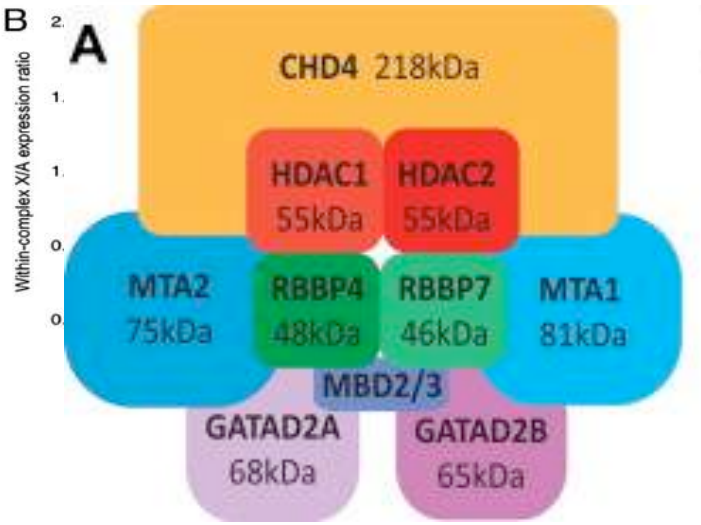
Analyses of RNA sequencing and proteomic data:

- Evidence for expression **halving** (i.e., no change in per-allele expression level) of X-linked genes during evolution, **with the exception of ~5% of genes**, that encode **members of large (≥ 7 member) protein complexes**.
- This class of genes is expected to be dosage-sensitive—expression of X-linked genes is similar to that of autosomal genes within the complex
- Many of these genes escape x inactivation (MORE NEXT WEEK)
- *(cf chromatin remodeler complexes, Crabtree et al, Cancer & Epigenetics lectures 2015)*

Ohno's Hypothesis Refuted?

Gene name*	Y homology	Max complex size†	Function annotation‡
PPP2R3B	Y homolog	3	Serine/threonine-protein phosphatase 2A regulatory subunit B
TBL1X	Y homolog	7	F-box-like protein involved in the recruitment of the **ubiquitin**/19S proteasome complex to nuclear receptor-regulated transcription units
RBBP7	-	16	Core histone-binding subunit, Component of several complexes which regulate chromatin metabolism
EIF1AX	Y homolog	3	Eukaryotic translation initiation factor 1A
SH3KBP1	-	7	SH3 domain-containing kinase-binding protein 1
USP9X	Y homolog	20	**Ubiquitin**-specific-processing protease FAF-X.
MED14	Y pseudogene	29	Mediator complex, a coactivator involved in the regulated transcription of nearly all RNA polymerase II-dependent genes
UBA1	Y homolog	40	**Ubiquitin**-activating enzyme E1
WAS	-	12	Effector protein for Rho-type GTPases. Regulates actin filament reorganization via its interaction with the Arp2/3 complex.
SMC1A	-	9	Central component of cohesin complex, required for the cohesion of sist chromatids after DNA replication.
RPS4	Y homolog	40	40S ribosomal protein S4, structural constituent of ribosome
MAGEE1	-	4	Hepatocellular carcinoma-associated protein 1
CHM	-	3	Rab proteins geranylgeranyltransferase component A 1
MORF4L2	-	27	Component of the NuA4 histone acetyltransferase complex
TRPC5	-	5	Transient receptor potential Ca2+ channel
PLS3	-	3	Actin-bundling protein
CUL4B	-	7	Core component of multiple cullin-RING-based E3 **ubiquitin**-protein ligase complexes
HCFC1	-	13	Host cell factor 1

*The best candidates (members of large complexes and with a Y homolog) are shown in bold.
†Most of the genes are involved in several complexes in the list from HPRD (see *Material and Methods*), only the size of the largest complex is indicated here.
‡From NextProt, the new database on human proteins developed by Swissprot (www.nextprot.org). Ubiquitin related genes are in bold.



NuRD complex

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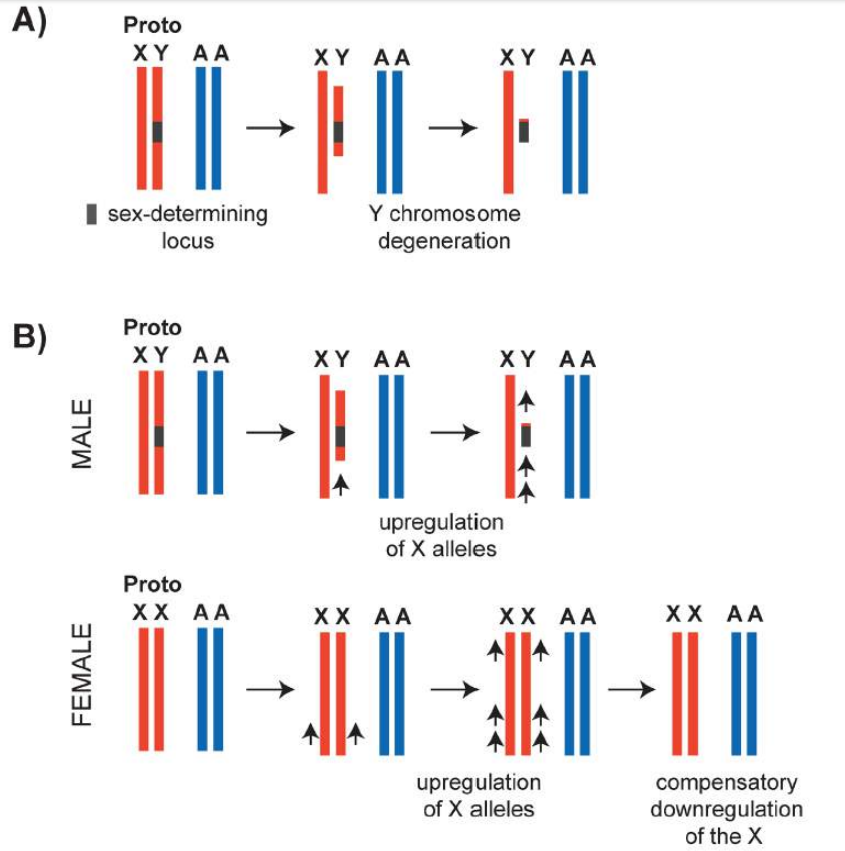
Testing Ohno's Hypothesis



Ohno hypothesized that due to Y chromosome degeneration, the remaining alleles on the X chromosome became potentially haploinsufficient. To compensate for this, the alleles on the X chromosome were transcriptionally upregulated.

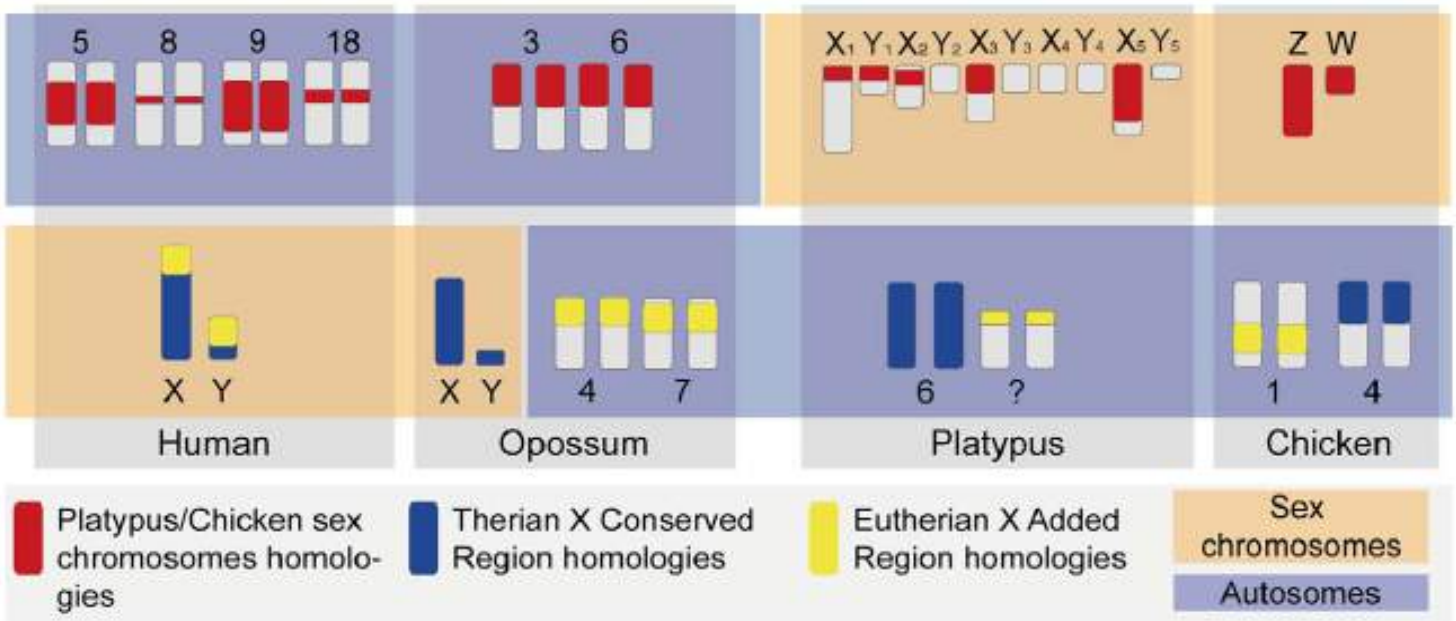
It was also hypothesized that the upregulation of X alleles were not limited to males, and also occurred in females. This caused a potential overtranscription of the X-linked genes in females, therefore female-specific downregulation occurred.

To test if X-upregulation really occurred, one should compare ancestral (autosomal) and present level of X-linked gene expression.

Since this is not possible, assuming that the function and expression of 1:1 orthologs are conserved, one can compare expression of 1:1 orthologs that are differentially located on X or autosomes.

Testing Ohno's Hypothesis

- Evaluate X up-regulation by comparing expression of X-linked genes in mammals to that of "ancestral" genes in chicken?
- Found no/very little up-regulation in mammals.
- Only marsupial X-linked genes are up-regulated compared to ancestral genes
- In placental mammals, genes resident on autosomal (nonsex) chromosomes that interact with X-linked genes have instead become downregulated.
- Similarly in C. elegans, no evidence for evolutionary up-regulation by measuring ancestral gene expression in another species



Ju nsation.

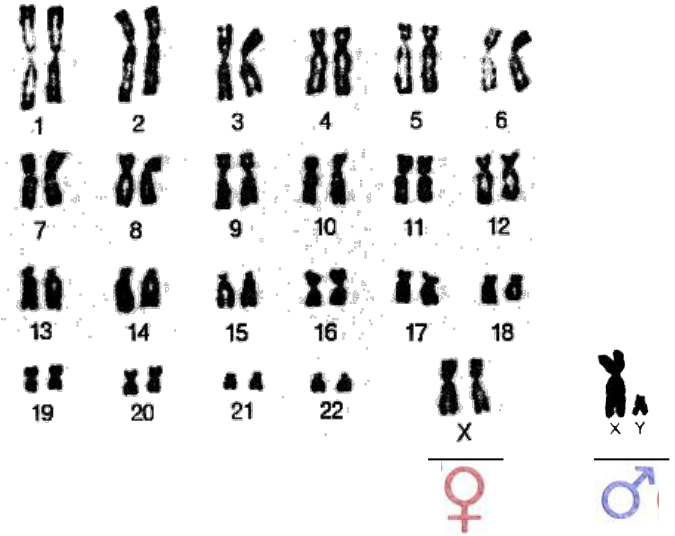
E. Heard, Lecture 5th, 2018

PLoS Biol. 10, e1001328 (2012).

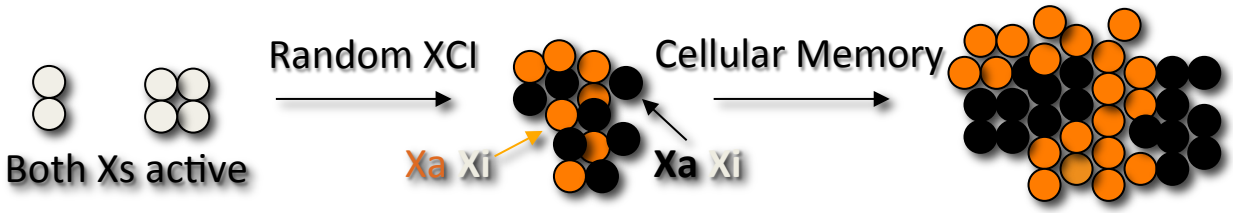
Does X-chromosome dosage matter during development?

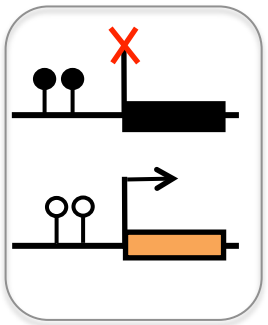
Does X-chromosome dosage matter during development?

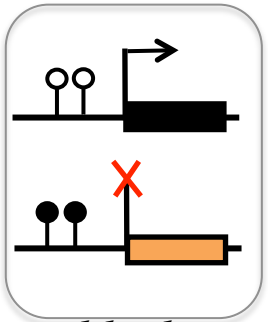
Silencing of almost all genes on one of the two X chromosomes
during early female development in mammals to achieve dosage compensation



X: 1300 genes
Y: ~100 genes
Sry (testis determinant factor)
Eif2s3y (spermatogenesis)







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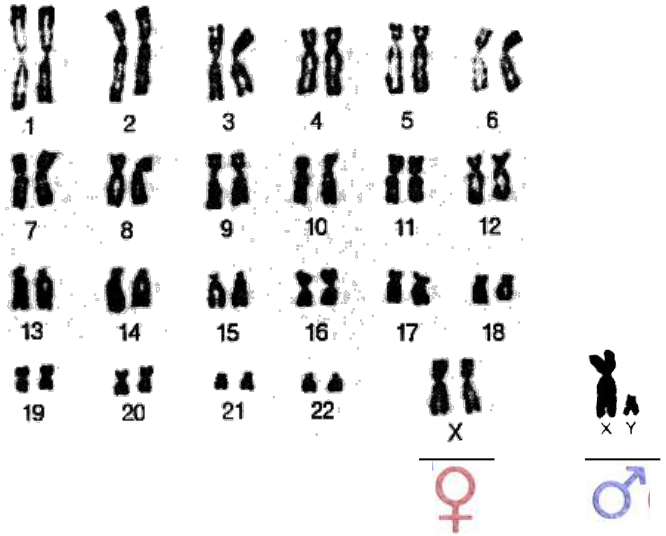
Does X-chromosome dosage matter during development?



X: 1300 genes
Y: ~100 genes
Sry (testis determinant factor)
Eif2s3y (spermatogenesis)

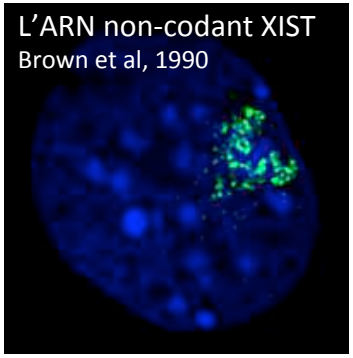
Also in human embryos:
Lethality in embryos with two active X chromosomes

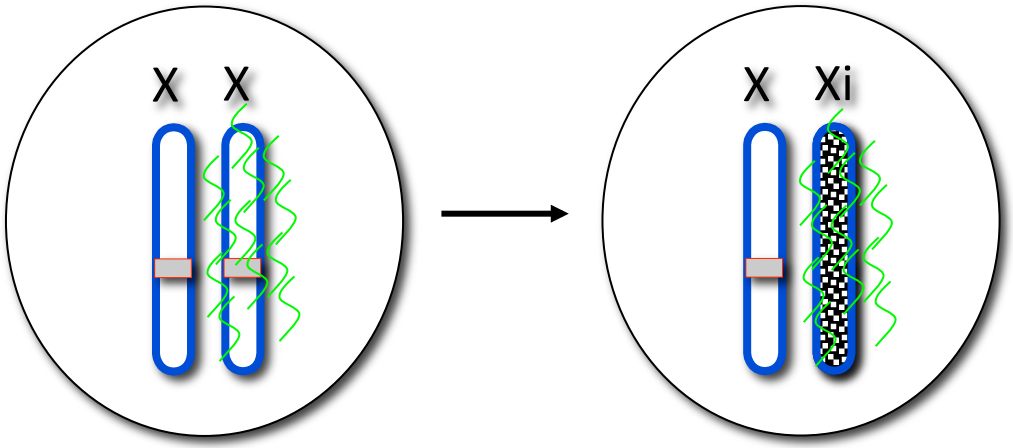
Ea

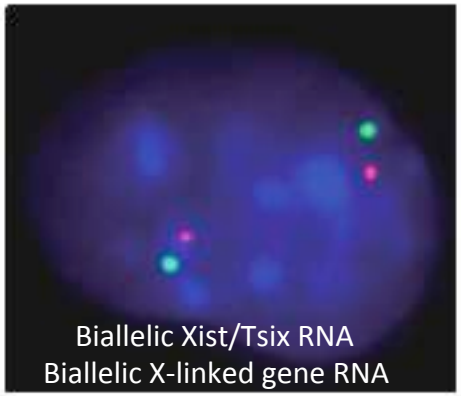
Initiating and maintaining X-Chromosome Inactivation

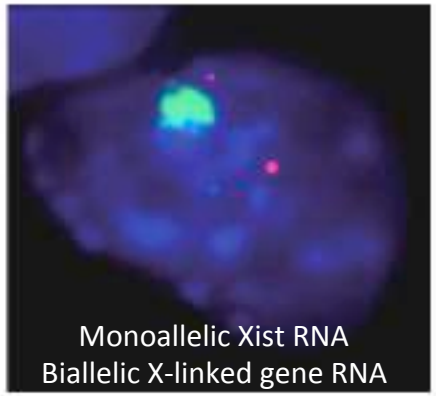
Initiation :
A non-coding RNA silences genes

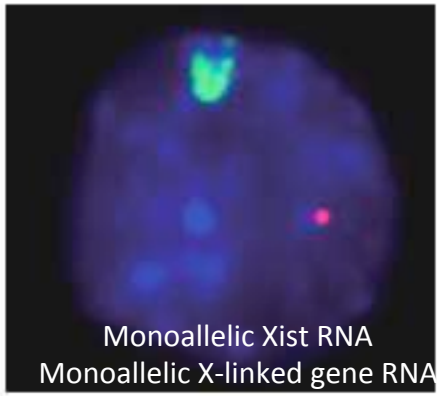
Maintenance:
Chromatin marks, Asynchronous replication, Nuclear organisation











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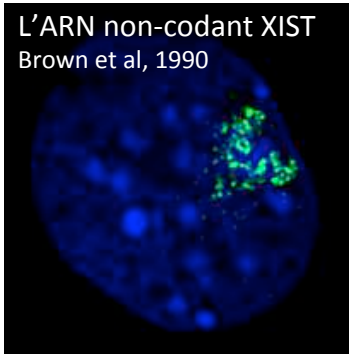
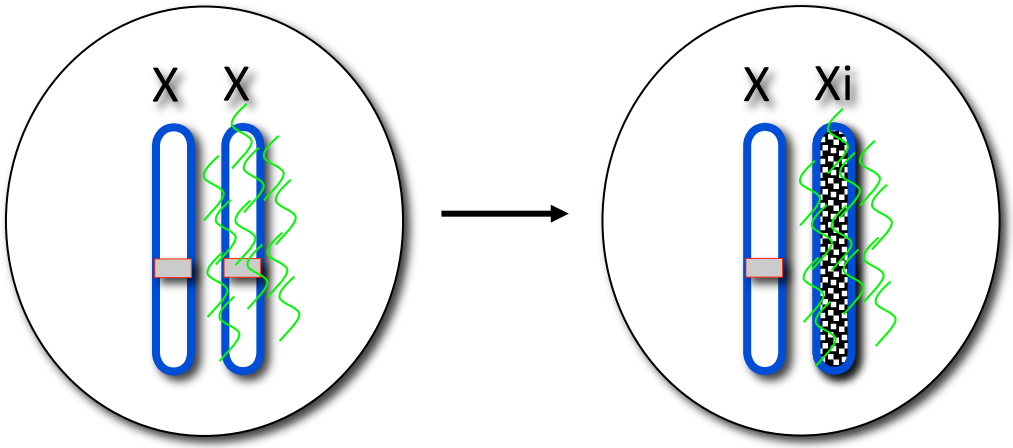
Initiating and maintaining X-Chromosome Inactivation

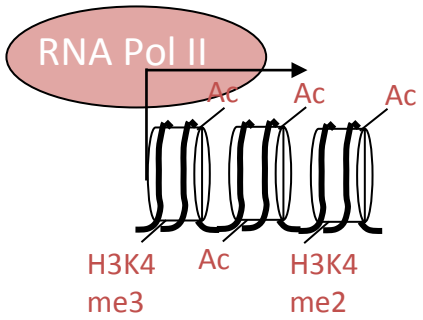
Initiation :
A non-coding RNA silences genes

Maintenance:
Chromatin marks, Asynchronous replication, Nuclear organisation

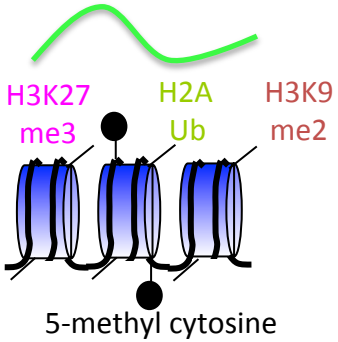




Active X chromosome



Inactive X chromosome



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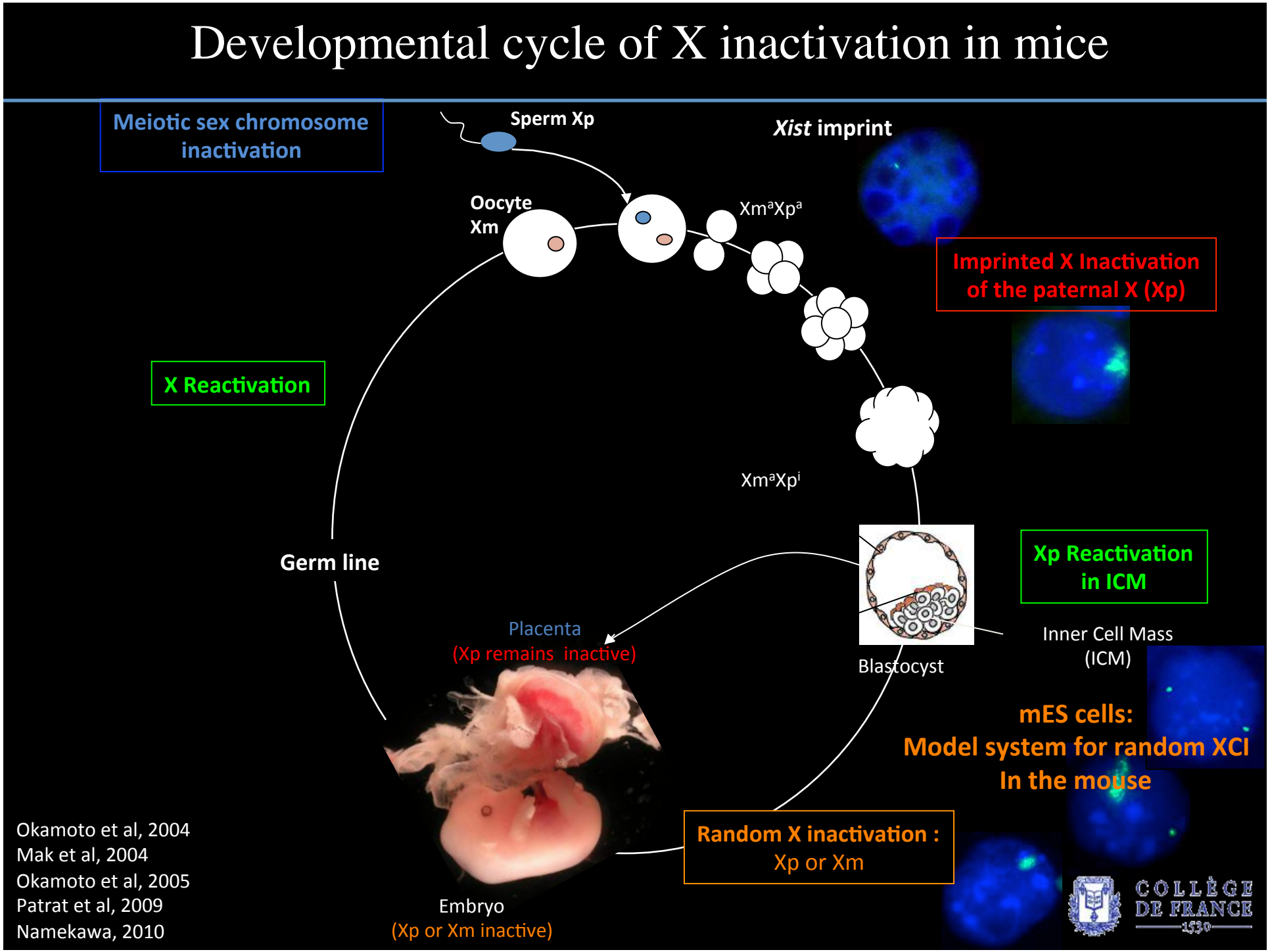
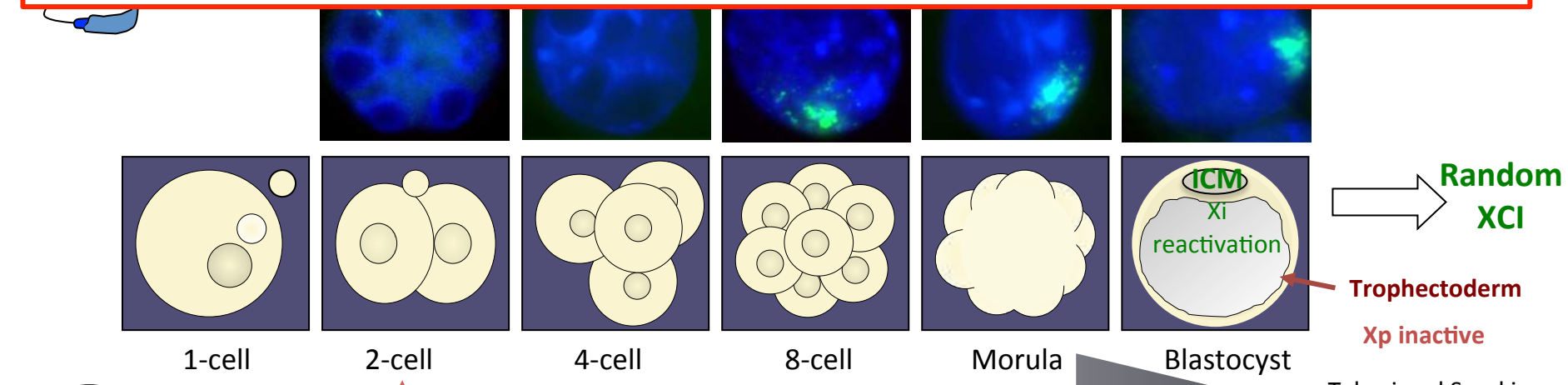
Developmental cycle of X inactivation in mice



Imprinted X inactivation during pre-implantation mouse development

Genes on the paternal and maternal X chromosomes are active at 1-cell stage: NO "pre-inactivation" of the paternal X

(Okamoto et al, 2005; Patrat et al, 2009; Borensztein et al, 2017)



Kay et al, 1994
Huyhn and Lee, 2003
Okamoto et al, 2004
Mak et al, 2004
Patrat et al, 2009

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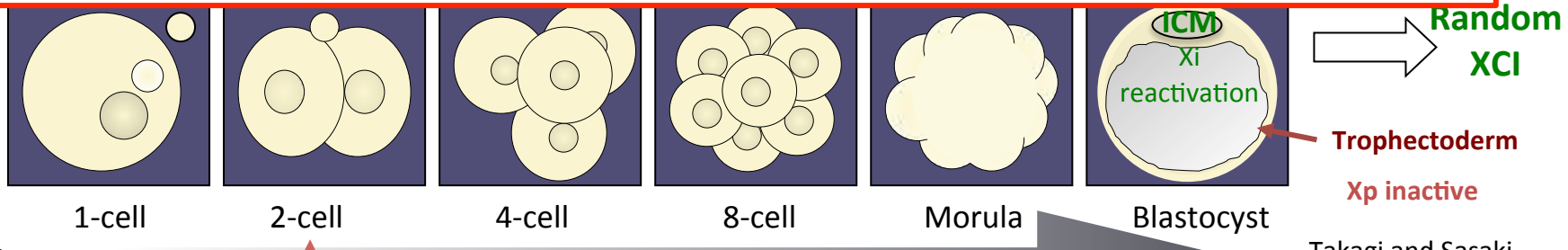
Imprinted X inactivation during pre-implantation mouse development

Cascade of gene silencing & epigenetic marking (chromatin changes)
The Xp seems to be silent by blastocyst stage (few genes analysed)
Then reactivates in the inner cell mass (stays silent in trophectoderm)
Then random XCI occurs in the embryo-proper…

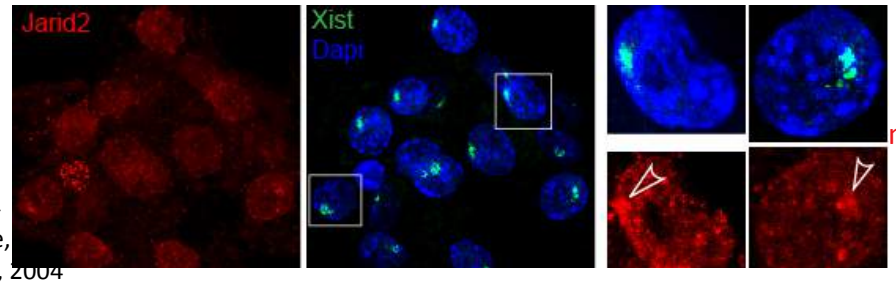


Takagi and Sasaki, 1975

Imprinted inactivation of X^P

Paternal *Xist* on
Xp active

Xist RNA coating of Xp
RNA Pol II exclusion



n

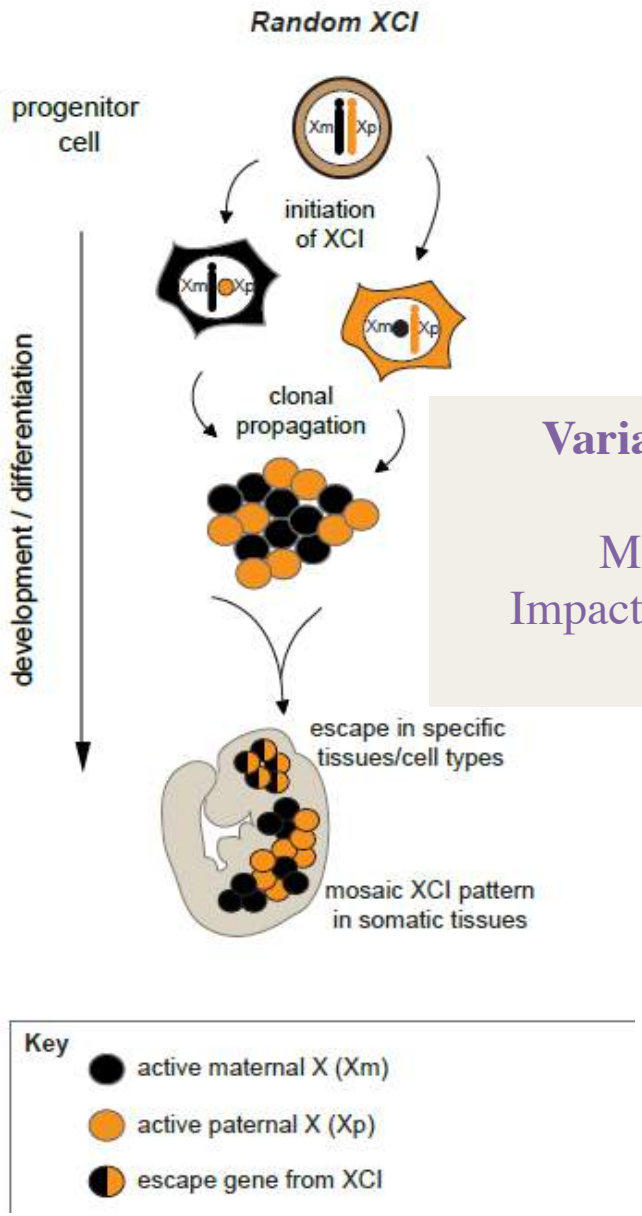
Polycomb complex recruitment
H3K27me3, H2Aub
MacroH2A association

H3K9 dimethyl

Kay et al, 1994
Huyhn and Lee,
Okamoto et al, 2004
Mak et al, 2004
Patrat et al, 2009

COLLÈGE DE FRANCE 1530

Tissue-specific and variable escape from X inactivation: Biallelic expression in some cells of female embryos



Variable X-linked gene dosage throughout life

Mechanisms (COURS III)

Impact on development and disease (COURS IV, V)

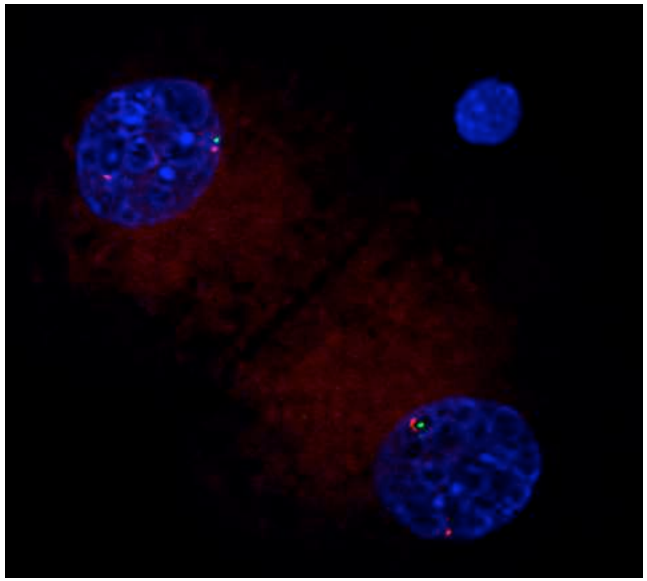
Defining X-chromosome wide kinetics of gene silencing in embryos

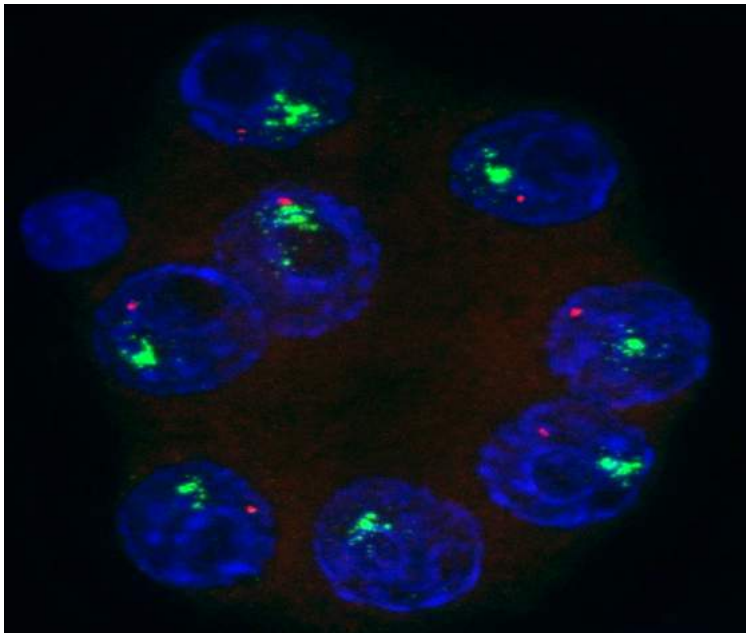
RNA FISH: single cell, gene by gene analysis

Nascent transcripts

Not allelic but Xist RNA identifies paternal X

Chromosome-wide analysis almost impossible

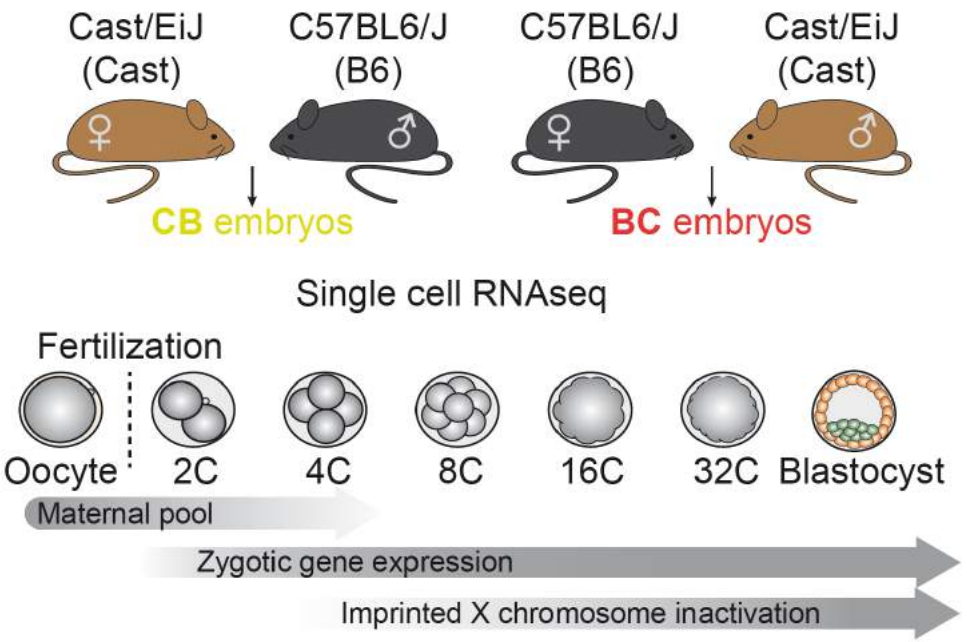


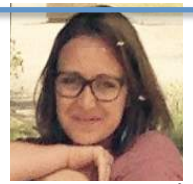


Okamoto et al, 2004
Okamoto et al, 2005
Patrat et al, 2009

Defining X-chromosome wide kinetics of gene silencing in embryos

Single cell RNA-seq analysis
(collaboration with A. Surani)

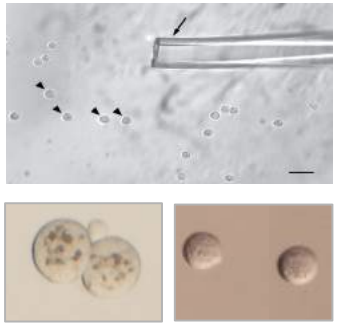




M. Borensztein

Allele-specific genome-wide analysis

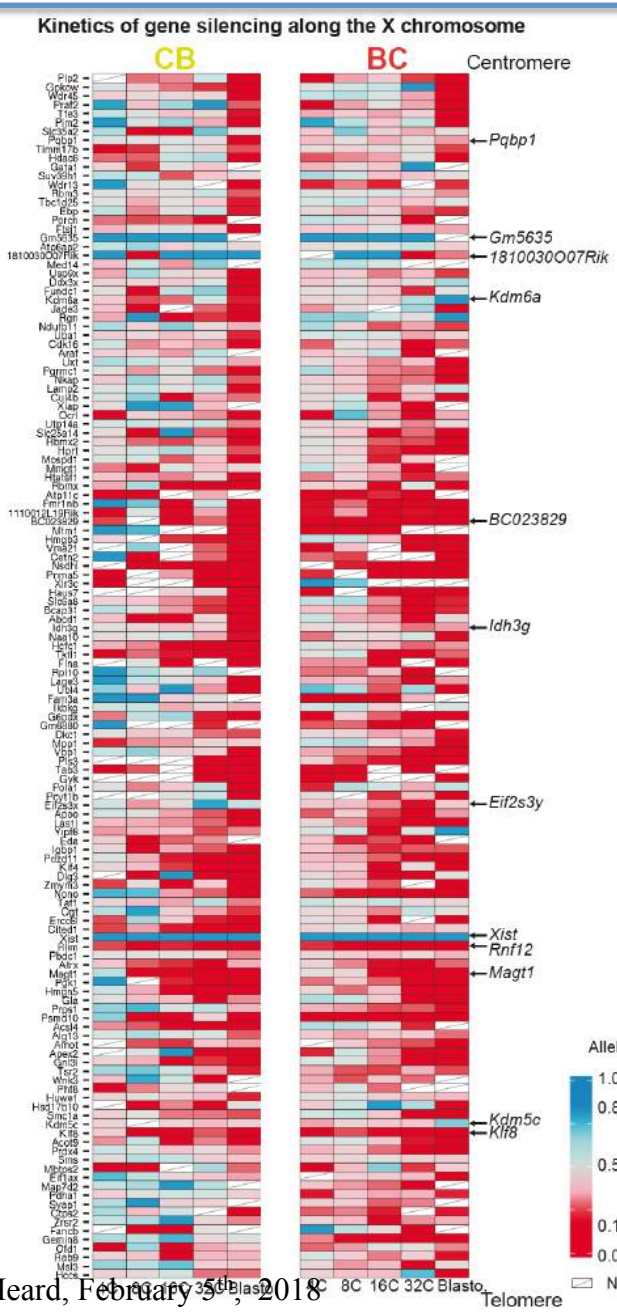
Inter-species crosses
=> F1 embryos
19 Millions SNPs; 1 SNP/100bp
1 SNP/650bp for the X
(Frazer *et al*, Nature, 2007)



Tang, F. *et al.* RNA-Seq analysis to capture the transcriptome landscape of a single cell. *Nat. Protoc.* **5,** 516–35 (2010).

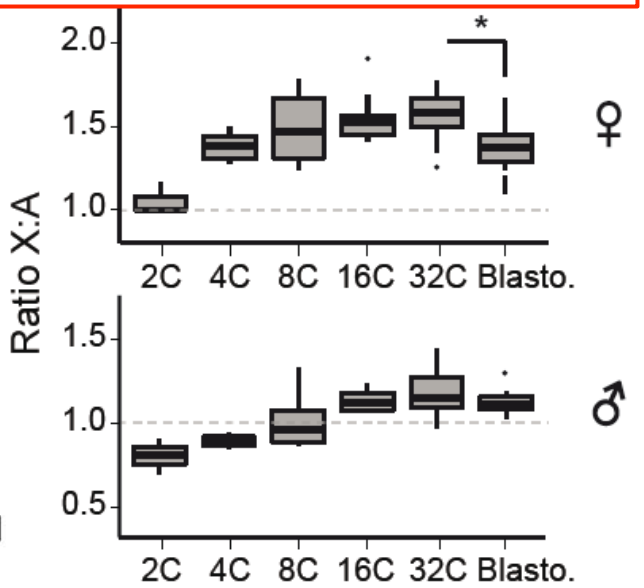
Deng, Q., Ramskold, D., Reinius, B. & Sandberg, R. Single-Cell RNA-Seq Reveals Dynamic, Random Monoallelic Gene Expression in Mammalian Cells. *Science* **343,** 193–196 (2014).

Allele-specific analysis of the timing and extent of XCI



- mRNA seq and nascent RNA FISH show quite similar kinetics
- X-linked gene silencing is triggered as early as 4-cell stage
- Most Xp genes are silenced by the 64-cell stage
- Different genes display very different kinetics
- The earliest silenced regions correspond to Xist RNA's predicted "entry sites"
- Strain-specific differences observed for XCI kinetics of some genes
- X:A dosage compensation (DC) in males by 16-cell stage
- In females DC not complete even by blastocyst stage

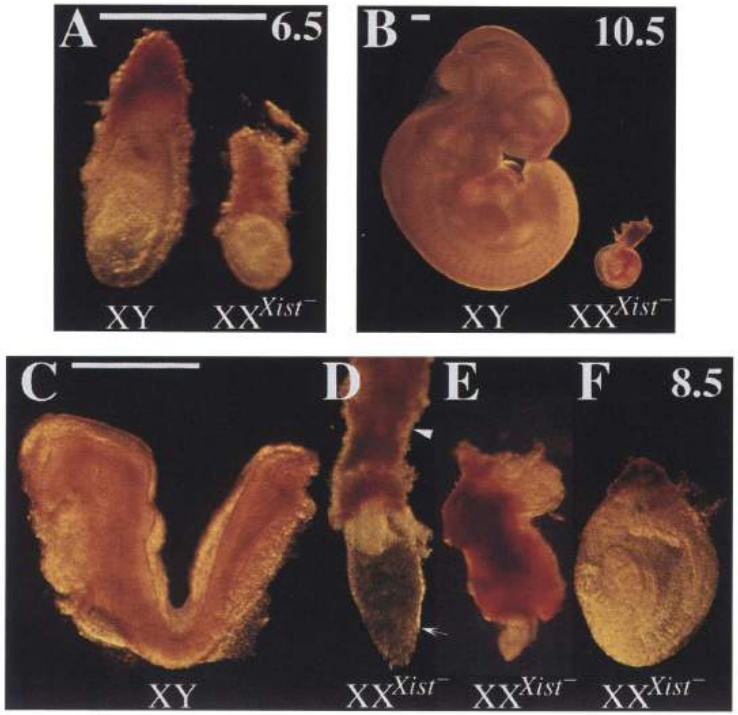
Role for Xist RNA in **initiation** of imprinted XCI?



M. Borensztein et al, NSMB, 2017

Female embryos lacking paternal *Xist* display early post-implantation embryonic lethality

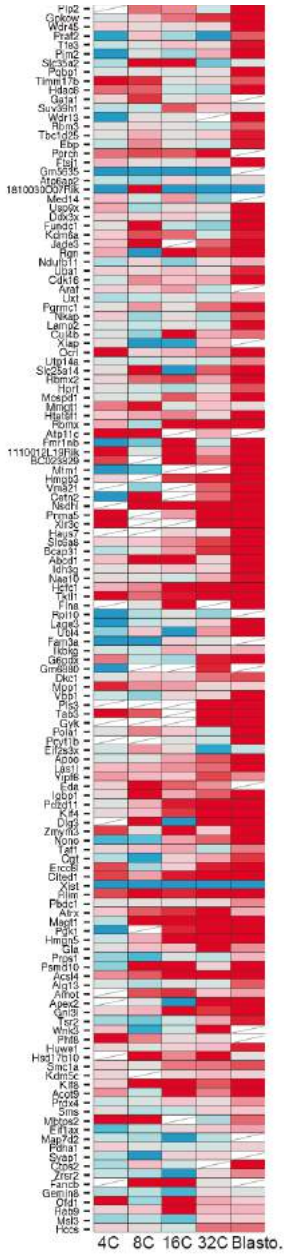
Severely reduced growth between E6.5 and E10.5 in *Xist* KO mutants

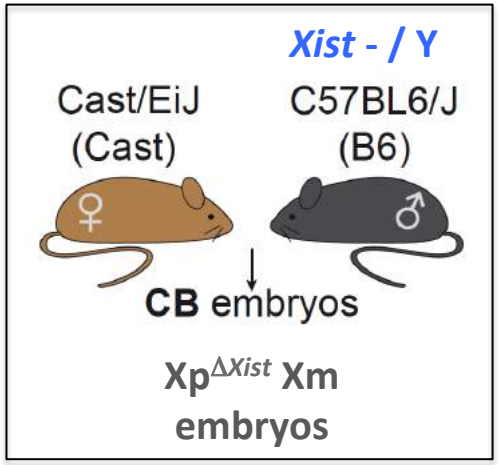


Lethality due to inappropriate gene expression from the normally inactive paternal X chromosome in extraembryonic tissues which supply embryo with nutrients and growth factors?

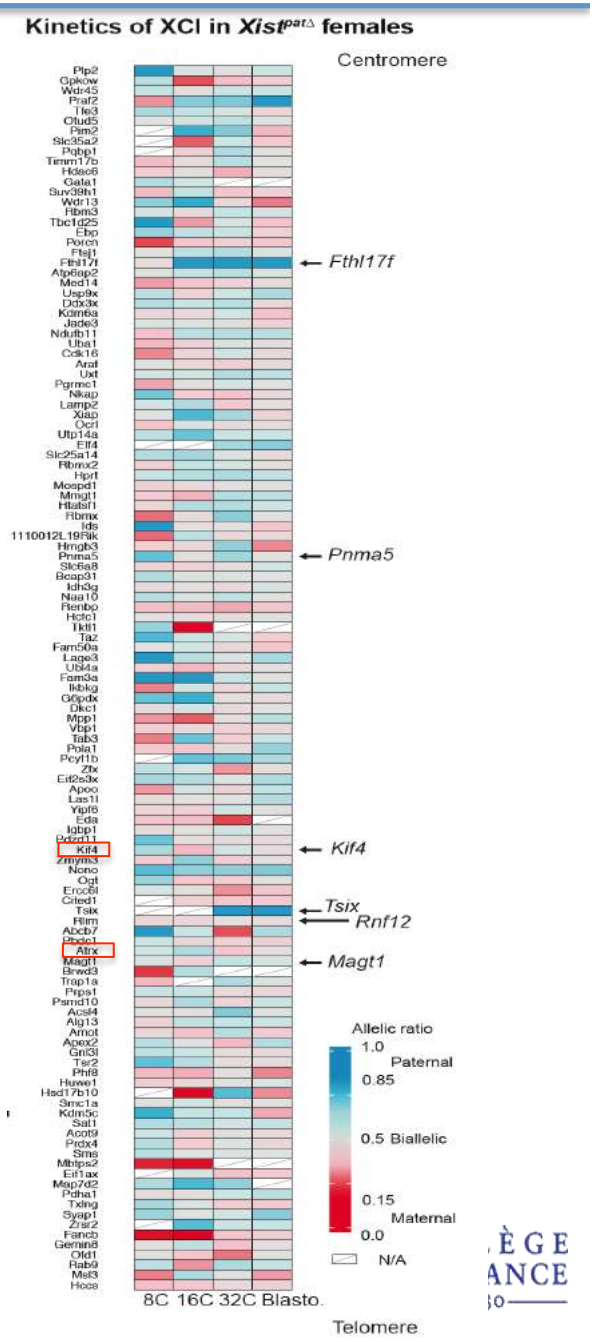
Marahrens, Y., Panning, B., Dausman, J., Strauss, W. & Jaenisch, R. Xist-deficient mice are defective in dosage compensation but not spermatogenesis. *Genes Dev.* **11,** 156–166 (1997).

Lack of paternal *Xist* leads to absence of initiation of XCI





- No paternal X chromosome gene silencing
- No maternal *Xist* up-regulation
- No Dosage Compensation

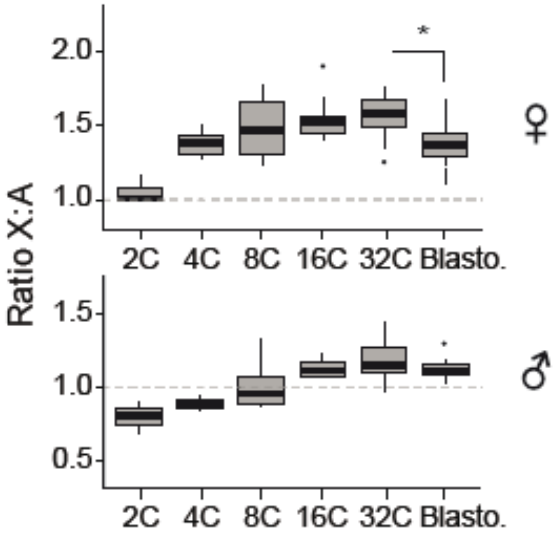


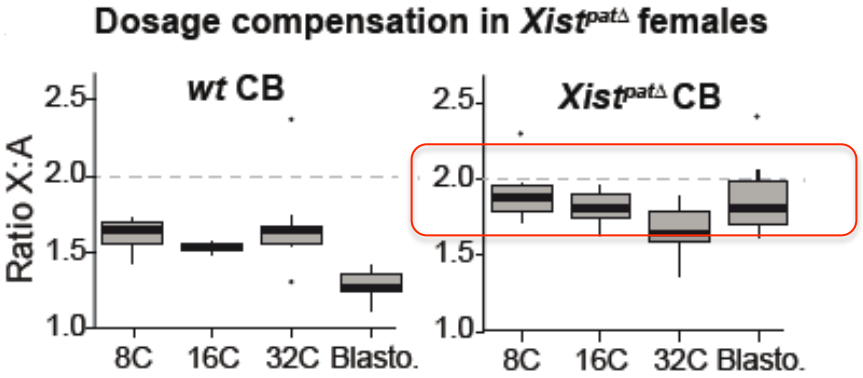
E. Heard, Fel

M. Borensztein et al, NSMB 2017

Absence of initiation of XCI leads to gene mis-regulation in early pre-implantation stages

Absence of dosage compensation



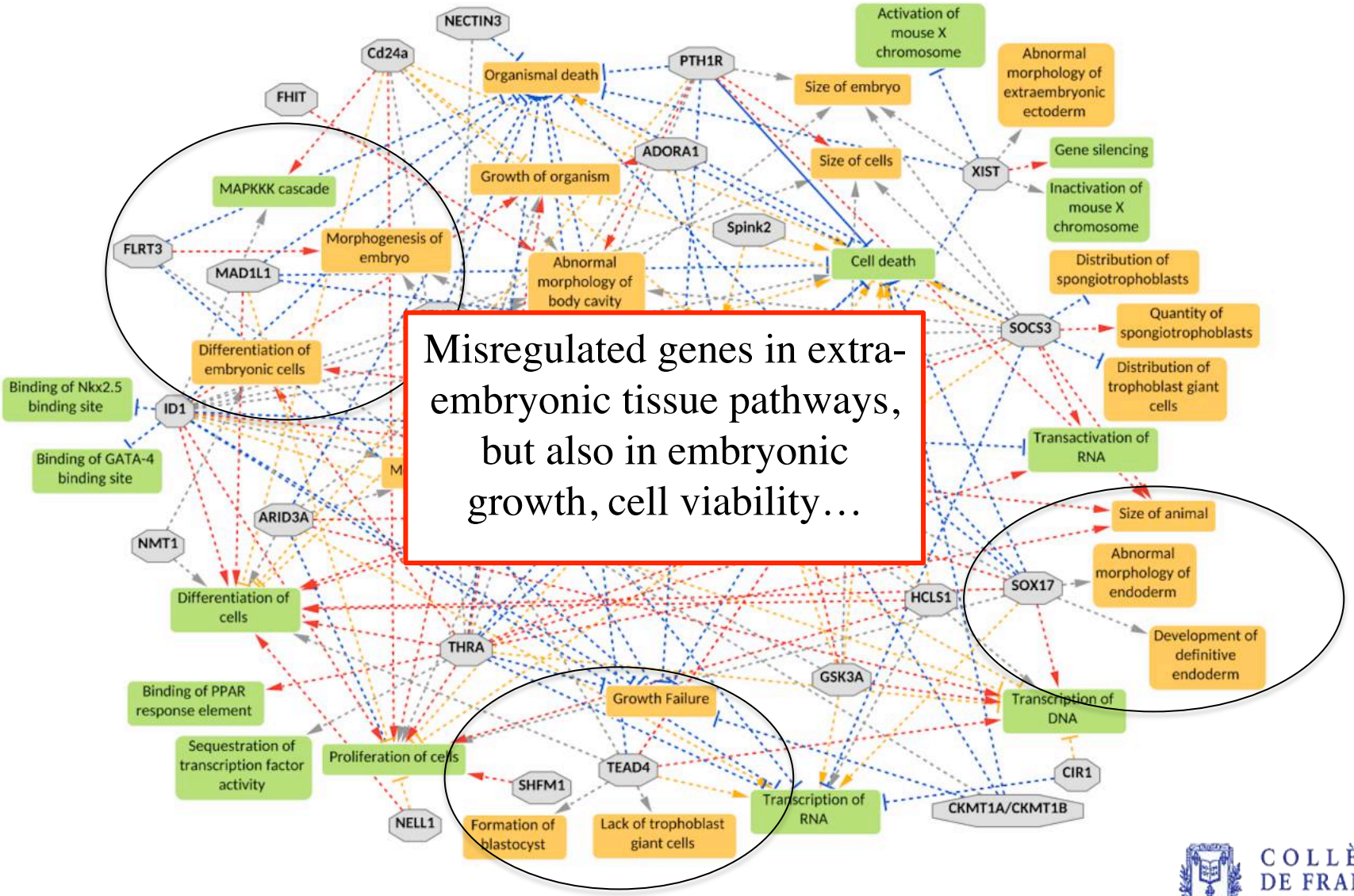


Genome-wide analysis:

8-cell and 32-cell stages: already see significant mis-regulation of both X-linked and autosomal genes

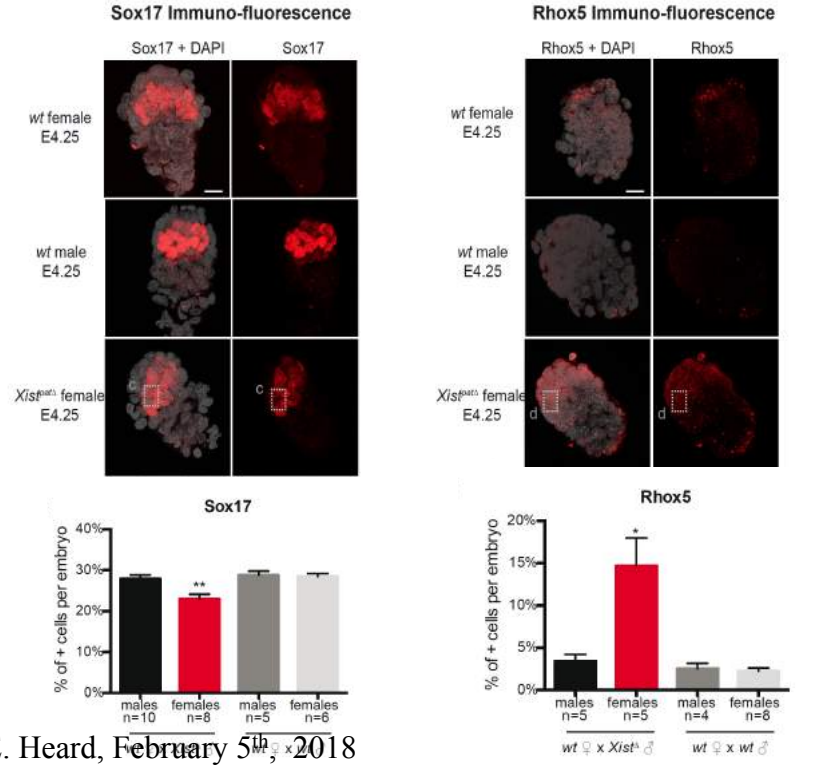
Developmental stages	Genes from the X chromosome				Genes from the autosomes			
	Upregulated		Downregulated		Upregulated		Downregulated	
	%	n	%	n	%	n	%	n
8-cell	5.4%	21	1.9%	19	94.6%	372	98.1%	972
16-cell	11.6%	10	1.0%	1	88.4%	76	99.0%	99
32-cell	4.2%	23	2.0%	11	95.8%	521	98.0%	543
Blastocyst	40.4%	19	4.4%	2	59.6%	28	95.6%	43

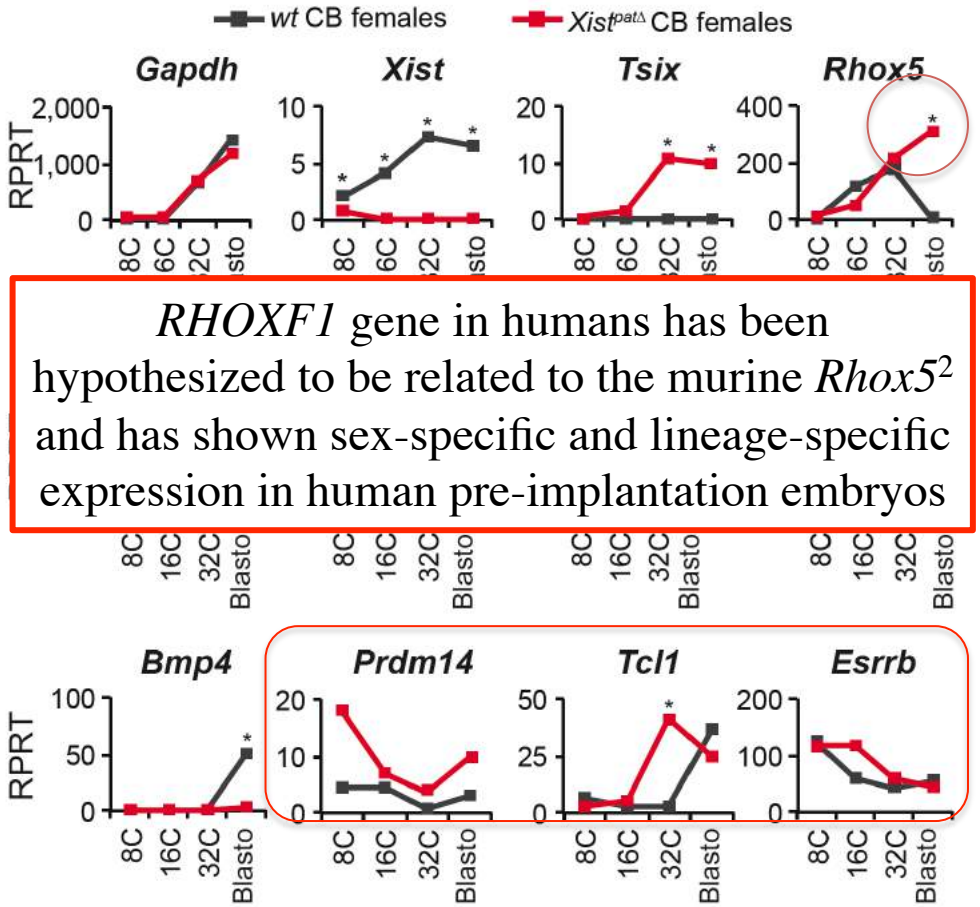
Which genes and pathways are disrupted in *Xist* mutant XX embryos? What are the immediate consequences of an absence of dosage compensation?



Aberrant patterns of gene expression in *Xist* mutant XX embryos

- **Aberrant decrease in extra-embryonic factor gene expression eg *Sox17***
- **Aberrant overexpression of pluripotency genes including *Prdm14, Esrrb* and *Tcl1***
  As in XX vs XO/XY ESCs - Schulz et al, 2014
- **Massive overexpression of *Rhox5:* absence of Xist RNA leads to *Rhox5* up-regulation**
  - Rhox5 (member of X-linked Rhox cluster) is normally imprinted and silent on maternal X and expressed (partially repressed) from paternal X
  - Overexpression of Rhox5/Pem1 impedes ESC differentiation (Cinelli et al, 2008)





RHOXF1 gene in humans has been hypothesized to be related to the murine *Rhox5*[2] and has shown sex-specific and lineage-specific expression in human pre-implantation embryos

Developmental catastrophe due to absence of X inactivation and dosage compensation in the mouse

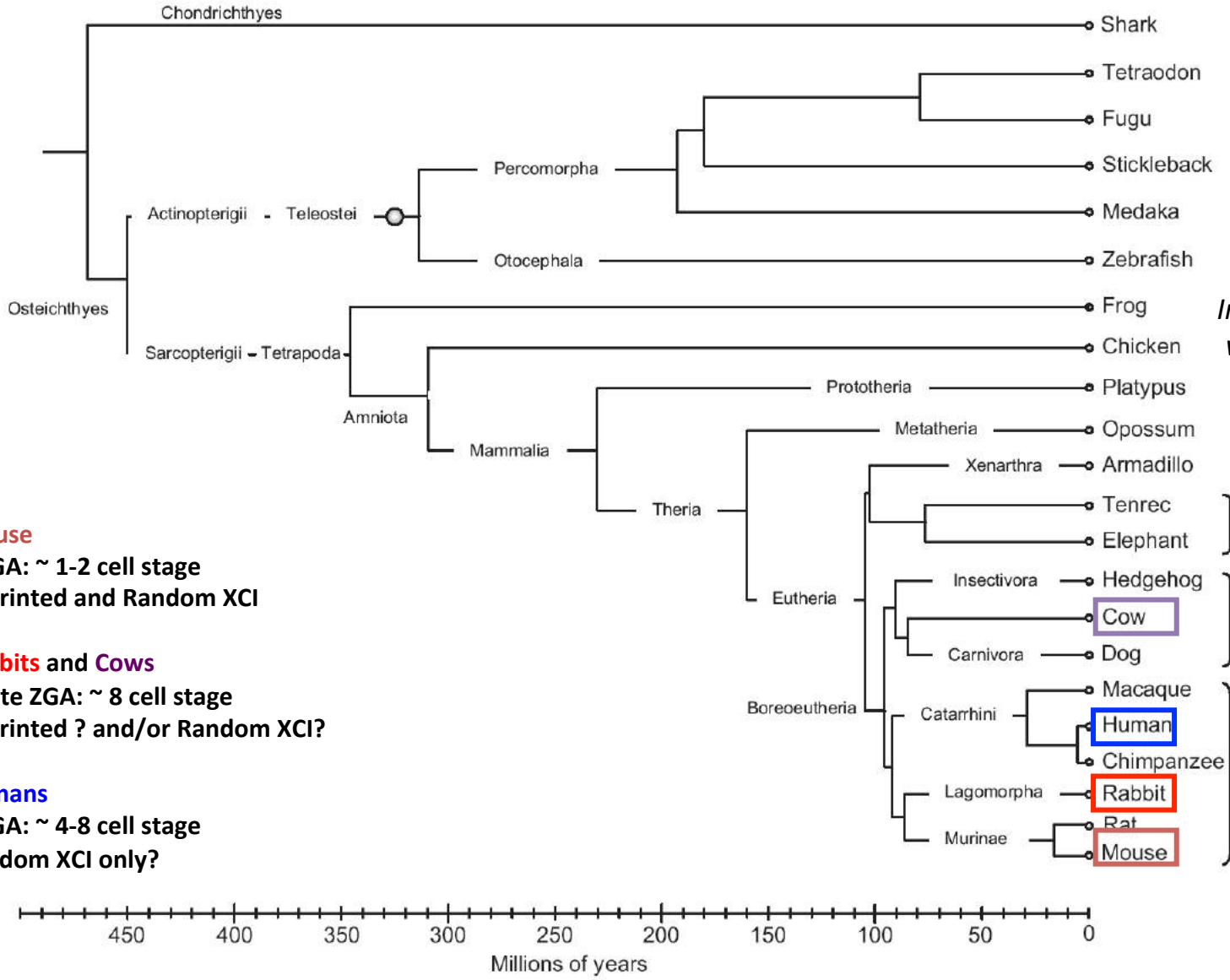
Insights into the molecular nature and degree of gene mis-regulation in the absence of X inactivation and dosage compensation in a developmental context in mammals:

- **aberrantly down regulated extra-embryonic factors (preventing proper trophectoderm and primitive endoderm formation?)**
- **aberrantly up-regulated expression of pluripotency factors (preventing epiblast formation?)**
- ***Rhox5* is massively up-regulated and may impede epiblast (embryo) formation**

Questions still open:

- Specific X-linked genes responsible for early defects that result in subsequent lethality? (eg *Rhox5* – others?)
- Are these the key X-linked targets of early X inactivation?
- Are different genes dosage sensitive at later stages of development?

How conserved are X-inactivation mechanisms during development in other mammals?



Mouse
- **ZGA: ~ 1-2 cell stage**

Imprinted and Random XCI

Rabbits and Cows
- **Late ZGA: ~ 8 cell stage**

Imprinted ? and/or Random XCI?

Humans
- **ZGA: ~ 4-8 cell stage**

Random XCI only?

In collaboration with C. Patrat, V. Duranthon

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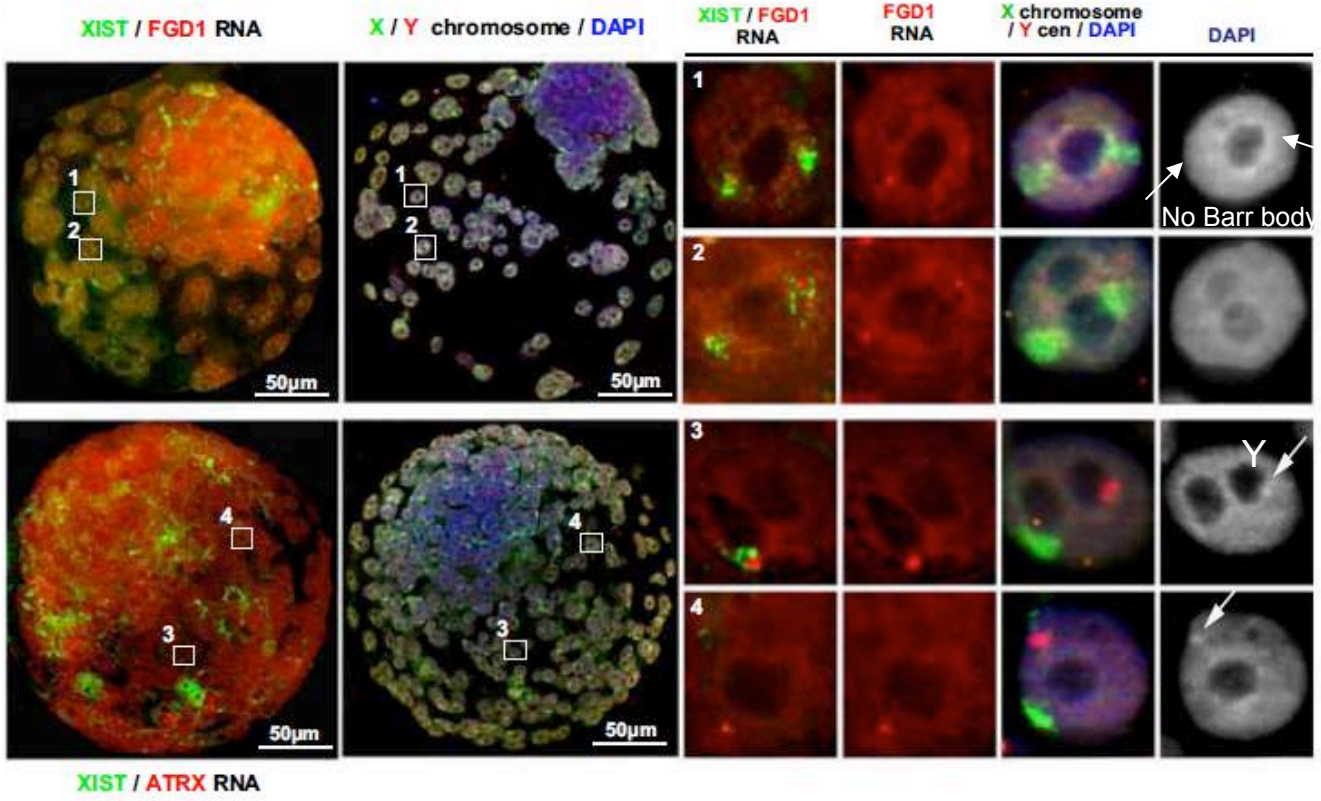
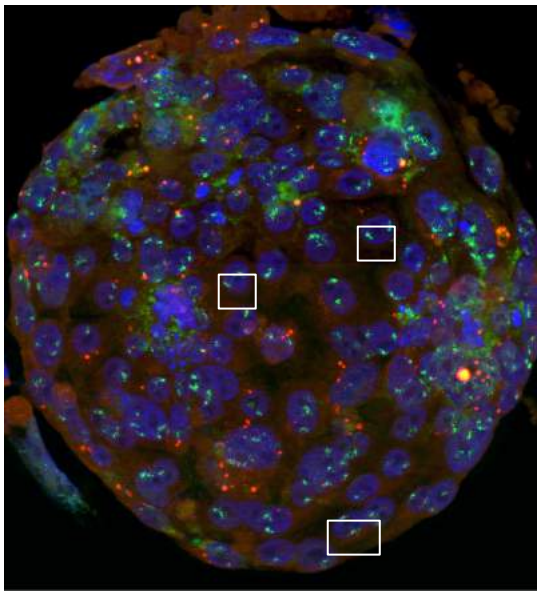
Human pre-implantation embryos: Constitutive XIST RNA up-regulation but no X inactivation?

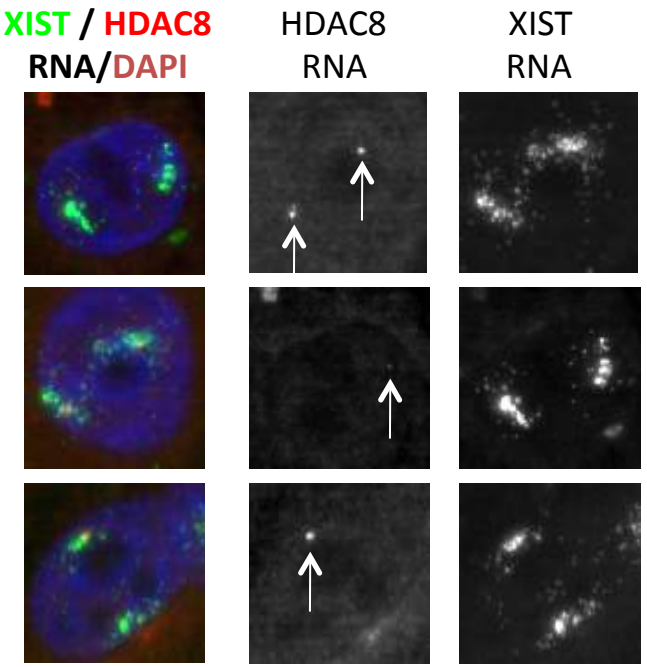


- No imprinted XIST regulation in human or rabbit embryos
- XIST up-regulation from both Xs then resolution to one X…
- In humans, XIST RNA accumulates in male and female embryos in TE and ICM cells but genes are biallelically expressed and no signs of H3K27me3 or a Barr body up to day 7

Okamoto et al, 2011, *Nature* 472 : 370-374

Human pre-implantation embryos: Constitutive XIST RNA up-regulation but late X inactivation?





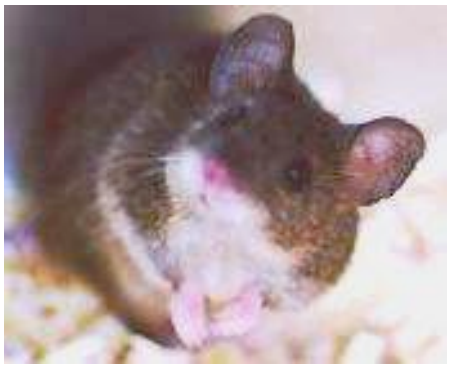
First signs of X inactivation after day 7?

- Gene silencing based on nascent RNA FISH
- H3K27me3 enrichment on Xi at E7.0 in TE cells
  (Teklenburg et al, 2012)

NO/LITTLE DOSAGE COMPENSATION IN EARLY HUMAN EMBRYOS AT THE BLASTOCYST STAGE…?!

COLLÈGE DE FRANCE 1530

Timing and modes of early development in mice, rabbits and humans: morphologically similar but temporally and molecularly diverse?



- No *XIST* imprinting
- Biallelic *XIST* expression, dispersed coating
- X-chromosome "dampening"= dosage comp. via partial XIST RNA silencing?
- Late onset of monoallelic X-gene silencing
- No *TSIX* across *XIST* in humans
- No "reactivation" in the ICM

Okamoto, Patrat et al, *Nature* 2011

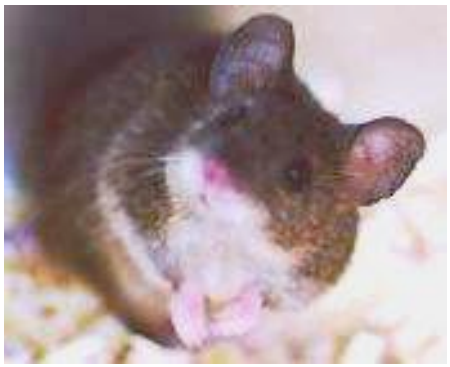
- *Xist* imprinting
- Monoallelic *Xist* expression
- XCI upon XIST RNA coating
- Xi "reactivation" in ICM
- XCI upon *Xist* up-regulation
- Murine *Xist* and XCI regulation

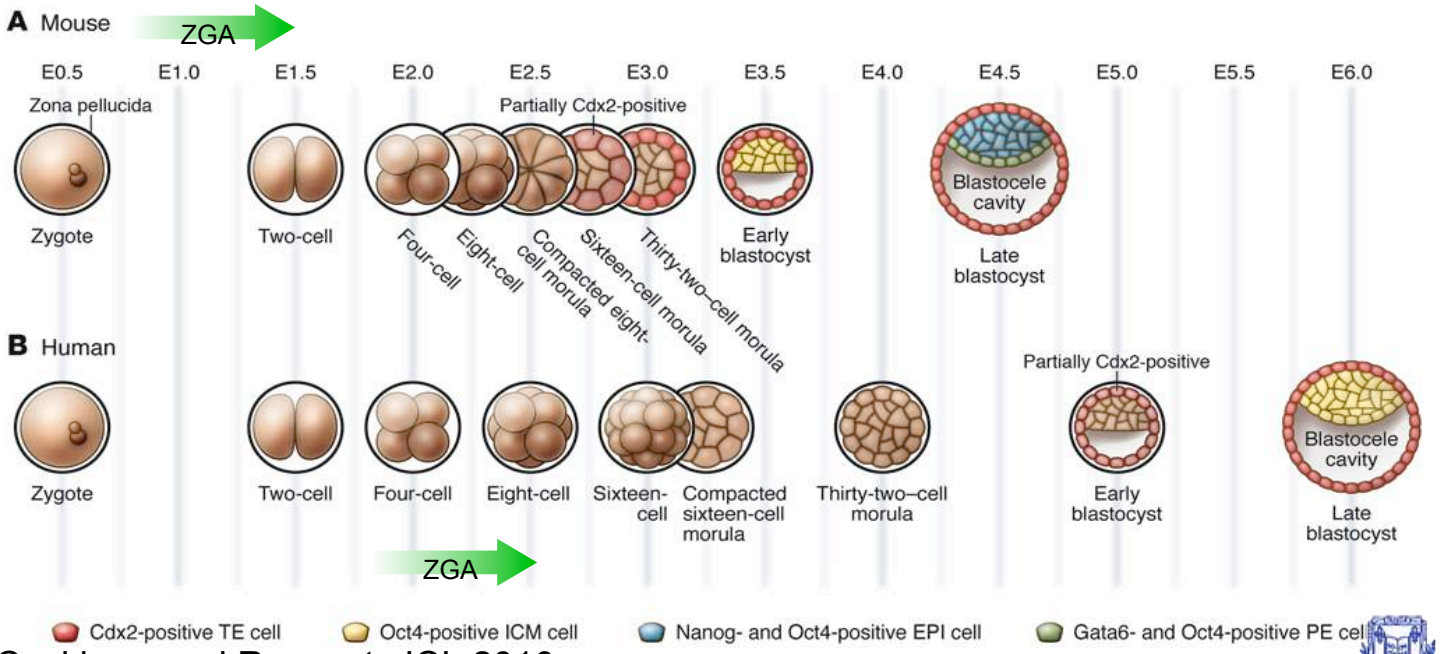
are tightly linked to pluripotency factors

Okamoto et al, *Science* 2004,
Okamoto et al, *Nature* 2005

Ikuhiro Okamoto

Timing and modes of early development in mice, rabbits and humans: morphologically similar but temporally and molecularly diverse?





Adapted from Cockburn and Rossant, JCI, 2010

E. Heard, February 5th, 2018

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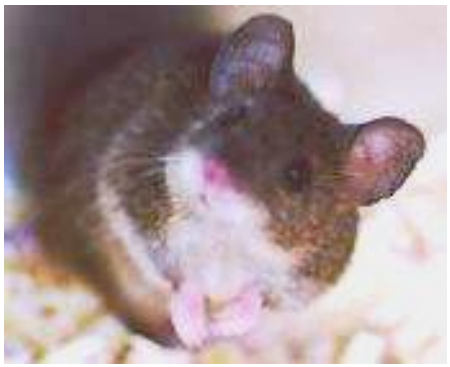
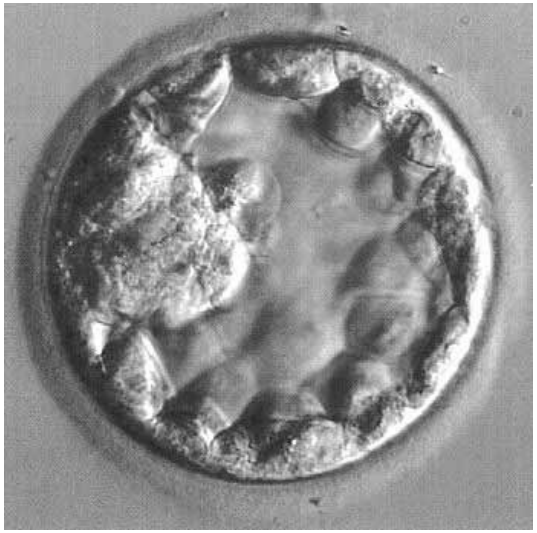
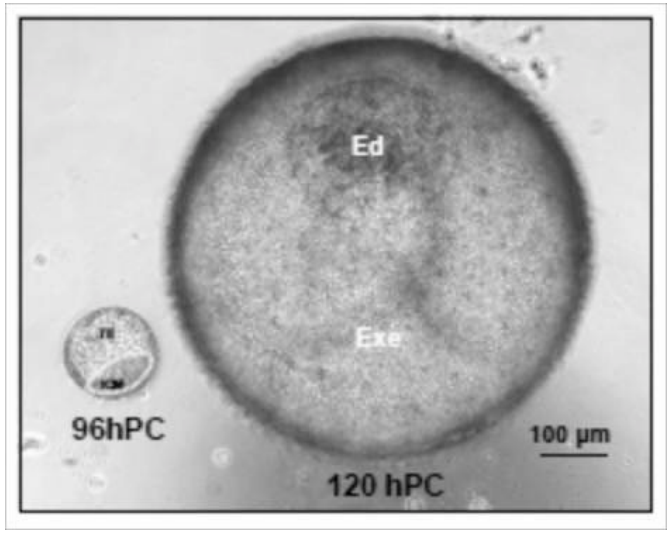
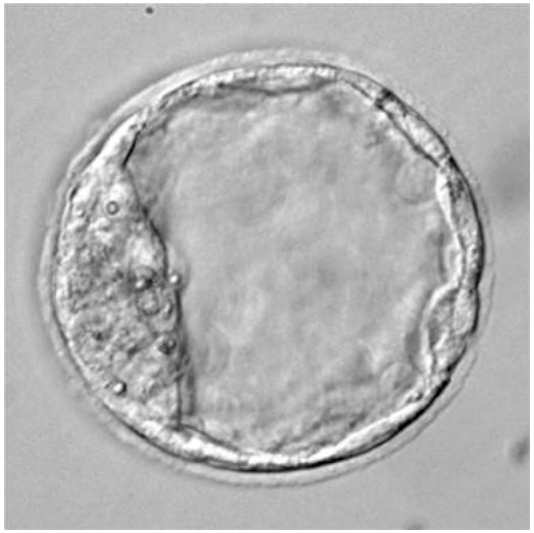
Different modes of initiation and timing of X inactivation in mice, rabbits and humans



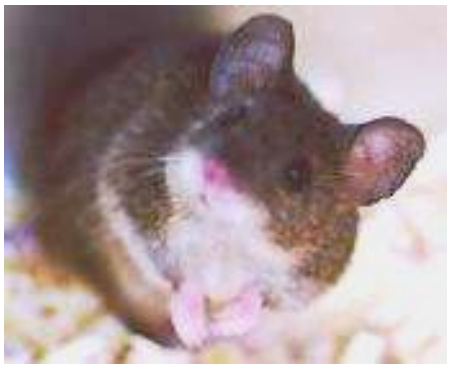






Different modes of initiation and timing of X inactivation in mice, rabbits and humans





During early female development
two *active* X chromosomes are present transiently?

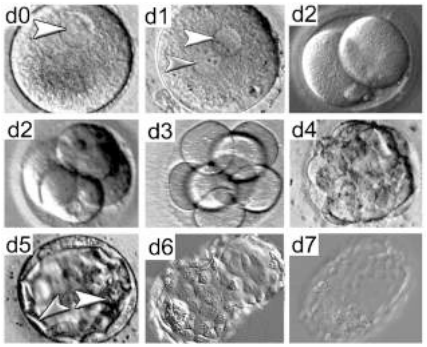
- for 1-2 cell cycles in mice
- for >2 cell cycles in rabbits
- for > 7 cell cycles in humans

⇒ **Double dose of X-linked gene products**....?

Yet in the mouse, no XCI leads to massive perturbations even after 3-4 cell cycles (by 32-cell stage)

How is a double dose tolerated – in particular during early human development?

Different modes of initiation and timing of X inactivation in mice, rabbits and humans



Accelerated growth of human, mouse, bovine **male** blastocysts

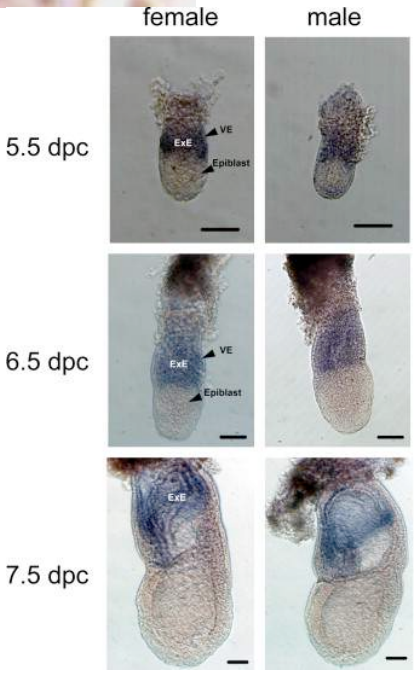
(Pergament et al, 1994; Ray et al, 1995; Tsunada et al, 1985; Zwingman et al, 1993; Perjins-Cole, 1987; Avery et al, 1992; Xu et al, 1992)

Delayed post-implantation development of **XX** mouse and rat embryos

(Scott and Holson, 1977; Burgoyne et al, 1995)

and differentiating XX vs XO ESCs

(Schultz et al, 2014)



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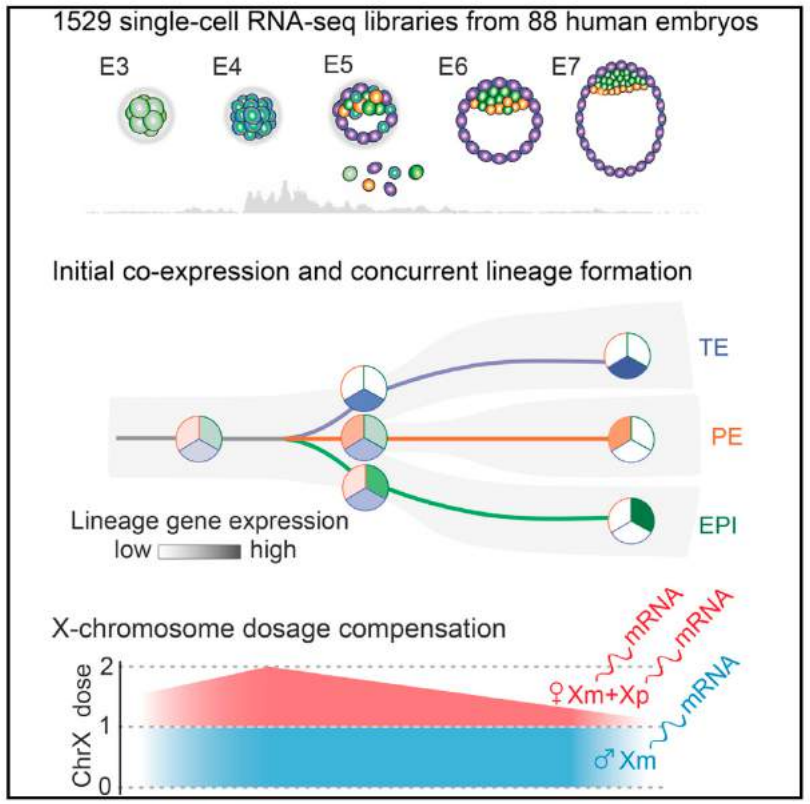
Single-Cell RNA-Seq examines X-Chromosome Expression Dynamics in Human Pre-implantation Embryos

No X inactivation but « dampening » of expression from both Xchromosomes?



Petropoulos et al (2016) *Cell* 165, 1-15.

- X-linked genes are expressed from both Xp and Xm based on nascent RNA FISH

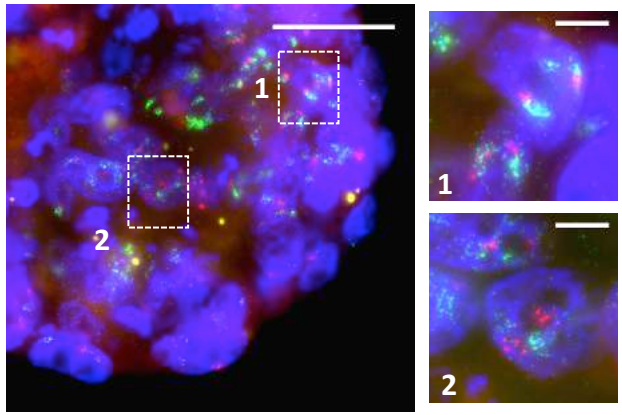
- X-linked gene mRNA levels are lower from each X in female compared to male embryos

- Dosage compensation may be achieved in human embryos via *partial* silencing of both X chromosomes by XIST RNA?
- Conflicting data/interpretations! (eg Vallot et al, 2017)
- Gene-by-gene analysis required using controls for genom-wide transcriptional changes and different techniques
- [illegible]nkey

What prevents XIST RNA from exerting its full silencing function in human embryos?

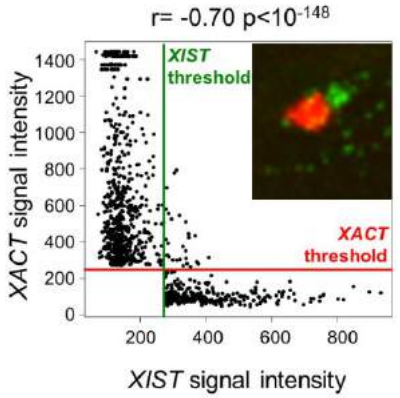
A role for the primate-specific XACT lncRNA in counter-acting XIST RNA mediated X-chromosome inactivation?

Female blastocyst (B3)

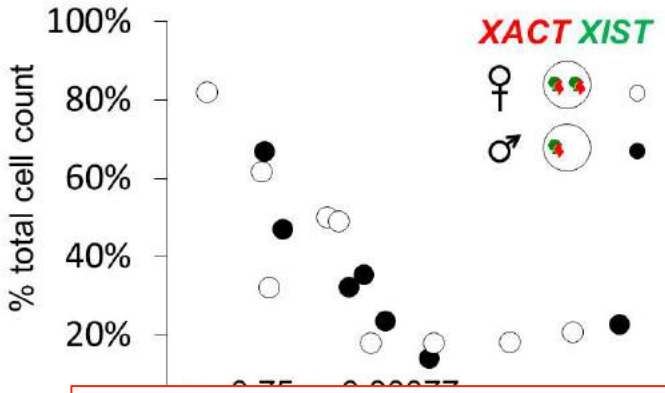


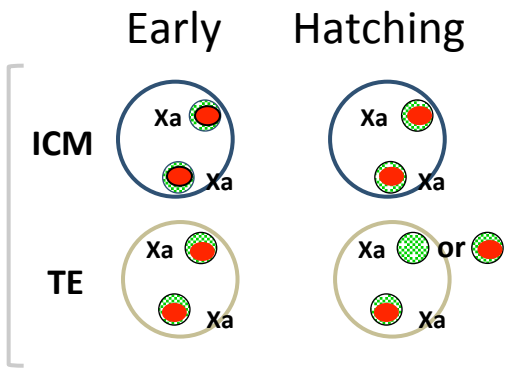
XIST/
XACT/
Dapi

Differential distribution in the nucleus



XIST and XACT RNA are mutually exclusive
The % of cells co expressing XIST and XACT decreases as blastocyst evolves



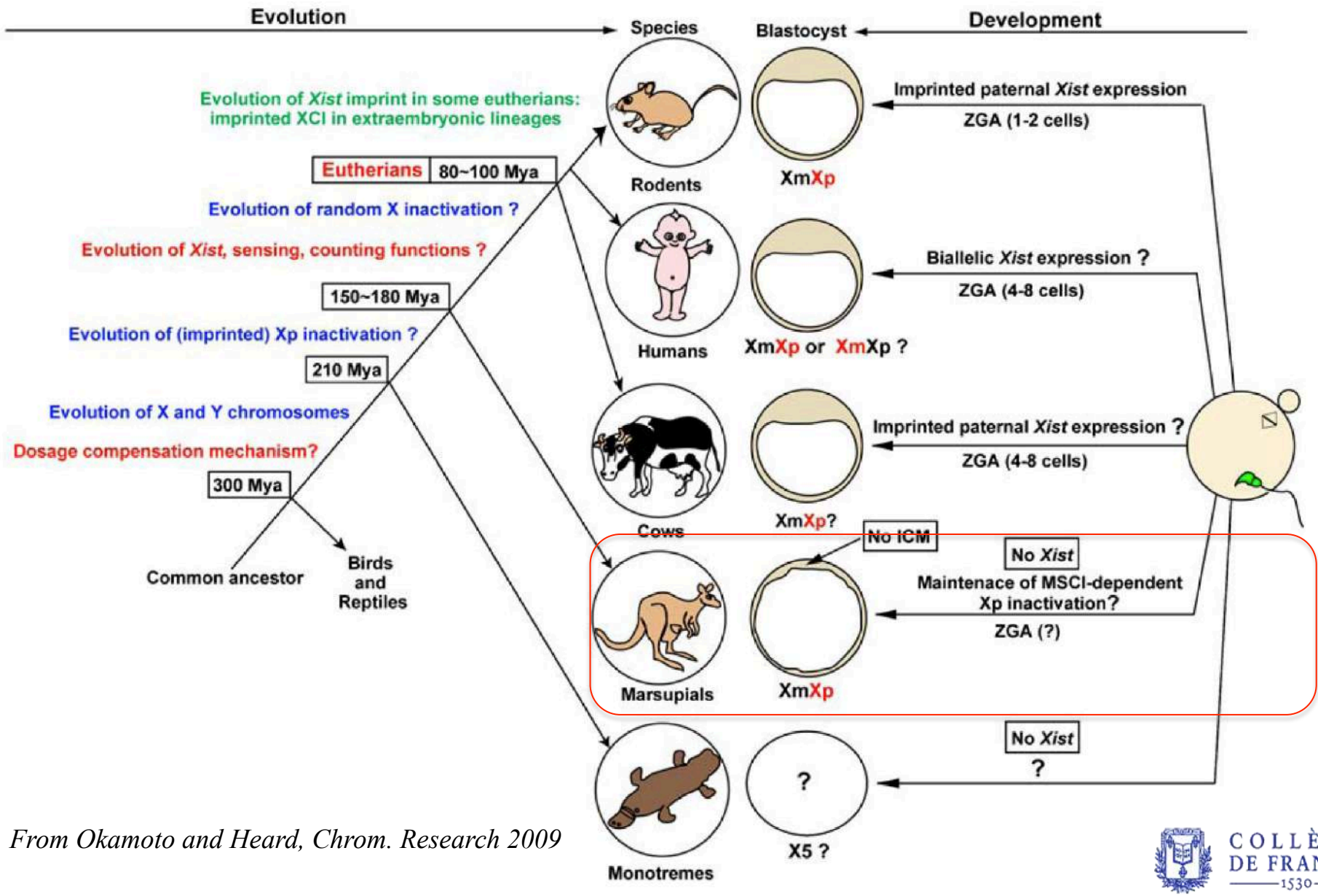


Potential role for XACT in counteracting XIST RNA coating and enabling complete and monoallelic XCI to occur?

C. Patrat and C. Vallot

Vallot et al., Cell Stem Cell, 2017

Evolutionary Dynamics of X inactivation



From Okamoto and Heard, Chrom. Research 2009

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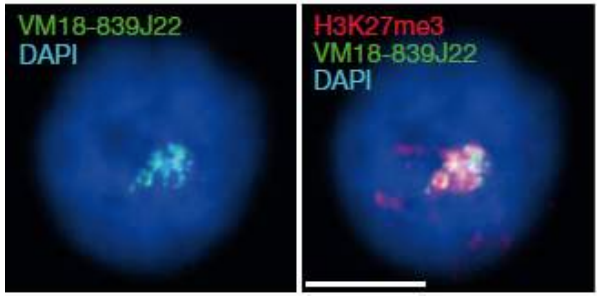
Evolutionary Dynamics of X inactivation

Rsx is a metatherian RNA with *Xist*-like properties in X-chromosome inactivation

Jennifer Grant[1], Shantha K. Mahadevaiah[1], Pavel Khil[2], Mahesh N. Sangrithi[1], Hélène Royo[1], Janine Duckworth[3], John R. McCarrey[4], John L. VandeBerg[5], Marilyn B. Renfree[6], Willie Taylor[1], Greg Elgar[1], R. Daniel Camerini-Otero[2], Mike J. Gilchrist[1] & James M. A. Turner[1]

Grant et al (2012) Nature 487, 254-258



James Turner
Colloque du 14 Mai, 2018
Collège de France

- Non-coding RNAs are more easy to evolve (often driven by retransposon relics – see COURS 2016)
- Easy to regulate dynamically in development
- Could be useful "triggers" or modulators for epigenetic processes

SUMMARY

- Dosage compensation for X-linked gene expression between sexes XX and XY (or XO) animals is clearly achieved in many animals (flies, worms, placental mammals) but not in others (snakes, etc). Very diverse strategies used to achieve dosage equality!
- Dosage does matter- failure to accomplish sex chromosome dosage compensate is lethal during mouse development: absence of dosage compensation perturbs both embryonic and extraembryonic developmental processes
- However dosage compensation is not necessarily chromosome-wide in many organisms
- Some genes are more dosage sensitive than others – and this may vary during development and in tissues (next week)
- X-chromosome up-regulation on the single X relative to autosomes is not a universal principle and does not necessary involve the whole X – just some dosage sensitive genes
- Sex determination and dosage compensation are triggered by the same pathway in Drosophila and C. elegans - not in placental mammals (XX dosage for XCI, Sry for sex)
- Targeting / modulating dosage compensation factors to the sex chromosomes involves DNA elements (C. elegans , Drosophila) and non-coding RNAs (Drosophila, mammals)
- And diverse chromatin and chromosomal complexes: **MORE NEXT WEEK!**

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2017-2018 :

"Le chromosome X -
paradigme de la génétique et l'épigénetique"

12 février, 2018

Cours III

Dynamique de l'hétérochromatine facultative

Dynamics of facultative heterochromatin