CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

**Année 2024-2025 :**

Nouvelles conaissances sur les mécanismes épigénétiques :
l'inactivation du chromosome X et d'autres exemples
d'expression monoallélique

Cours II, 19 mai 2025

*La génétique et l'épigénétique de l'inactivation du chromosome X et d'autres exemples d'expression monoallélique*

E. Heard, May 19th, 2025

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# COURS 2025

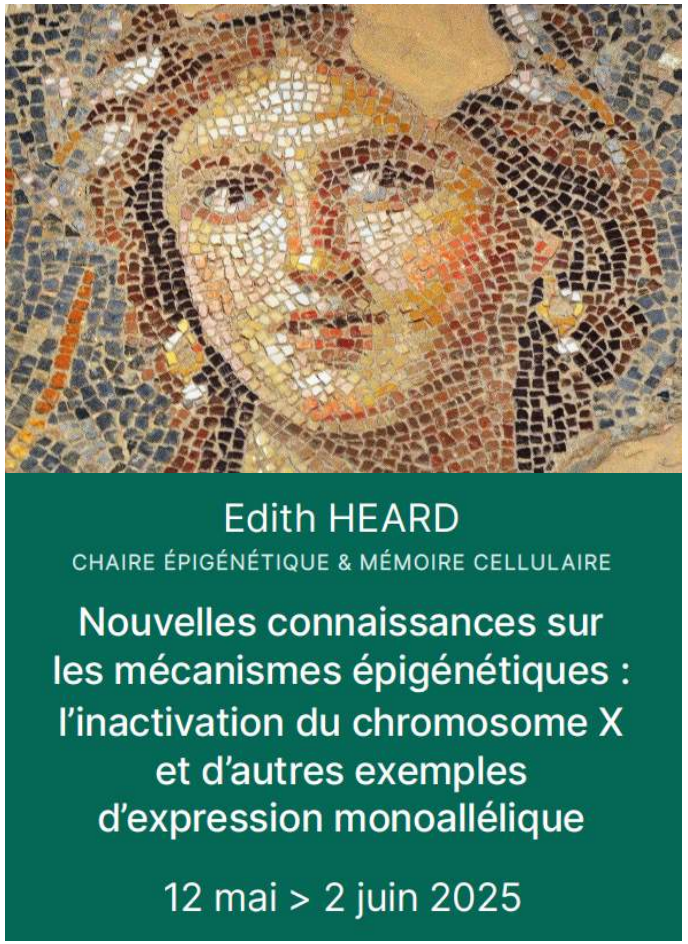
12 mai 2025
Découverte de l’inactivation du chromosome X (lyonisation)

19 mai 2025
**La génétique et l’épigénétique de l’inactivation du chromosome X et d’autres exemples d’expression monoallélique**

26 mai 2025
Évolution de l’inactivation du chromosome X et dynamique développementale

2 juin 2025
Implications de l’inactivation du chromosome X pour la biologie féminine

10-11 juin 2025 Colloque



# The Discovery of X-Chromosome Inactivation

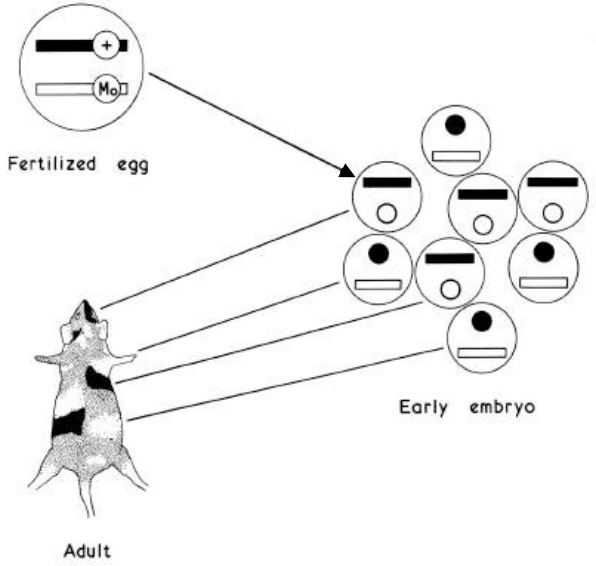
Mary Lyon

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

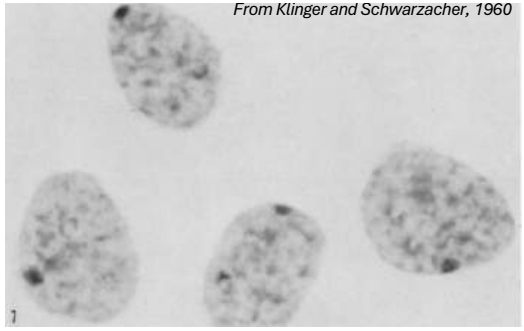
XO mice are normal fertile females => female mice needs only one X chromosome to develop normally (Welshons and Russell, 1959)


*Adapted from Mary Lyon, Henry Stewart Talks*




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

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




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



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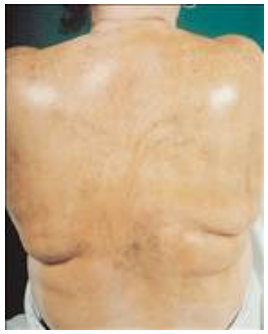
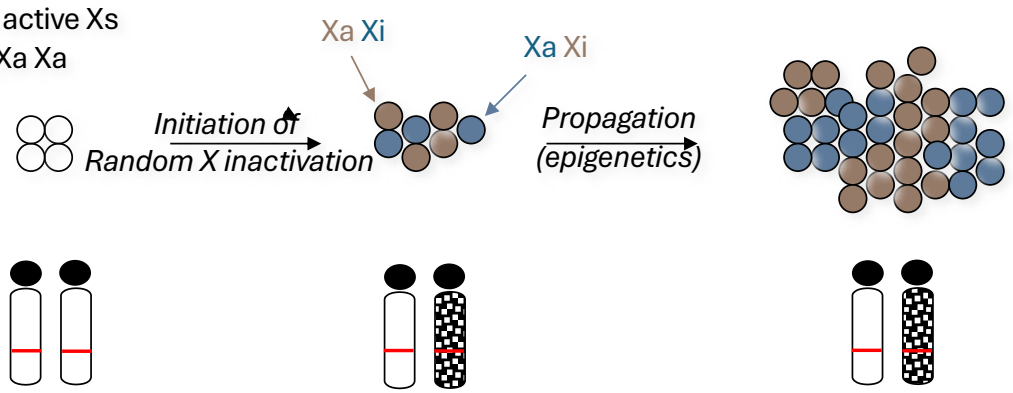
XO mice are normal fertile females => female mice needs only one X chromosome to develop normally (Welshons and Russell, 1959)

Evidence for X inactivation found in other mammals (humans, cats…)



*Cambiaghi et al, 2000*


Lyon (1962) Am. J. Hum. Genet 14:135-148.

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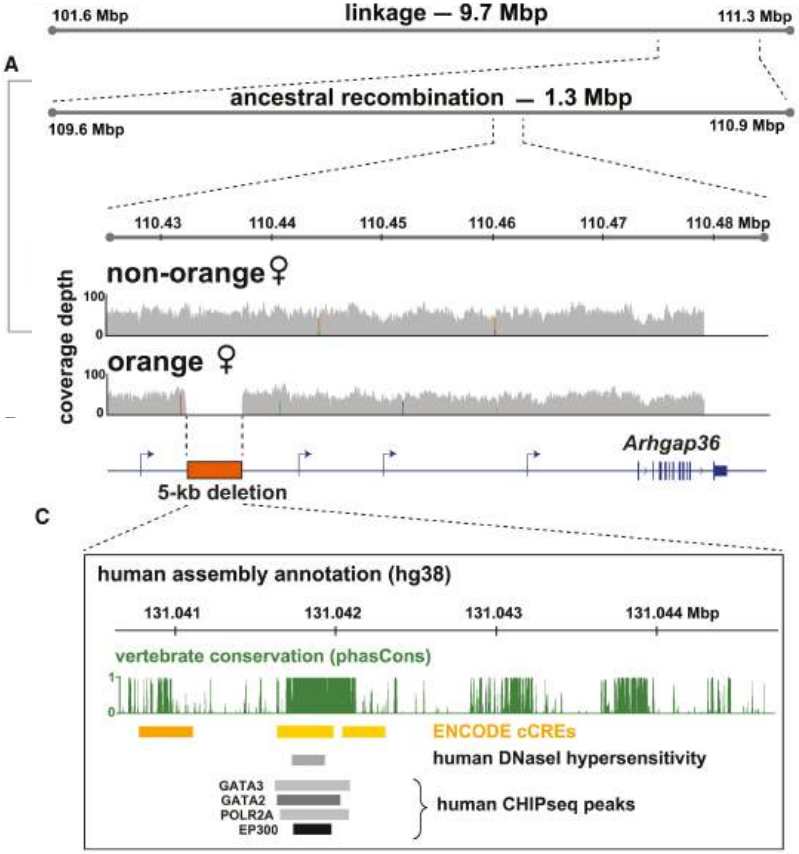
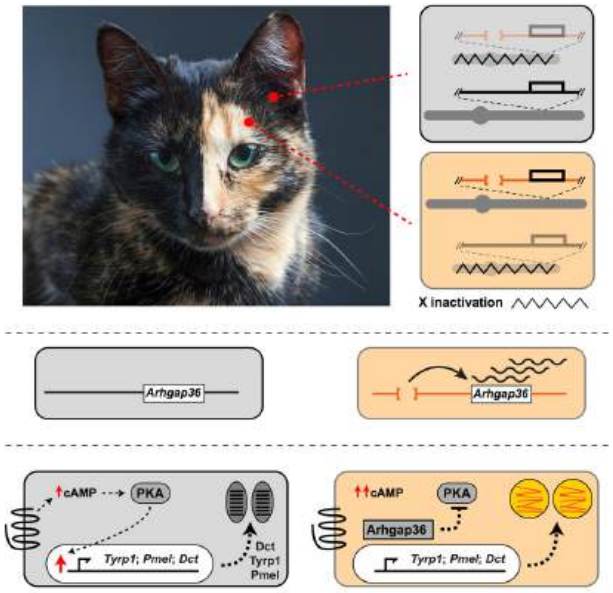
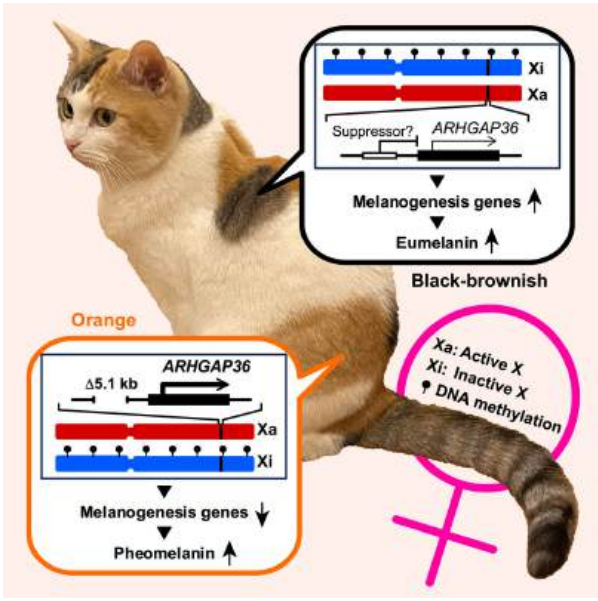
# X-linked coat colour genetics and X-Chromosome inactivation




Kaelin et al, Curr Biol 2025
*Molecular and genetic characterization of sex-linked orange coat color in the domestic cat.*




Toh et al, Curr Biol 2025
*A deletion at the X-linked ARHGAP36 gene locus is associated with the orange coloration of tortoiseshell and calico cats*



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# 1. Summary of LAST WEEK (COURS I)

- Discovery of the Barr body (Barr 1949), identified as the inactive X chromosome (Ohno 1959)
- Discovery of X inactivation (Lyon 1961) – also Ohno (1959) and Russell (1961)
- Evidence that XCI is present across several mammals including humans (Lyon 1962)
- Proposal that a single X chromosome remains active per diploid cell based on cells with more than two X chromosomes (Lyon 1962), with or without a Y.
- Proposal that there must be a part of the X chromosome that is non-inactivated (ie both alleles express) with a homologous region on the Y – thus does not require dosage compensation (accounts for XO severity in humans, (Lyon 1962): ESCAPEES
- Discovery that XCI can spread into autosomal regions (Russell, Cattanach 1960's and 70's) (when X portion contains the Xic)
- Mapping of the region that is required to trigger X inactivation (Rastan and Robertson 1985); Brown et al 1990) - the X-inactivation centre.

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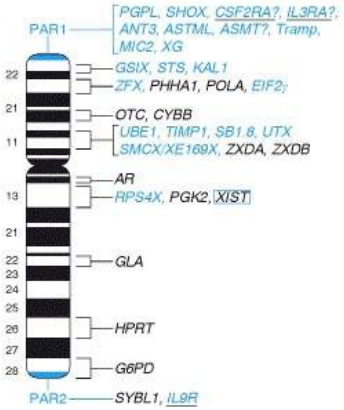
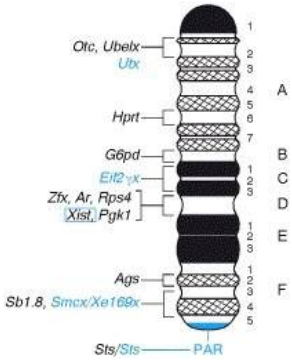
Pseudoautosomal regions (PARs) and Escapees:
PARs contain and express the "same" genes from both X and Y, other non-PAR genes also escape XCI
Now know that >25% genes expressed from the human inactive X (escapees)




E. Heard, May 19th

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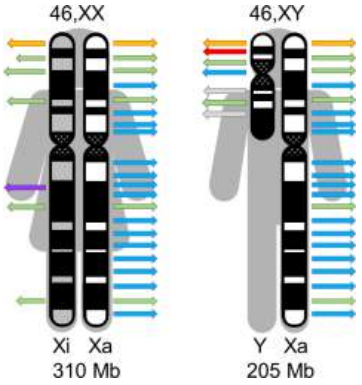


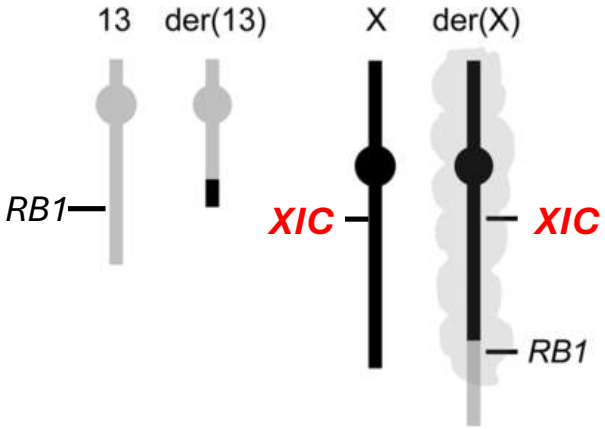


The der(X) is inactivated in a subset of the cells

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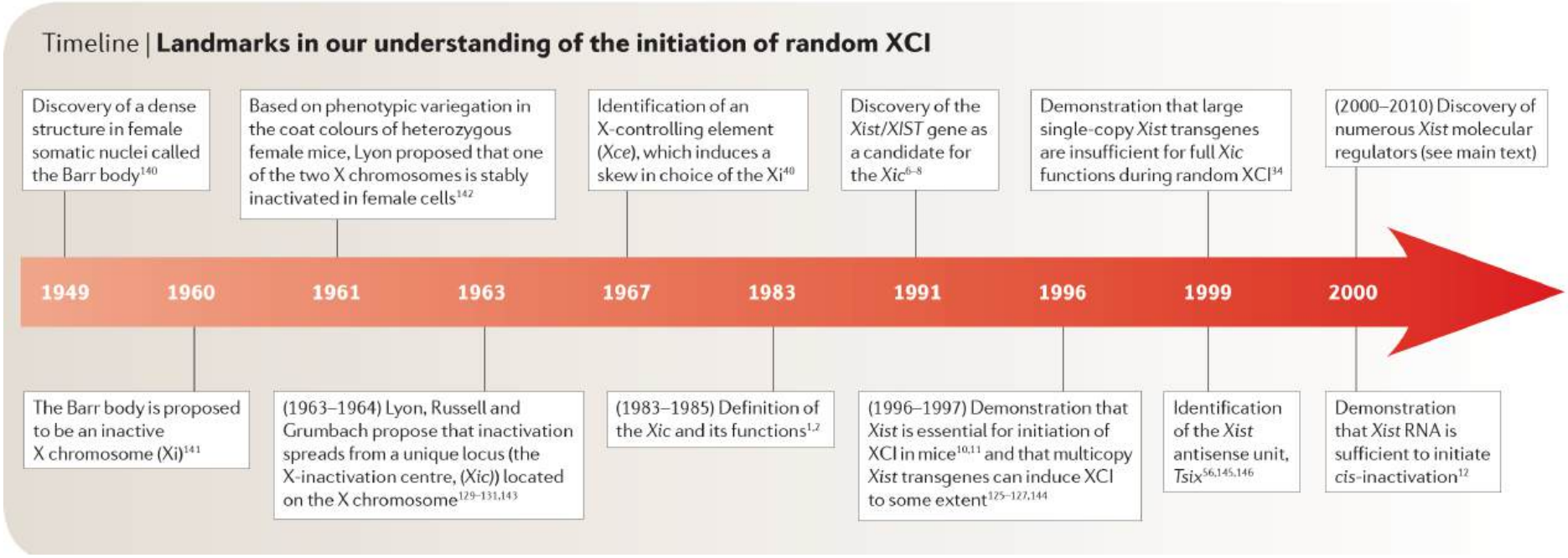
**Major limitations** to studying the onset of XCI in the decades following the discovery of XCI:

- The small size of the embryo and the limited amount of material available at the peri-implantation stages when X-inactivation occurs: IN VITRO SYSTEMS



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**1978 – first use of teratocarcinoma stem cells & in 1985 of XX embryonic stem cells to study X inactivation upon *in vitro* differentiation**

In ES and some EC cell lines with two intact X chromosomes, both X chromosomes are active while the cells are maintained in a pluripotent state, but if the cells are allowed to differentiate, X-inactivation occurs.
Such cell lines have facilitated investigation of the different steps in X-inactivation and reactivation and have been particularly useful in the genetic dissection of the Xic.

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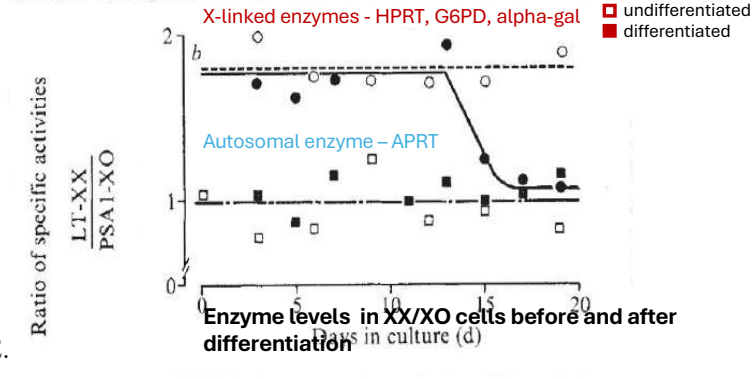
**1978 – first use of teratocarcinoma stem cells & in 1985 of XX embryonic stem cells to study X inactivation upon *in vitro* differentiation**

## X–chromosome inactivation during differentiation of female teratocarcinoma stem cells *in vitro*


Gail R. Martin
Department of Anatomy and Cancer Research Institute, University of California, San Francisco, California 94143
Charles J. Epstein, Bruce Travis, Georgianne Tucker & Shaul Yatziv*
Departments of Pediatrics and of Biochemistry and Biophysics, University of California, San Francisco
David W. Martin Jr, Shirley Clift & Sara Cohen
Departments of Medicine and of Biochemistry and Biophysics, University of California, San Francisco



*Evidence is presented that both X chromosomes are genetically active in clonal cultures of undifferentiated female mouse teratocarcinoma stem cells derived from a spontaneous ovarian tumour. As the cells differentiate in vitro one of the X chromosomes becomes inactivated.*


E. **Enzyme levels in XX/XO cells before and after differentiation**

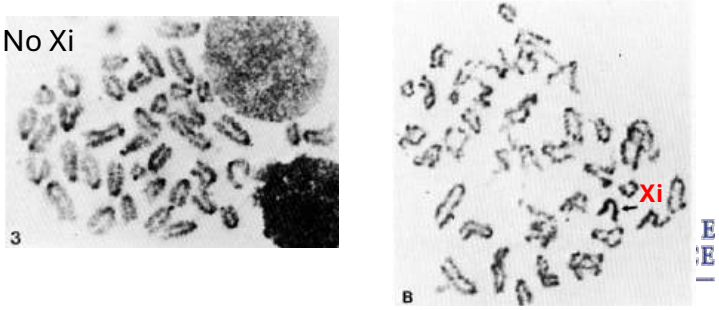

*J. Embryol. exp. Morph. 90, 379–388 (1985)*
*Printed in Great Britain © The Company of Biologists Limited 1985*




## X-chromosome deletions in embryo-derived (EK) cell lines associated with lack of X-chromosome inactivation


SOHAILA RASTAN
*Division of Comparative Medicine, Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ, U.K.*
AND ELIZABETH J. ROBERTSON
*Department of Genetics, University of Cambridge, Downing Street, Cambridge, U.K.*


Cytological Xi assay (Kanda staining)



# 2. Summary of LAST WEEK (COURS I)

**Major limitations** to studying the onset of XCI in the decades following the discovery of XCI:

- The small size of the embryo and the limited amount of material available at the peri-implantation stages when X-inactivation occurs.
- The large size of the candidate Xic region (10-20 Mbp) – TODAY's Lecture
- The lack of markers that enable identification of the inactive X from the active homolog in the same nucleoplasm (X-linked proteins, cytological assays heterochromatin, replication timing)

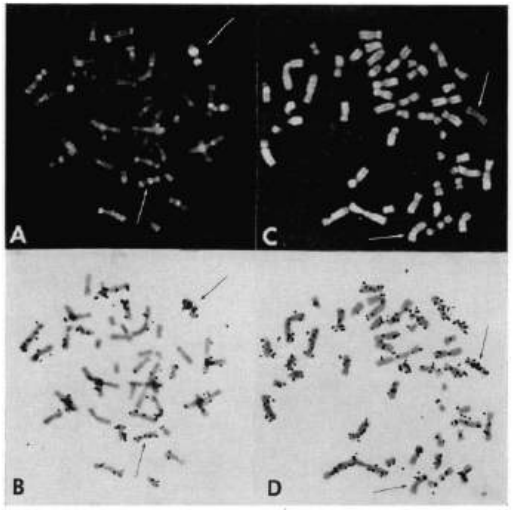



FIG. 1.—Detection of DNA synthesis by 33258 Hoechst fluorescence (*A* and *C*) and autoradiography (*B* and *D*). *A*, Late-replicating regions (*upper arrow*) of human metaphase chromosomes appear as localized foci of bright fluorescence when female leukocytes are grown according to the T-pulse protocol. *B*, Late-replicating X (*upper arrow*) and early-replicating X (*lower arrow*), autoradiography. *C*, Suppressed fluorescence of late-replicating regions compared to earlier-replicating regions when cells are grown according to the B-pulse protocol. *D*, Late-replicating X (*upper arrow*) is distinguished from earlier-replicating X (*lower arrow*).

Am J Hum Genet 28:213-227, 1976

**Analysis of Deoxyribonucleic Acid Replication in Human X Chromosomes by Fluorescence Microscopy**

H. F. WILLARD[1, 2] AND S. A. LATT[1]

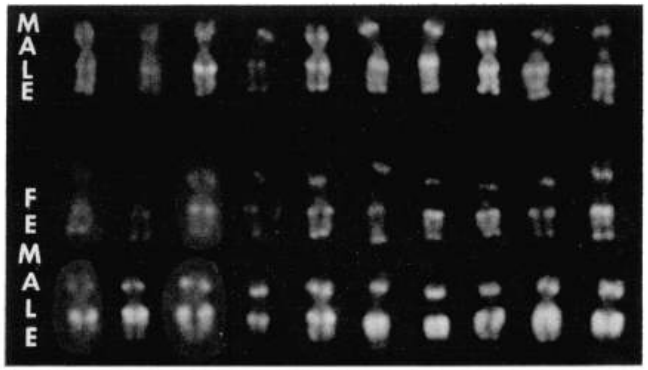



FIG. 4.—33258 Hoechst fluorescence of male and female human X chromosomes from cells grown according to the T-pulse protocol. The fluorescence pattern of the male X, *top row;* early-replicating female X, *middle row;* and late-replicating female X from the same cells, *bottom row*. Note that the former two are indistinguishable.

Active X

Active X

Inctive X



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# Introduction to Course II

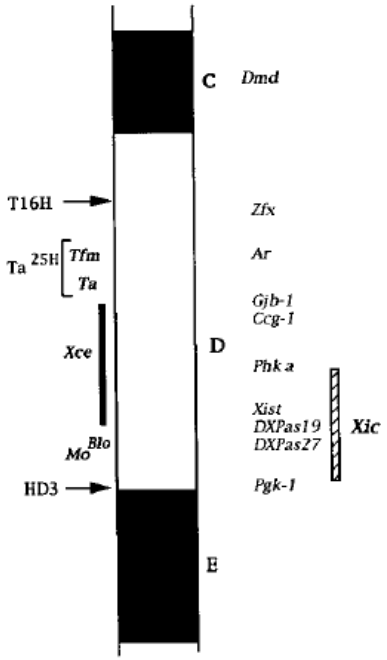
**Genetic control of an epigenetic process: the Xic, the ncXist RNA, Xist's regulatory landscape**

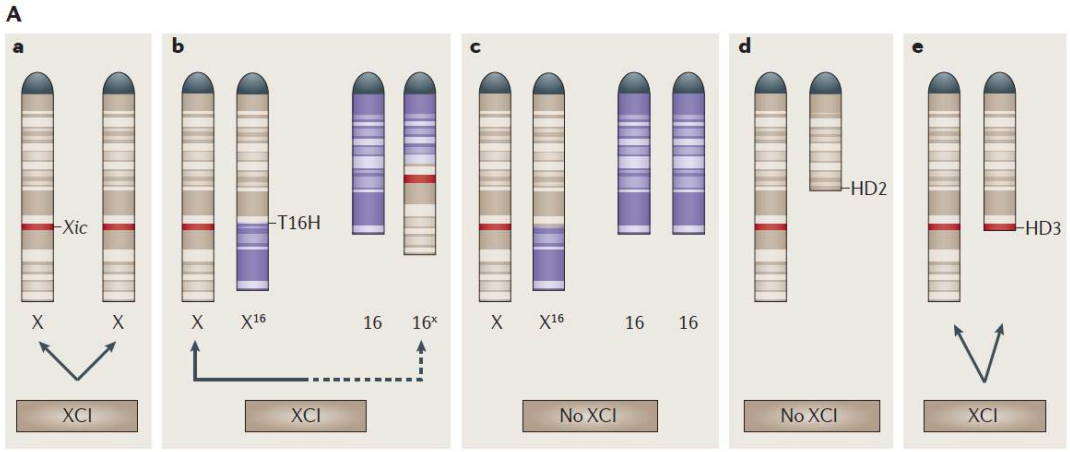
- Mapping and dissection of the Xic and its role in initiation of XCI
- Concepts of Counting, Choice and Sensing
- (Serendipitous) discovery of the *Xist* gene and its unique features as the potential Xic
- Through deletion and transgenesis defining that *Xist* is necessary but not sufficient for full initiation of random XCI => Xic and Xist's regulatory landscape
- (Developmental dynamics, imprinted XCI, evolutionary conservation in cours III)

**A new era of modern epigenetics – and the epigenetic memory of the Xi**

- DNA Methylation and other chromatin marks
- Spatio-temporal organisation of the inactive X chromosome
- Other examples of monoallelic expression
- Revisiting the roles of Xist RNA beyond early development

# Identification and Mapping of the Human and Mouse X-Inactivation Centers




**Key concepts in X-chromosome inactivation**

Before the discovery of the many molecular actors in X inactivation, some key concepts relating to the steps necessary for inactivation to occur were already proposed in the 1960's and 70's. Although theoretical, these notions became, to an extent, dogmatic over the years: -

**X inactivation is a *3-step* process involving**

**(1) Initiation (counting, choice, competence/sensing) – Xic and Xce**

**(2) Spreading (in cis; relay elements / way stations)**

**(3) Maintenance (epigenetic memory)**



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# The X-Inactivation Center (Xic)

Studies of mouse embryos with altered X chromosomes revealed the X-inactivation center (Xic) is essential for initiating inactivation.

- Random XCI only occurs in cells with two Xic-bearing X chromosomes, suggesting mutual sensing between them.
- This mutual interaction is linked to the process of competence or sensing.
- In XX cells, either X can be randomly inactivated — this is known as choice.
- Counting refers to the influence of autosome number on how many X chromosomes are inactivated.
- These processes — competence, choice, and counting — are genetically and molecularly interconnected.

Counting:

- Cells measure the X-to-autosome (X/A) ratio.
- Ensures only one active X per diploid autosome set.
- Based on studies of individuals with abnormal numbers of X chromosomes

Choice:

- Refers to random selection of which X chromosome is inactivated.
- Usually equal chance for maternal or paternal X unless Xic mutations are present.
- Once one X is chosen, the other is protected from inactivation.

Sensing / Competence:

- A state allowing XCI only when more than one X chromosome is present.
- Involves recognition of multiple Xs and the X/A ratio.
- Now considered distinct from counting due to mutant studies.

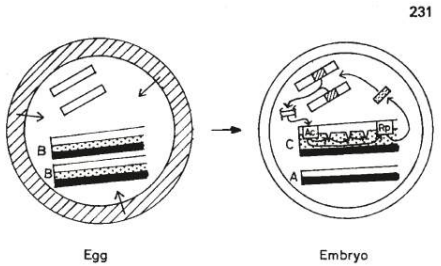



**Fig. 2** A possible model for activation of one *X* chromosome by autosomes. A molecule from the autosomal pair initiates gene activity by the activation centre, Ac, which in turn activates the non-histone protein genes (projections) and also a repressor (Rp) which represses the autosomal genes. For meanings of A, B and C see Fig. 1.

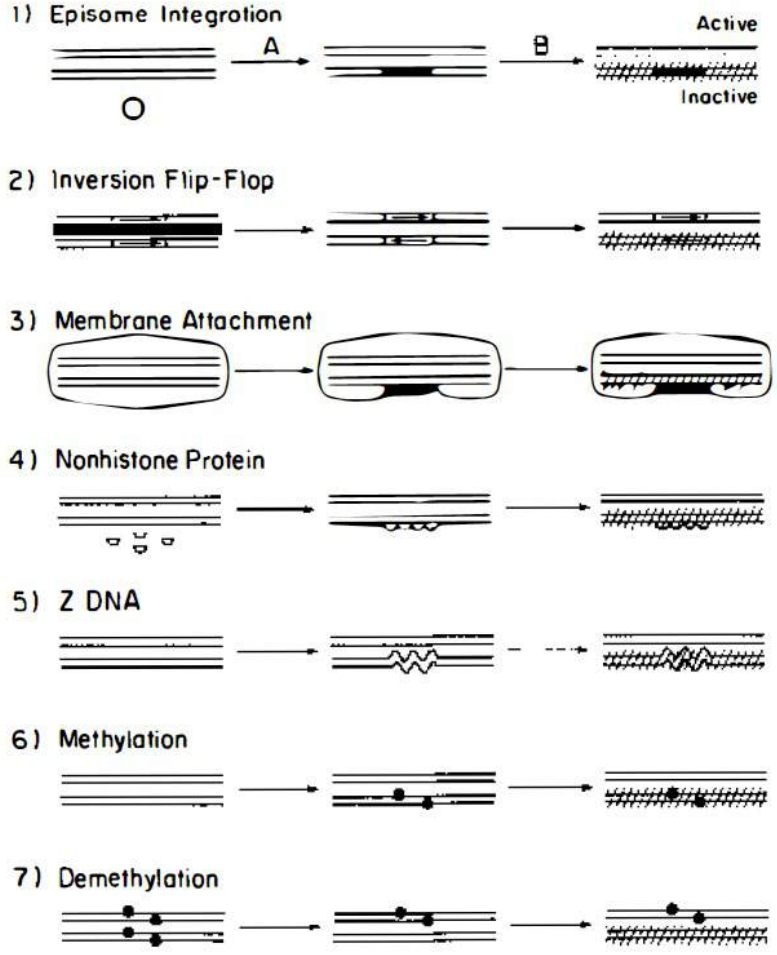
# Early models for X inactivation: Initiation, Spreading and Maintenance

The models differ mainly with respect to step A, which is the **initiation** step.

Step B is the event that follows the initial event and results in the observable **spreading** of condensation and genetic inactivity to cover most genes on the X chromosome and the subsequent **maintenance** of the inactive state.

Gartler and Riggs (1983) Ann. Rev. Genet. 1983. 17:155-90

COURS 2013




Comings, 1968

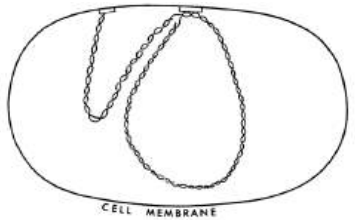
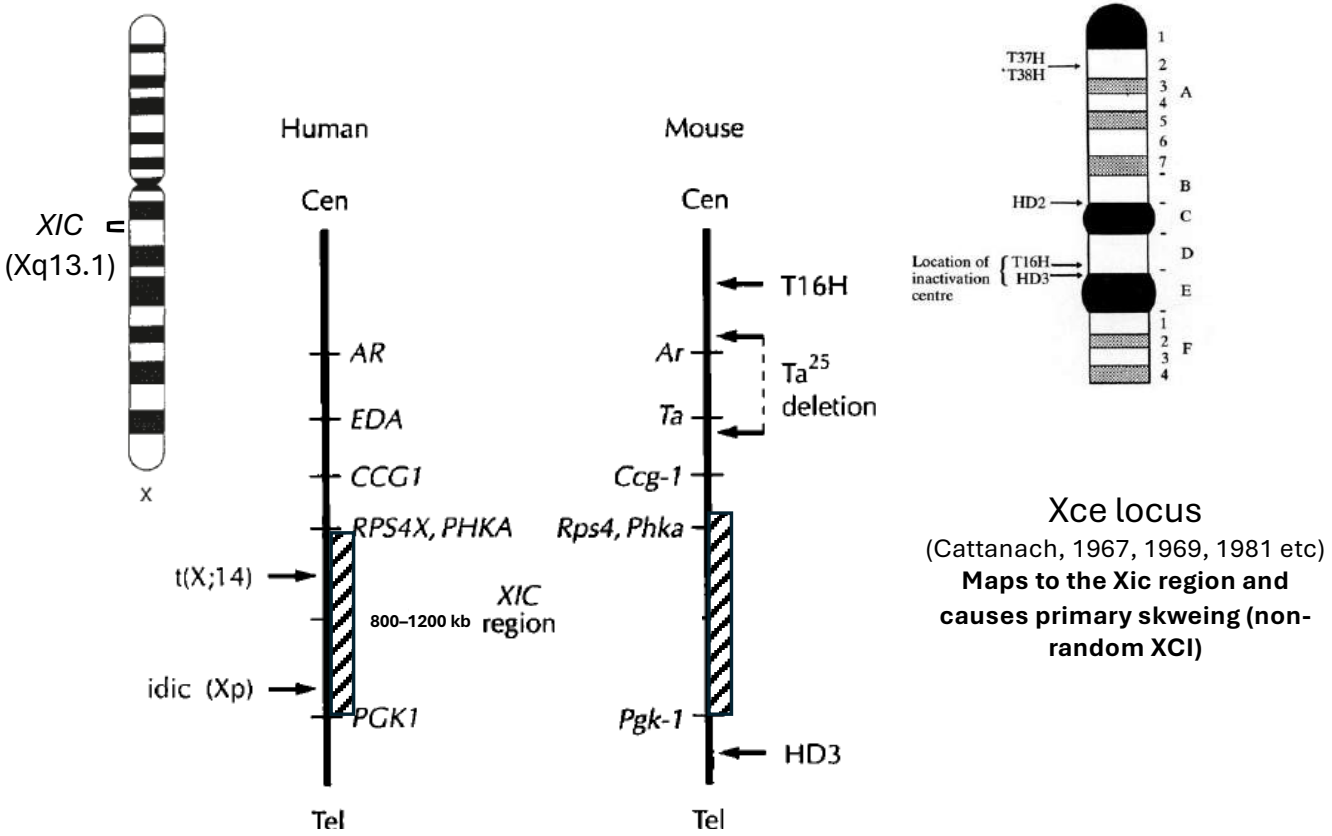



FIG. 1.—The bacterial replicon as proposed by Jacob *et al.* (1963). Before initiation of DNA synthesis, the circular bacterial chromosome is attached at a specific place on the chromosome (replicator) and to a specific location on the bacterial cell membrane (mesosome or mesosome-like structure). DNA synthesis is initiated at this point of attachment to the membrane, and replication always occurs in proximity to the membrane. The segregation of the two daughter strands is controlled by their attachment to the cell membrane (shown here in exaggerated separation).

# The hunt for the X-Inactivation Center (human and murine)




Xce locus

(Cattanach, 1967, 1969, 1981 etc)

**Maps to the Xic region and causes primary skweing (non-random XCI)**

Human XIC locus maps to an 800–1200 kb region lying within the PHA–PGK1 region in band Xq13 (Brown et al., 1991 ; Leppig et al., 1993 ; Lafreniere & Willard, 1993).
The corresponding syntenic region in the mouse, which appears to have been subject to several chromosomal inversions, is somewhat smaller (Debrand et al., 1998).

# Discovery of human *XIST*

*(when luck favours prepared minds…!)*

**A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome**

**Carolyn J. Brown, Andrea Ballabio*, James L. Rupert, Ronald G. Lafreniere, Markus Grompe*, Rossana Tonlorenzi* & Huntington F. Willard†**

Department of Genetics, Stanford University School of Medicine, Stanford, California 94305, USA
* Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030, USA

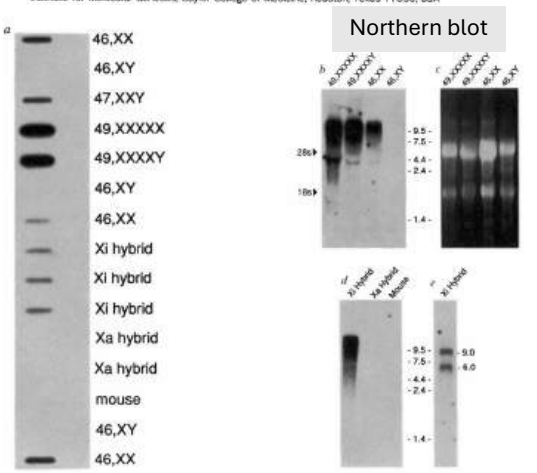
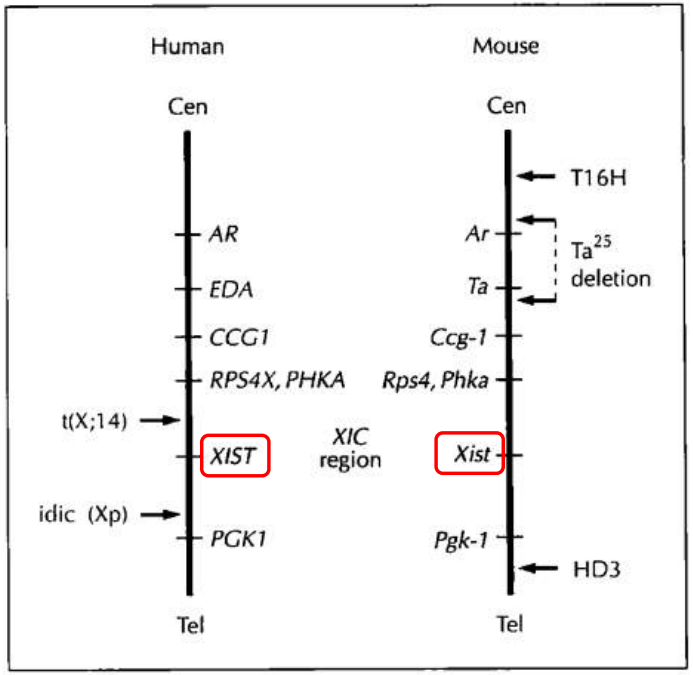



FIG. 1 Expression of the *XIST* gene in males, females, and somatic cell hybrids. *a*, Slot blot of total cellular RNA isolated from human lymphoblastoid cell lines or mouse–human somatic cell hybrids retaining either the active or inactive human X chromosome, hybridized with the 14A *XIST* cDNA probe. The probe hybridizes only to RNA samples from cell lines which contain an $X_i$. *b*, Northern blot of total cellular RNA from male and female cell lines, hybridized with the 14A cDNA probe. The probe detects a large, heterogeneous message only in cell lines which contain an $X_i$. Apparent bands below the position of 28S and 18S rRNA are created by the huge abundance of rRNA present. *c*, Ethidium bromide-stained RNA before northern transfer for the filter in *b*. *d*, Northern blot of poly(A)$^+$ RNA from human/mouse hybrids retaining either the $X_a$ or the $X_i$ and a mouse cell line control. The 14A *XIST* probe hybridizes to transcripts only in the hybrid with an inactive X chromosome. *e*, The $X_i$ hybrid lane of *d* stripped and reprobed with a control cDNA probe (SB1.8) identifying a 9- and a 6-kb mRNA (ref. 17) to show that the mRNA is intact.

Carolyn Brown
Stanford Univ., USA

Hunt Willard
Stanford Univ., USA




"We have searched for additional genes expressed from the X, particularly among those that map near the interval of the X inactivation centre (X/C) on the human X chromosome. We report here the isolation and characterization of a novel gene, XIST, expressed from X; but not from Xa chromosomes, which by virtue of its localization on the X to the same interval as XIC, represents a candidate for a gene either involved in or strongly influenced by X inactivation."

Brown CJ, Ballabio A, Rupert JL, et al. "A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome." Nature. 1991;349(6304):38–44.

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Department of Genetics, Stanford University School of Medicine, Stanford, California 94305, USA
* Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030, USA

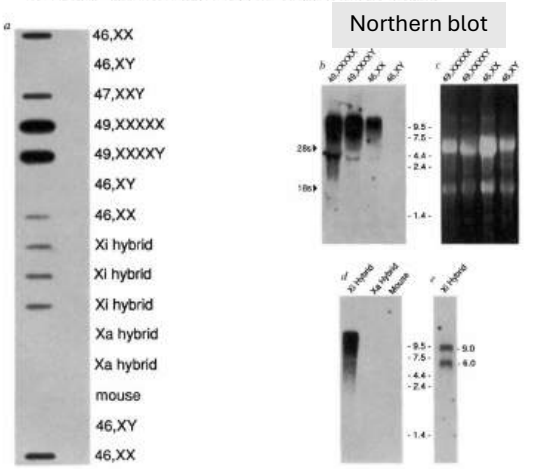



FIG. 1 Expression of the *XIST* gene in males, females, and somatic cell hybrids. *a*, Slot blot of total cellular RNA isolated from human lymphoblastoid cell lines or mouse–human somatic cell hybrids retaining either the active or inactive human X chromosome, hybridized with the 14A *XIST* cDNA probe. The probe hybridizes only to RNA samples from cell lines which contain an $X_i$. *b*, Northern blot of total cellular RNA from male and female cell lines, hybridized with the 14A cDNA probe. The probe detects a large, heterogeneous message only in cell lines which contain an $X_i$. Apparent bands below the position of 28S and 18S rRNA are created by the huge abundance of rRNA present. *c*, Ethidium bromide-stained RNA before northern transfer for the filter in *b*. *d*, Northern blot of poly(A)$^+$ RNA from human/mouse hybrids retaining either the $X_a$ or the $X_i$ and a mouse cell line control. The 14A *XIST* probe hybridizes to transcripts only in the hybrid with an inactive X chromosome. *e*, The $X_i$ hybrid lane of *d* stripped and reprobed with a control cDNA probe (SB1.8) identifying a 9- and a 6-kb mRNA (ref. 17) to show that the mRNA is intact.

Carolyn Brown
Stanford Univ., USA

Hunt Willard
Stanford Univ., USA

TABLE 1 Summary of *XIST* expression

| Cell-line | Karyotype | Expression RT-PCR | Slot blot | Northern |
|---|---|---|---|---|
| Chromosomally normal | | | | |
| 7 females | 46, XX | +(7) | +(6) | +(6) |
| 10 males | 46, XY | −(10) | −(6) | −(6) |
| Chromosomally aberrant | | | | |
| GM6061B | 49, XXXXX | + | + | + |
| GM1202 | 49, XXXXY | + | + | + |
| 106 | 45, XO | − | − | − |
| 107 | 69, XXY | − | − | − |
| D64.0 | 47, XXY | + | + | + |
| GM10074 (ref. 19) | 47, Y, t(X;14) +der(14) | + | ND | ND |
| A.G. (ref. 19) | 45, X/46, X, idic(Xp) | + | ND | ND |
| Somatic cell hybrids | | | | |
| t60-12 | Active X | − | − | − |
| AHA11aB1 | Active X | − | − | − |
| t11-4Aaz5 | Inactive X | + | + | + |
| t48-1a-1Daz4a | Inactive X | + | + | + |
| t81-az1D | Inactive X | + | + | + |
| t86-B1maz1b-3a | Inactive X | + | + | ND |
| LT23-lE2Buv5Cl26 | Inactive X | + | ND | ND |

"We have searched for additional genes expressed from the X, particularly among those that map near the interval of the X inactivation centre (X/C) on the human X chromosome. We report here the isolation and characterization of a novel gene, XIST, expressed from X; but not from Xa chromosomes, which by virtue of its localization on the X to the same interval as XIC, represents a candidate for a gene either involved in or strongly influenced by X inactivation."



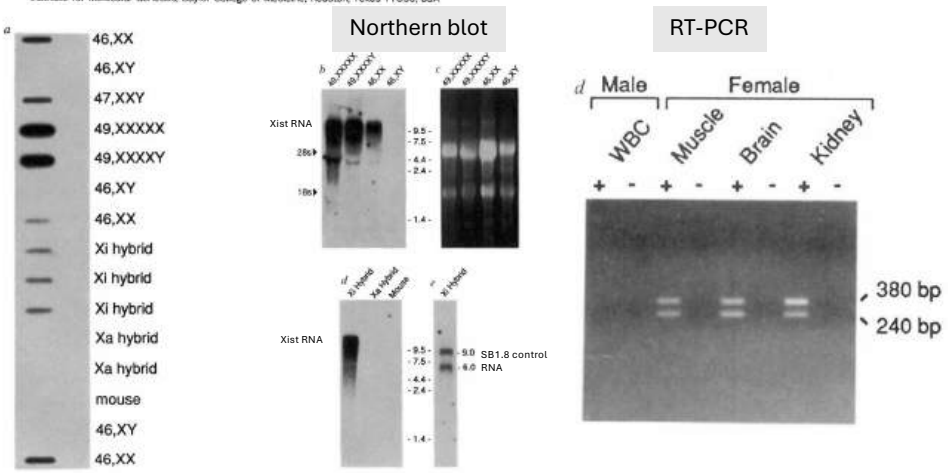
Brown CJ, Ballabio A, Rupert JL, et al. "A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome." Nature. 1991;349(6304):38–44.

# Discovery of human *XIST*

**A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome**

**Carolyn J. Brown, Andrea Ballabio*, James L. Rupert, Ronald G. Lafreniere, Markus Grompe*, Rossana Tonlorenzi* & Huntington F. Willard†**

Department of Genetics, Stanford University School of Medicine, Stanford, California 94305, USA
* Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030, USA

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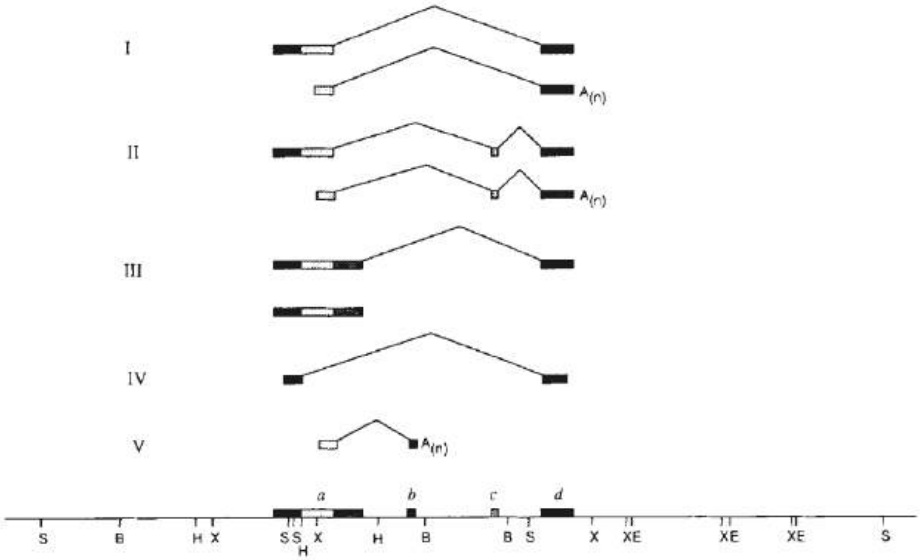
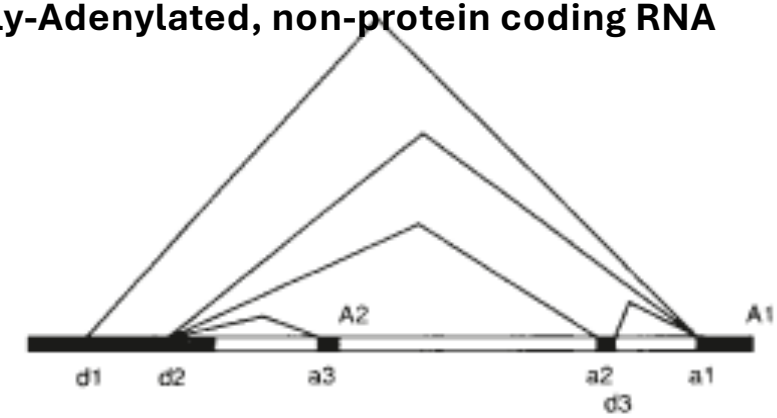



**Multiple alternatively spliced forms of a long, poly-Adenylated, non-protein coding RNA**




E. Heard, May 19th, 2025

Brown CJ, Ballabio A, Rupert JL, et al. "A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome." Nature. 1991;349(6304):38–44.

# Discovery of human and mouse *Xist* genes

Andrea Ballabio
(Baylor College,
Houston, Texas, USA)

Phil Avner
(Pasteur Institute
Paris, France)

## Characterization of a murine gene expressed from the inactive X chromosome

**Giuseppe Borsani, Rossana Tonlorenzi*, M. Christine Simmler†, Luisa Dandolo†, Danielle Arnaud†, Valeria Capra*, Markus Grompe, Antonio Pizzuti, Donna Muzny, Charles Lawrence‡, Huntington F. Willard§, Philip Avner† & Andrea Ballabio‖**

Institute for Molecular Genetics and ‡ Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030, USA
† Unite de Genetique Moleculaire Marine, Institut Pasteur, 25 rue du Docteur Roux, F-75724 Paris Cedex 15, France
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**IN mammals, equal dosage of gene products encoded by the X chromosome in male and female cells is achieved by X inactivation. Although X-chromosome inactivation represents the most extensive example known of long range *cis* gene regulation, the mechanism by which thousands of genes on only one of a pair of identical chromosomes are turned off is poorly understood. We have recently identified a human gene (*XIST*) exclusively expressed from the inactive X chromosome[1]. Here we report the isolation and characterization of its murine homologue (*Xist*) which localizes to the mouse X inactivation centre region and is the first murine gene found to be expressed from the inactive X chromosome. Nucleotide sequence analysis indicates that *Xist* may be associated with a protein product. The similar map positions and expression patterns for *Xist* in mouse and man suggest that this gene may have a role in X inactivation.**

To isolate the murine *Xist* homologue, we hybridized the human *XIST* cDNA clone 14A to a cDNA library in λZAPII from thymus RNA of female mice (Stratagene). The longest of the isolated cDNA clones, MR20 (3.1 kilobases (kb)), gave results on the backcross panel indistinguishable from those found for the X-linked sequences detected with the human probe 14A (Fig. 1*b* and *c*) and consequently mapped it between the *Ccg-1/Phka* and *Pgk-1* loci. Independent confirmation of the

## Conservation of position and exclusive expression of mouse *Xist* from the inactive X chromosome

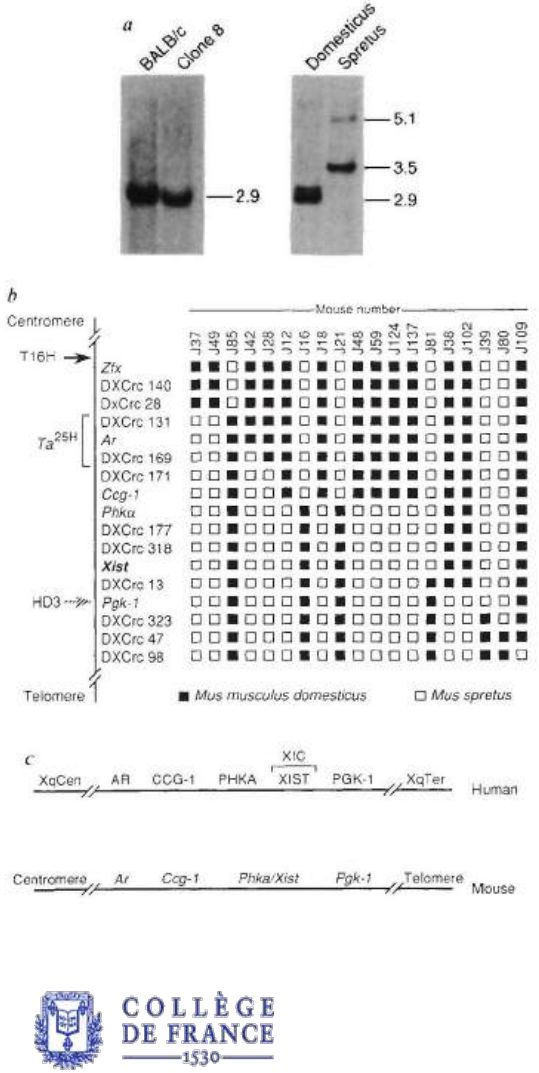
**Neil Brockdorff, Alan Ashworth*, Graham F. Kay, Penny Cooper, Sandy Smith, Veronica M. McCabe, Dominic P. Norris, Graeme D. Penny, Dipika Patel & Sohaila Rastan†**

Section of Comparative Biology, MRC Clinical Research Centre, Harrow HA1 3UJ, UK
* Chester Beatty Laboratories, The Institute of Cancer Research, London SW3 6JB, UK
† To whom correspondence should be addressed

**X-CHROMOSOME inactivation in mammals is a regulatory phenomenon whereby one of the two X chromosomes in female cells is genetically inactivated, resulting in dosage compensation for X-linked genes between males and females[1]. In both man and mouse, X-chromosome inactivation is thought to proceed from a single *cis*-acting switch region or inactivation centre (XIC/Xic)[2–5]. In the human, XIC has been mapped to band Xq13 (ref. 6) and in the mouse to band XD (ref. 7), and comparative mapping has shown that the XIC regions in the two species are syntenic[8]. The recently described human *XIST* gene maps to the XIC region[6] and seems to be expressed only from the inactive X chromosome[9]. We report here that the mouse *Xist* gene maps to the Xic region of the mouse X chromosome and, using an interspecific *Mus spretus/Mus musculus domesticus* $F_1$ hybrid mouse carrying the T(X;16)16H translocation, show that *Xist* is exclusively expressed from the inactive X chromosome. Conservation between man and mouse of chromosomal position and unique expression exclusively from the inactive X chromosome lends support to the hypothesis that *XIST* and its mouse homologue are involved in X-chromosome inactivation.**

We have used a 1.3- kilobase (kb) human probe, generated by the polymerase chain reaction from the published human *XIST* sequence, to screen an oligo(dT)-primed complementary

Sohail Rastan
(MRC Clinical
Research Centre
London, UK)






COLLÈGE DE FRANCE 1530

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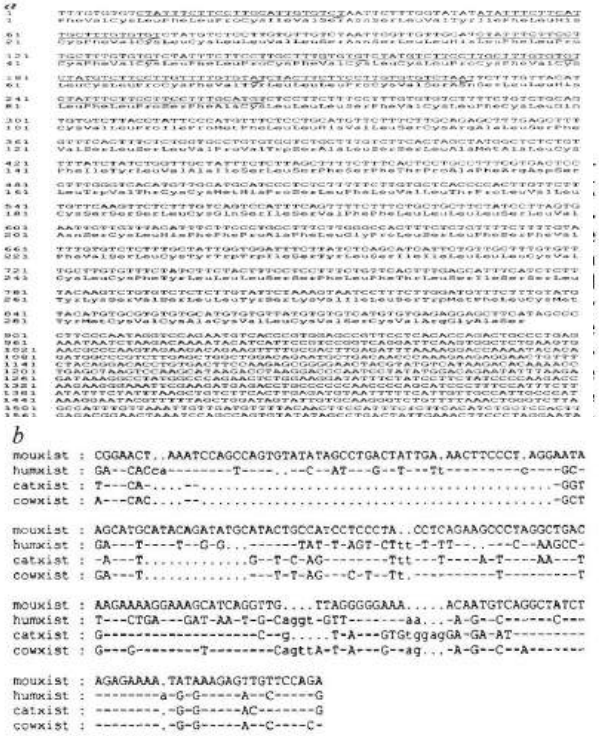

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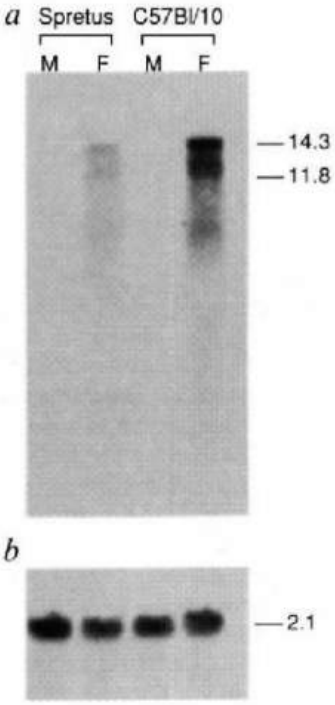


Sohail Rastan
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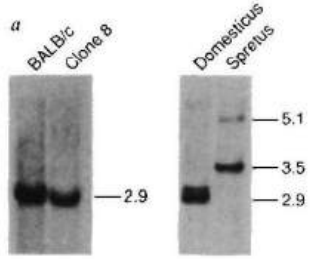


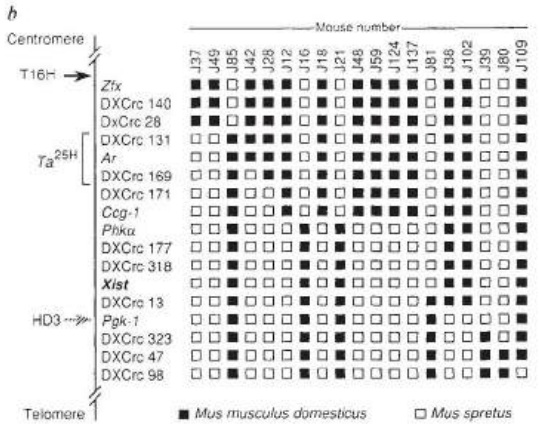


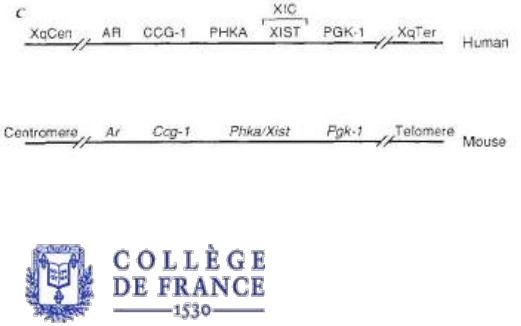


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1530

# Discovery that Xist RNA is nuclear and associates with the inactive X chromosome from which it is expressed

Carolyn Brown
Stanford Univ., USA

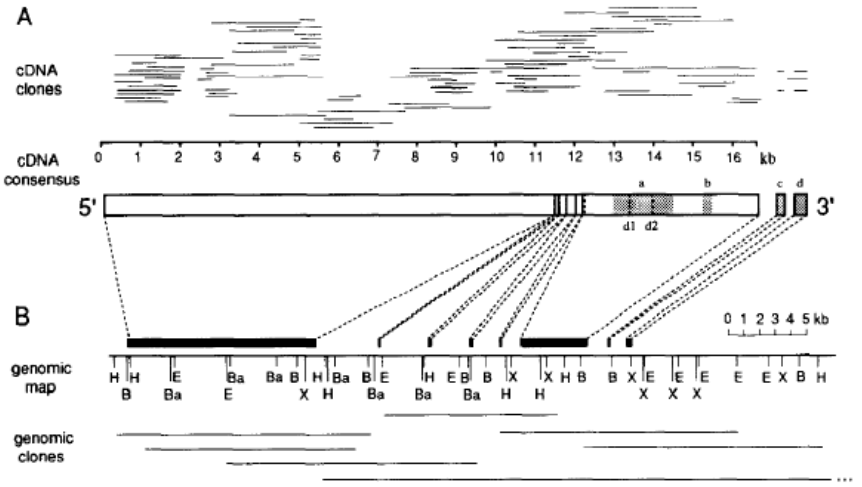
Hunt Willard
Stanford Univ., USA

Cell, Vol. 71, 527–542, October 30, 1992, Copyright © 1992 by Cell Press

## The Human *XIST* Gene: Analysis of a 17 kb Inactive X-Specific RNA That Contains Conserved Repeats and Is Highly Localized within the Nucleus

Carolyn J. Brown,*† Brian D. Hendrich,*†
Jim L. Rupert,*† Ronald G. Lafrenière,*†
Yigong Xing,‡ Jeanne Lawrence,‡
and Huntington F. Willard*†

- The XIST gene is unique among X-linked genes in being expressed exclusively from the Inactive X chromosome.
- Human XIST cDNAs containing at least eight exons and totalling 17 kb have been isolated and sequenced within the region on the X chromosome known to contain the X inactivation center.
- The *XIST* gene includes several tandem repeats, the most 5' of which are evolutionarily conserved.
- The gene does not contain any significant conserved ORFs and thus does not appear to encode a protein, suggesting that XIST may function as a structural RNA within the nucleus.
- Consistent with this, fluorescence in situ hybridization experiments demonstrate localization of XIST RNA within the nucleus to a position indistinguishable from the X inactivation-associated Barr body.




D

| EXON No. | EXON SIZE (bp) | SPLICE | SEQUENCE of 5' EXON | Intron Sequence | SEQUENCE of 3' EXON |
|---|---|---|---|---|---|
| 1 | 11,364 | 1/2 | TTAGAATACT | gtaagtacta....tgtgttccag | GATCCCATTG |
| 2 | 64 | 2/3 | GCTCCTCTTG | gtaatgacag....tcttcttaag | GACATTCTGA |
| 3 | 137 | 3/4 | GGAGAAAAAG | gtagtttggg....ctcttttgag | ATCTTCCTCA |
| 4 | 209 | 4/5 | ACACGTCAAG | gtgcgtaa.....tttttatag | CTCTTCATTG |
| 5 | 164 | 5/6 | GCTGAATGAA | gtaagttgtt.....nd | TGTGTATTTC |
| 6 | 4,543# | 5/6i | GCTGAATGAA | gtaagttgtt.....tatctaaag^ | TGTGTCTTAC |
| 7 | 146 | 7/8* | AAGCGAAAAG | gtttgtctat.....ctttcacag | TTTCTGGCAT |
| 8 | 377 | | | | |

E. Heard, May 19th, 2025

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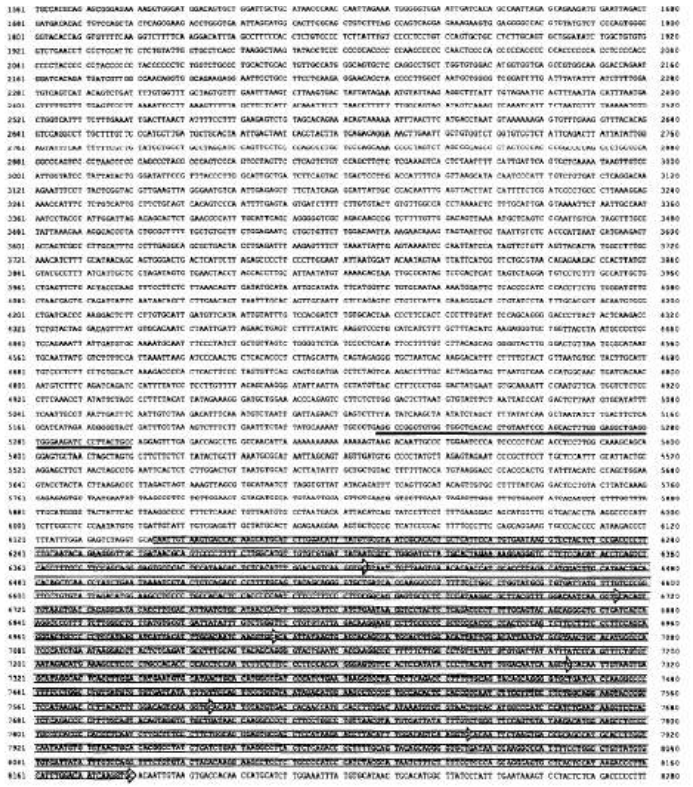
 Human Molecular Genetics, 1993, Vol. 2, No. 6 663–672

## Evolutionary conservation of possible functional domains of the human and murine *XIST* genes

Brian D.Hendrich[1,2], Carolyn J.Brown[1] and Huntington F.Willard[1]*
[1]Department of Genetics and Center for Human Genetics, Case Western Reserve University School of Medicine, Cleveland, OH 44106-4955 and [2]Department of Genetics, Stanford University, Stanford, CA 94305, USA



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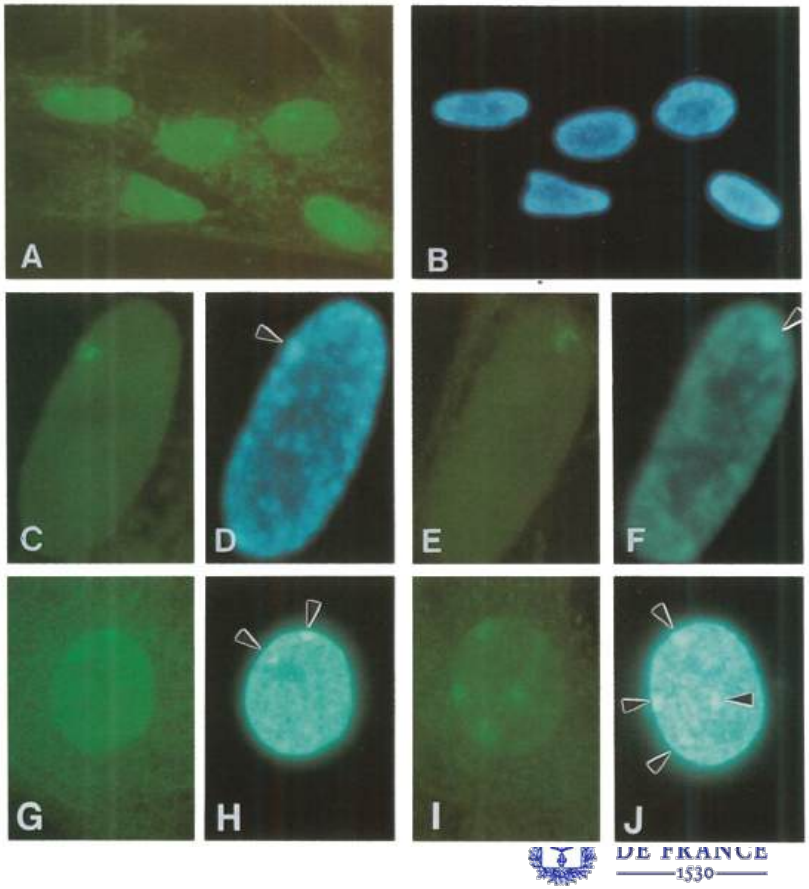
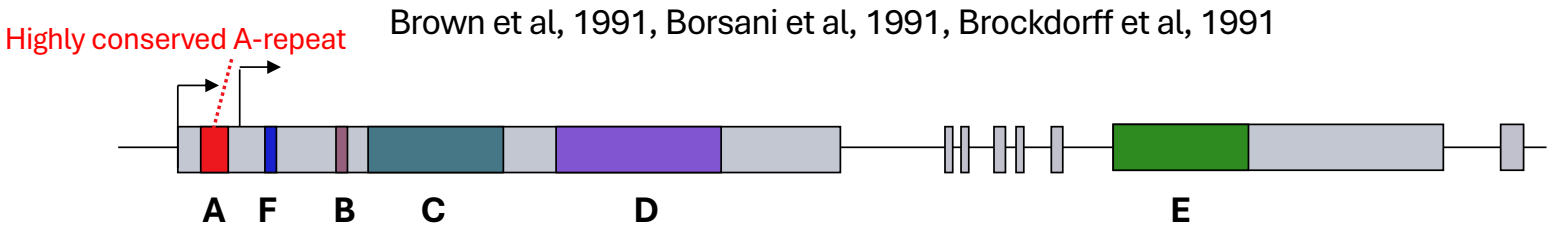
## RNA fluorescence *in situ* hybridisation (FISH)

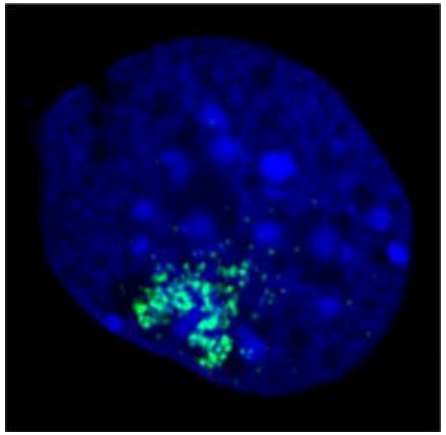
Fluorescently labelled single-stranded probe (DNA or RNA) is hybridised to cells without denaturation (only detect ssRNA not dsDNA

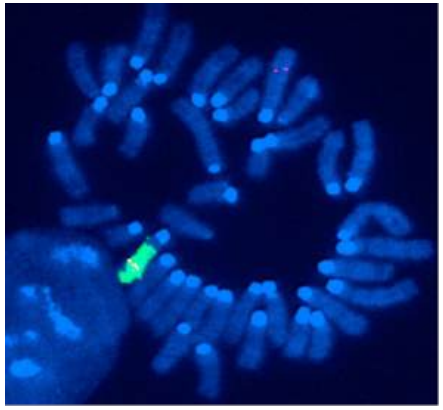



Jeanne Lawrence
UMass medical schoole
Mass., USA

# XIST (X-Inactive-Specific-Transcript)





- Xist maps in the Xic region in humans and mice
- Poor sequence conservation between mammals - except for some repeats A-F – only in eutherians not marsupias (*more next week*)
- 17 000 - 19 000 nt, alternative spliced, 5'- capped, 3' polyA tail, untranslated, nuclear transcript
- Xist RNA is expressed from and "coats" the inactive X chromosome in *cis*
- *Xist* is **essential** for X inactivation in *cis,* based on Xist KOs *(*Brockdorff and Jaenisch labs)
- Is *Xist* is **sufficient** for X inactivation in *cis ?* (Transgenesis experiments…)
- When is *Xist* is **expressed** during development ?
- How is Xist regulated ? (expressed only in XX cells or cells with >1 X; only from one X)?
- How does Xist RNA exerts its X-inactivation functions? (how does it coat the X? how does it silence genes?

*RNA FISH in female mouse cell (image E. Heard 2001)*

# Expression of *Xist* during development and ESC differentiation suggested a role in Initiation of X inactivation

Xist RNA is up-regulated upon differentiation of ES cells and only in XX not XY (or XO) cells

*Xist* is expressed from the 2-4 cell stage in female embryos (more next week)

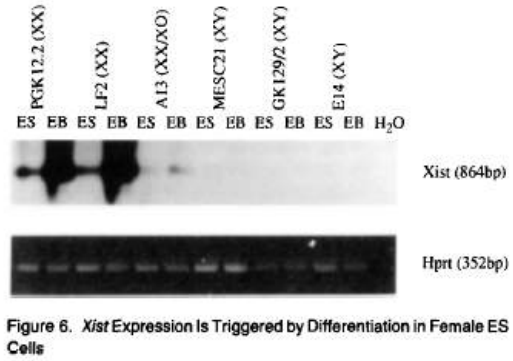
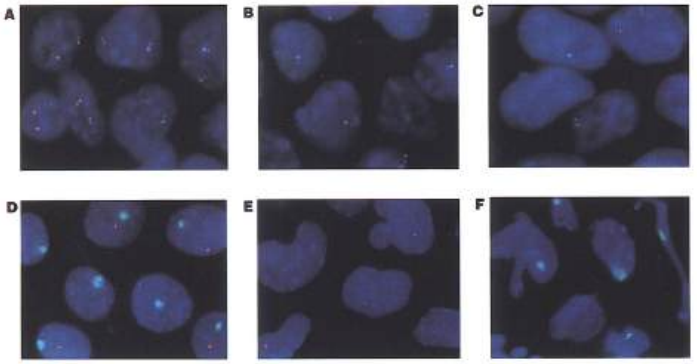



Figure 6. *Xist* Expression Is Triggered by Differentiation in Female ES Cells

Kay et al, Cell 1993

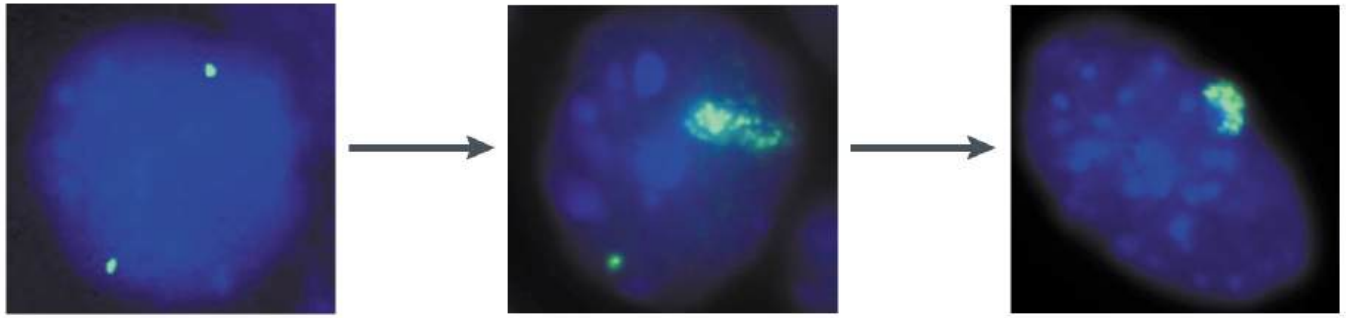



Panning and Jaenish, Genes and Dev 1995

Undifferentiated XX ES Cells

Day 2-4 differentiation

Late differentiation & somatic cells



*Xist* nascent transcription loci

Xa: nascent *Xist* transcripts
Future Xi: Xist mRNA coating

Xa: *Xist* is silent
Xi: Xist mRNA coating

Heard and Avner, Nature Reviews Genetics (2001)

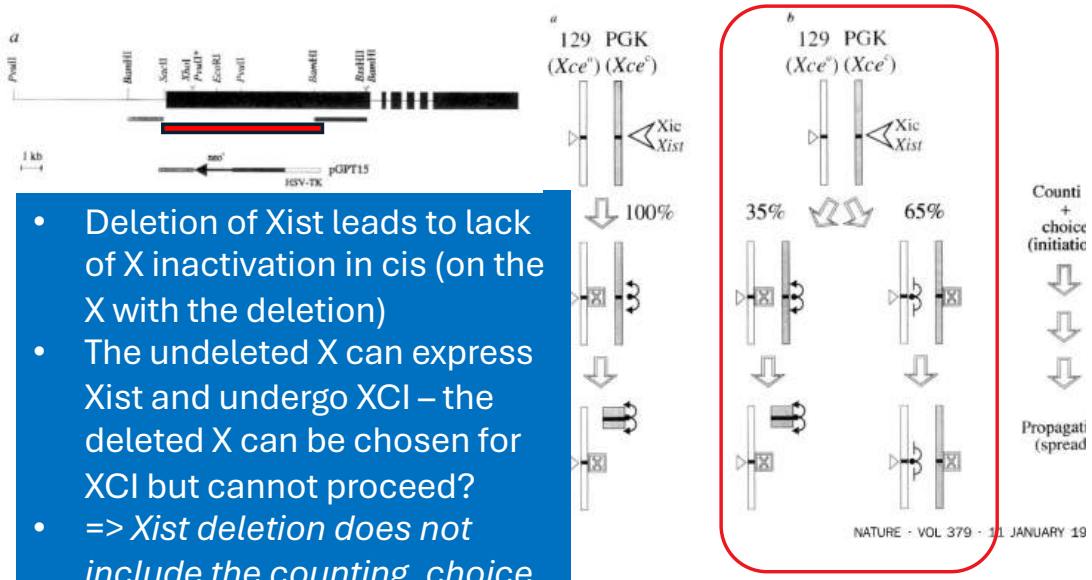
# Deletion of murine *Xist* demonstrates that it is essential for X inactivation

## Requirement for *Xist* in X chromosome inactivation

**Graeme D. Penny, Graham F. Kay*, Steven A. Sheardown, Sohaila Rastan* & Neil Brockdorff†**

Section of Comparative Biology, MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

**The *Xist* gene has been proposed as a candidate for the X inactivation centre, the master regulatory switch locus that controls X chromosome inactivation. So far this hypothesis has been supported solely by indirect evidence. Here we describe gene targeting of *Xist*, and provide evidence for its absolute requirement in the process of X chromosome inactivation.**




- Deletion of Xist leads to lack of X inactivation in cis (on the X with the deletion)
- The undeleted X can express Xist and undergo XCI – the deleted X can be chosen for XCI but cannot proceed?
- *=> Xist deletion does not include the counting, choice and sensing elements of the Xic?*

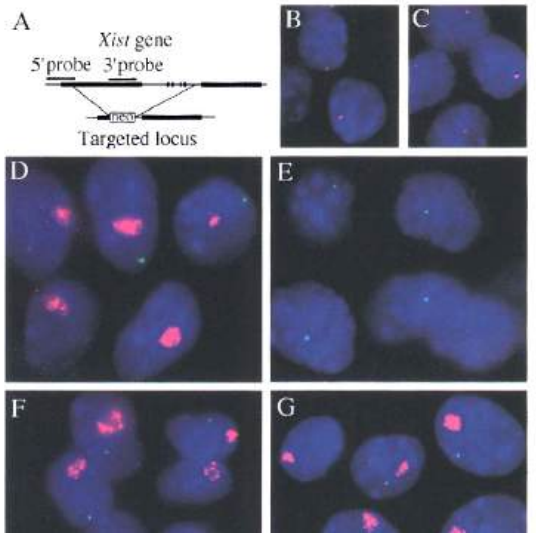
E. Heard, May 19th, 2025

## *Xist*-deficient mice are defective in dosage compensation but not spermatogenesis

**York Marahrens,[1] Barbara Panning,[1] Jessica Dausman,[1] William Strauss,[2] and Rudolf Jaenisch[1,3,4]**

[1]Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142 USA; [2]Department of Medicine, Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts 02215 USA; [3]Massachusetts Institute of Technology, Cambridge, Massachusetts 02142 USA

**The X-linked *Xist* gene encodes a large untranslated RNA that has been implicated in mammalian dosage compensation and in spermatogenesis. To investigate the function of the *Xist* gene product, we have generated male and female mice that carry a deletion in the structural gene but maintain a functional *Xist* promoter. Mutant males were healthy and fertile. Females that inherited the mutation from their mothers were also normal and had the wild-type paternal X chromosome inactive in every cell. In contrast to maternal transmission, females that carry the mutation on the paternal X chromosome were severely growth-retarded and died early in embryogenesis. The wild-type maternal X chromosome was inactive in every cell of the growth-retarded embryo proper, whereas both X chromosomes were expressed in the mutant female trophoblast where X inactivation is imprinted. However, an XO mouse with a paternally inherited *Xist* mutation was healthy and appeared normal. The imprinted lethal phenotype of the mutant females is therefore due to the inability of extraembryonic tissue with two active X chromosomes to sustain the embryo. Our results indicate that the *Xist* RNA is required for female dosage compensation but plays no role in**




re 2. Phenotypic comparison of wild-type mutant littermates at 6.5, 8.5, and 10.5 days station. Bar scale, 0.5 mm. (*A*) Whole view of 6.5 wild-type conceptus (*left*) and a mutant rmate; (*B*) whole view of an E10.5 wild-type ryo (*left*) and a mutant embryo with yolk sac trophoblast; (*C*) side view of E8.5 wild-type ryo; (*D–F*) view of mutant E8.5 conceptuses. owhead] Ectoplacental cone; [arrow] yolk sac.

Deletion of Xist leads to early death (abnormalities in both extraembryonic and embryonic lineages already visible at E6.5 )

# *Xist* is necessary for initiation of X inactivation, but is it sufficient?

Phil Avner

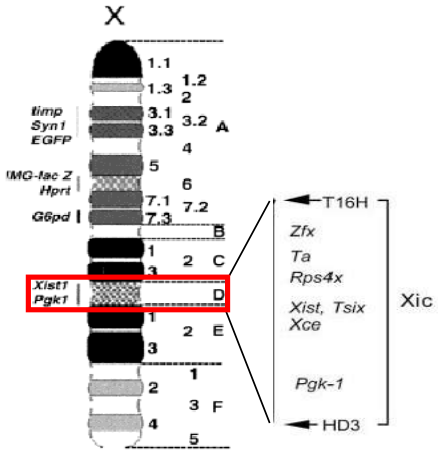
## Transgenes at ectopic sites can define sequences not only necessary but <u>sufficient</u> to induce X inactivation

Mapping and cloning the X-inactivation centre for transgenesis assays

*A 10 Mbp candidate region*

Hunt Willard
Stanford Univ., USA

Chromosome X = ~ 130 million bp



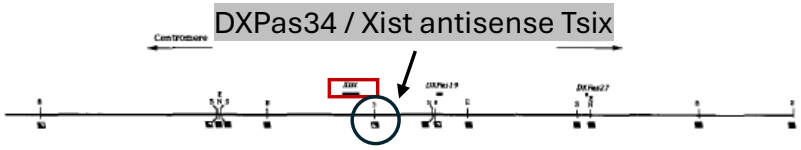

GENOMICS 15, 559–569 (1993)

**Physical Mapping and YAC Contig Analysis of the Region Surrounding *Xist* on the Mouse X Chromosome**


EDITH HEARD,[1] MARIE-CHRISTINE SIMMLER, ZOIA LARIN,* CLAIRE ROUGEULLE, BEATRICE COURTIER, HANS LEHRACH,† AND PHILIP AVNER

*Inité de Génétique Moléculaire Murine, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France; *Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom; †Genome Analysis Laboratory, Imperial Cancer Research Fund Laboratories, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, United Kingdom*


DXPas34 / Xist antisense Tsix




Functional Analysis of the *DXPas34* Locus, a 3′ Regulator of *Xist* Expression


E. DEBRAND, C. CHUREAU, D. ARNAUD, P. AVNER, AND E. HEARD*

*Unité de Génétique Moléculaire Murine, URA CNRS 1947, Institut Pasteur, Paris 75015, France*



Received 4 May 1999/Returned for modification 7 July 1999/Accepted 25 August 1999



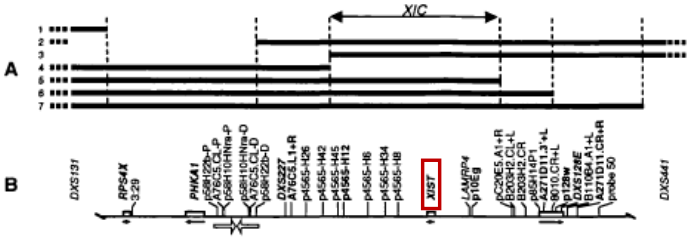


*Human Molecular Genetics, 1993, Vol. 2, No. 8 1105–1115*


**2.6 Mb YAC contig of the human X inactivation center region in Xq13: physical linkage of the *RPS4X*, *PHKA1*, *XIST* and *DXS128E* genes**


Ronald G.Lafrenière, Carolyn J.Brown, Sue Rider[1], Jamel Chelly[1], Patricia Taillon-Miller[2], A.Craig Chinault[2], Anthony P.Monaco[1] and Huntington F.Willard*

Department of Genetics and Center for Human Genetics, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106, USA, [1]Imperial Cancer Research Fund Laboratories, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK, [2]Center for Genetics in Medicine, Washington University School of Medicine, St Louis, MO 63130 and [3]Institute for Molecular Genetics and Human Genome Center, Baylor College of Medicine, Houston, TX 77030, USA


Long-range mapping using rare cutting restriction enzymes (often methylation sensitive)
Establishment of a contig of YACs (yeast artificial chromosomes) spanning the Xic



Heard et al , Genomics 1993

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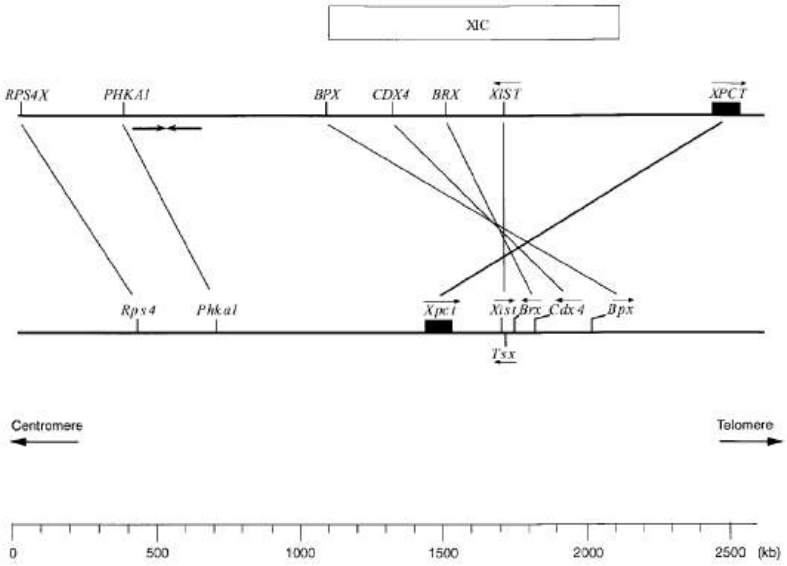
# Long range comparative maps revealed an inversion of the *Xpct* gene and that the candidate XIC region is smaller in mouse than human

Mapping and cloning the X-inactivation centre for transgenesis assays

GENOMICS 48, 296–303 (1998)
ARTICLE NO. GE975173

**Cloning and Localization of the Murine *Xpct* Gene Evidence for Complex Rearrangements during the Evolution of the Region around the *Xist* Gene**

Emmanuel Debrand,[1] Edith Heard, and Philip Avner





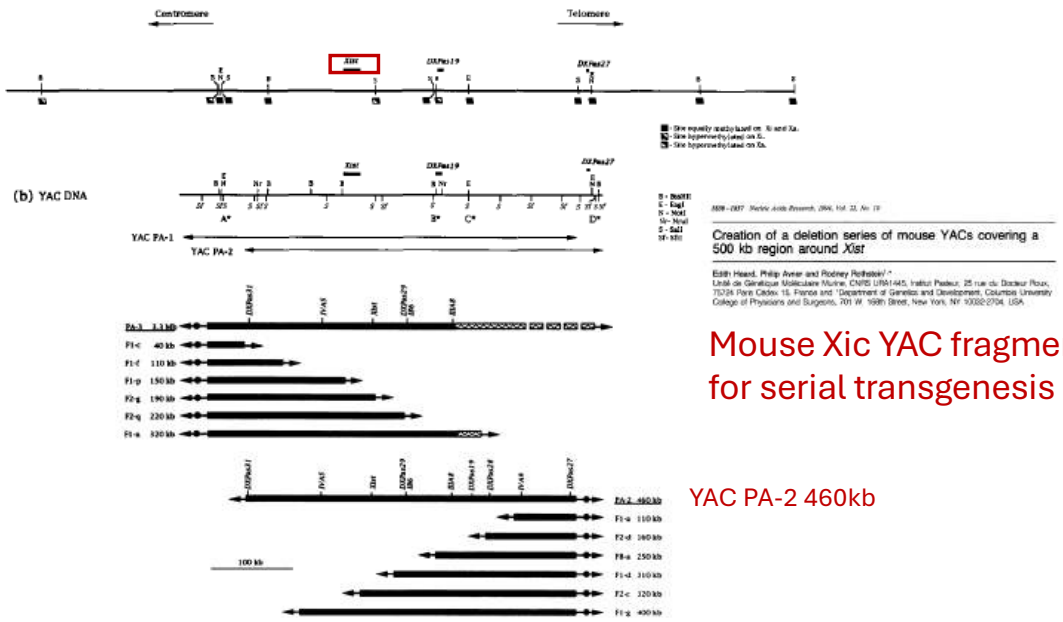

Debrand et al , Genomics 1997

# *Xist* is necessary for initiation of X inactivation, but is it sufficient?

Transgenes at ectopic sites can define sequences not only necessary but <u>sufficient</u> to induce X inactivation

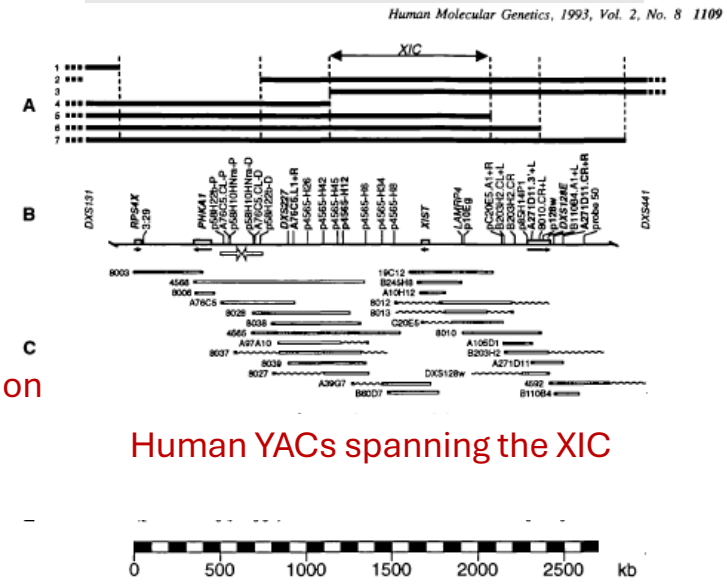
What is the minimal region required as a single copy at an ectopic site to trigger normal XCI?

MOUSE Xic candidate region




Mouse Xic YAC fragmentation for serial transgenesis

HUMAN XIC candidate region




Human YACs spanning the XIC



Heard et al Genomics 1993, Heard et al, Nucleic Acids Research 1994

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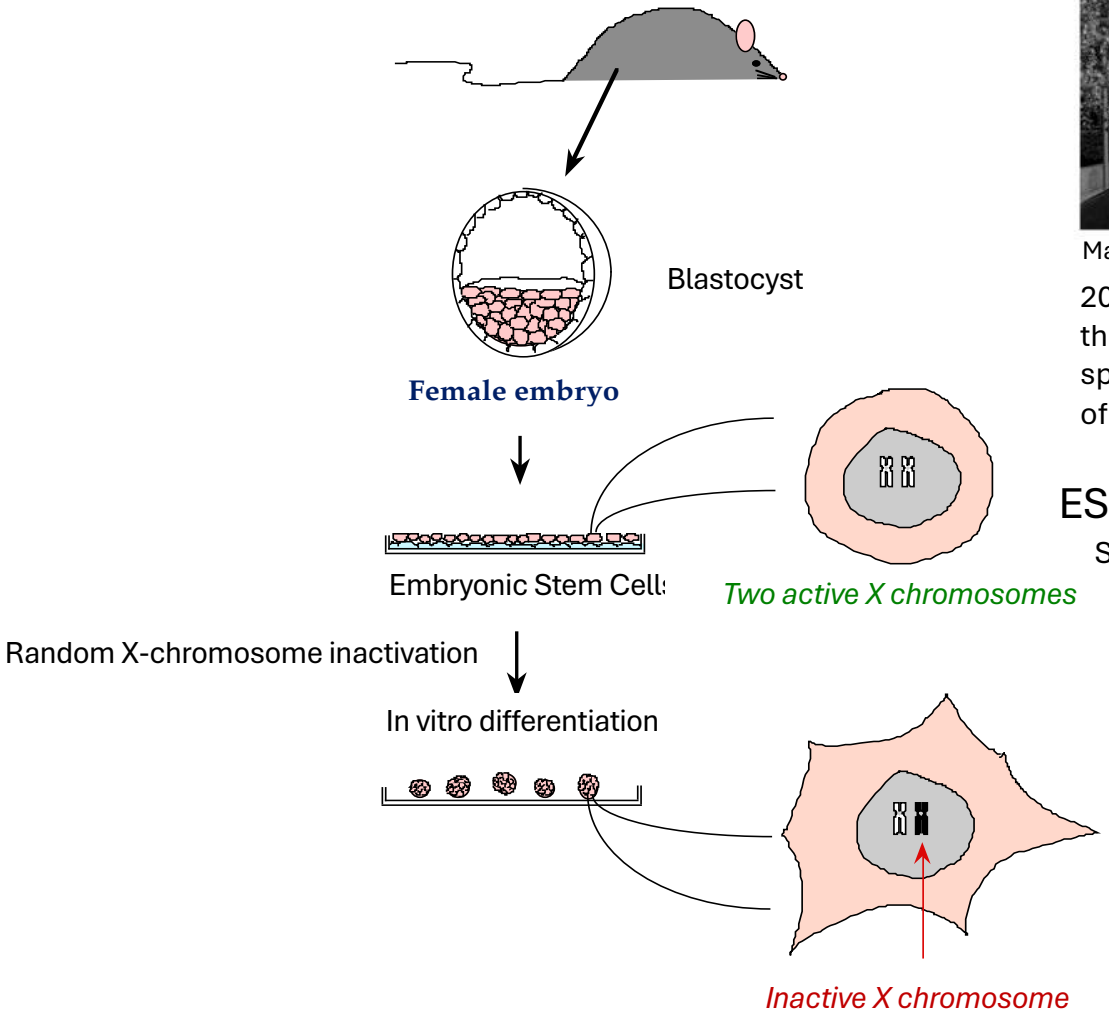
# Candidate Xic transgenesis in mouse Embryonic Stem Cells




Mario R. Capecchi, Sir Martin J. Evans, Oliver Smithies

2007 Nobel Prize in Physiology or Medicine "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells'.

## ES cells: a model system to study XCI

Sohaila Rastan and Liz Robertson1983, 1985



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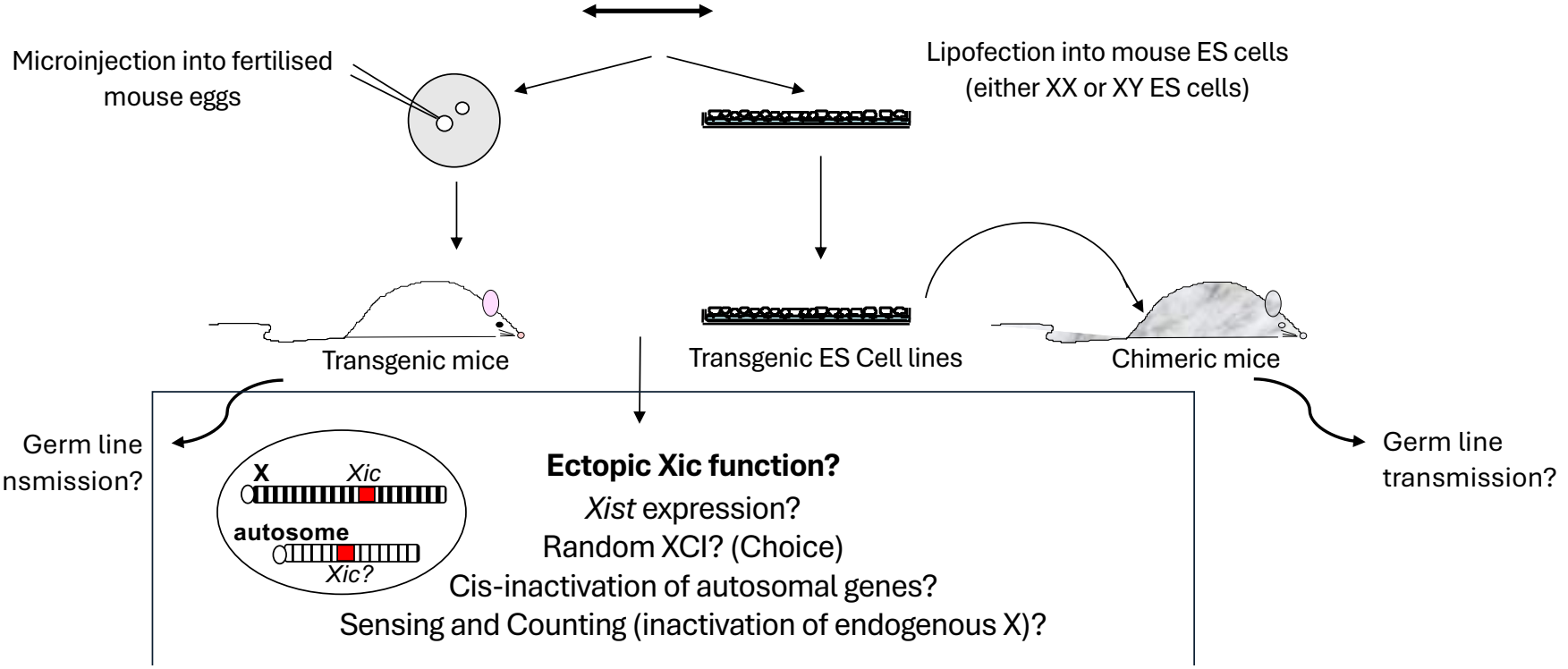
# Defining the minimal region necessary and sufficient to trigger X inactivation

Transgenes can help define the minimal region required to recapitulate Xic functions, as well as revealing how *Xist* is regulated: during differentiation , only in female cells, monoallelically?


Purified DNA of yeast artifical chromosomes from Xic region (mouse or human)
Used to make transgenes in mice and in ES cells



# Defining the region necessary and sufficient to trigger X inactivation

Transgenes can help define the minimal region required to recapitulate Xic functions, as well as how *Xist* is regulated: during differentiation , only in female cells, monoallelically?

(my post-doctoral years in the laboratory of Dr. Phil Avner, Institut Pasteur)

Charles Babinet
(1940-2008)

# Mice produced by pronuclear injection:
# Single *Xist* transgenes do not trigger XCI in *cis* or in *trans* (ie of the X)

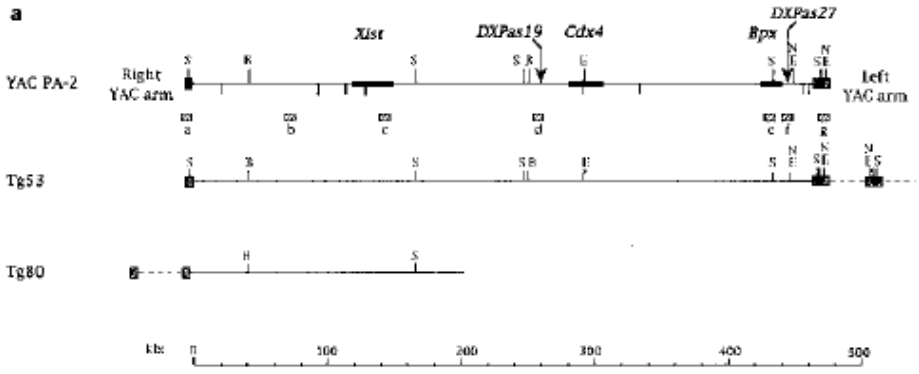


Human Molecular Genetics, 1996, Vol. 5, No. 4 441–450

## Transgenic mice carrying an *Xist*-containing YAC

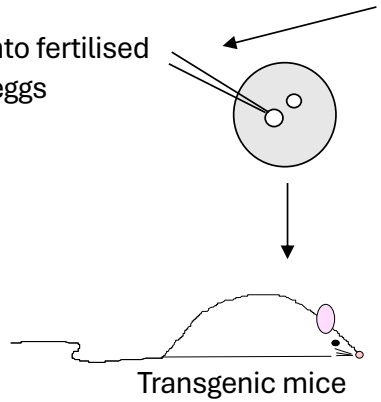
Edith Heard[1,*], Chantal Kress[2], Fabien Mongelard[3], Béatrice Courtier[1], Claire Rougeulle[1], Alan Ashworth[4], Claire Vourc'h[3], Charles Babinet[2] and Philip Avner[1]

To test whether sequences spanning several hundred kilobases and including *Xist* from the Xic region are capable of initiating inactivation, we have created a series of transgenic mice using a 460 kb yeast artificial chromosome (YAC). Analysis in these mice of the expression of Xist, of a LacZ reporter gene and of two genes in the region that are normally silent on the inactive X chromosome, suggests that **essential sequences for *Xist* expression and X-inactivation may be absent in these transgenic animals.**




Purified DNA of yeast artifical chromosomes from Xic region (mouse or human)
Used to make transgenes in mice and in ES cells

Microinjection into fertilised mouse eggs



Transgenic mice

Autosomal $A^{tg\ YAC}$
Only single copy YAC Tg mice
No sign of cis-inactivation
Or any abnormalities....

Heard et al, Hum. Mol. Gen, 1996



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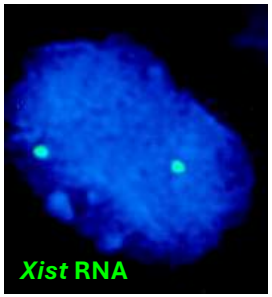
# Single *Xist* transgenes do not trigger XCI in *cis* or in *trans* (ie of the X)

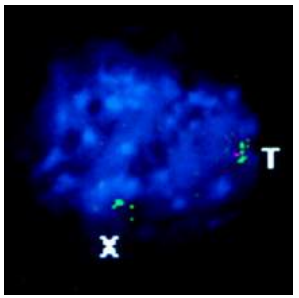
**Undifferentiated**
**Transgenic male ES cells**




Single copy YAC Tg

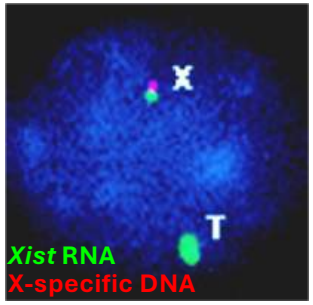
7 independent single copy lines

**Differentiated**
**ES cells**



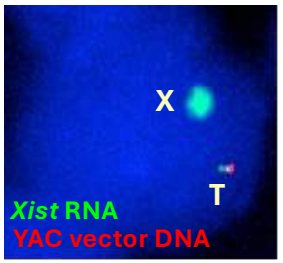

No counting
No cis-inactivation

# Multicopy *Xist* transgenes CAN induce XCI in *cis* (and to a much lesser extent of the X)

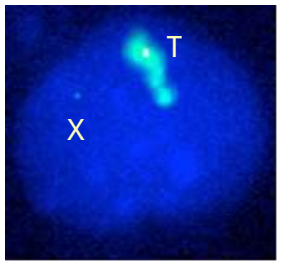



Multicopy YAC Tg

6 independent multicopy lines




Very rare Xi accumulation




Transgenic *Cis*-inactivation

Heard et al, Hum. Mol. Gen, 1996
Heard et al, Mol. Cell Biol., 1999
Heard et al, PNAS, 1999



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# ES cells with autosomal multicopy h*XIST* YAC transgenes can trigger XCI

*Proc. Natl. Acad. Sci. USA*
Vol. 96, pp. 6841–6846, June 1999
Genetics

**Human *XIST* yeast artificial chromosome transgenes show partial X inactivation center function in mouse embryonic stem cells**

EDITH HEARD*†, FABIEN MONGELARD‡, DANIELLE ARNAUD*, CORINNE CHUREAU*, CLAIRE VOURC'H‡, AND PHILIP AVNER*

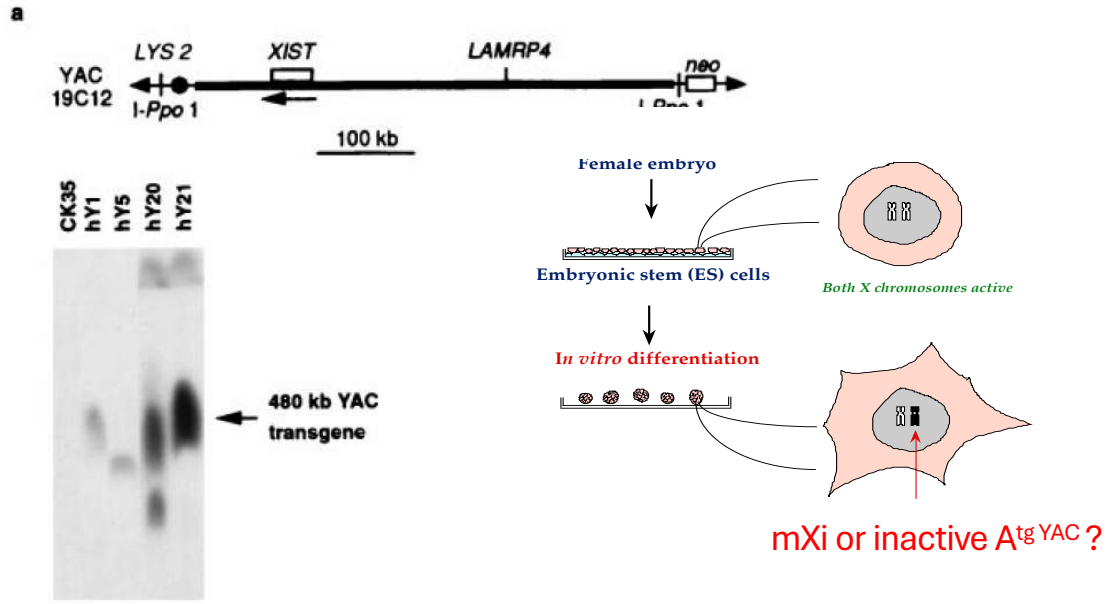
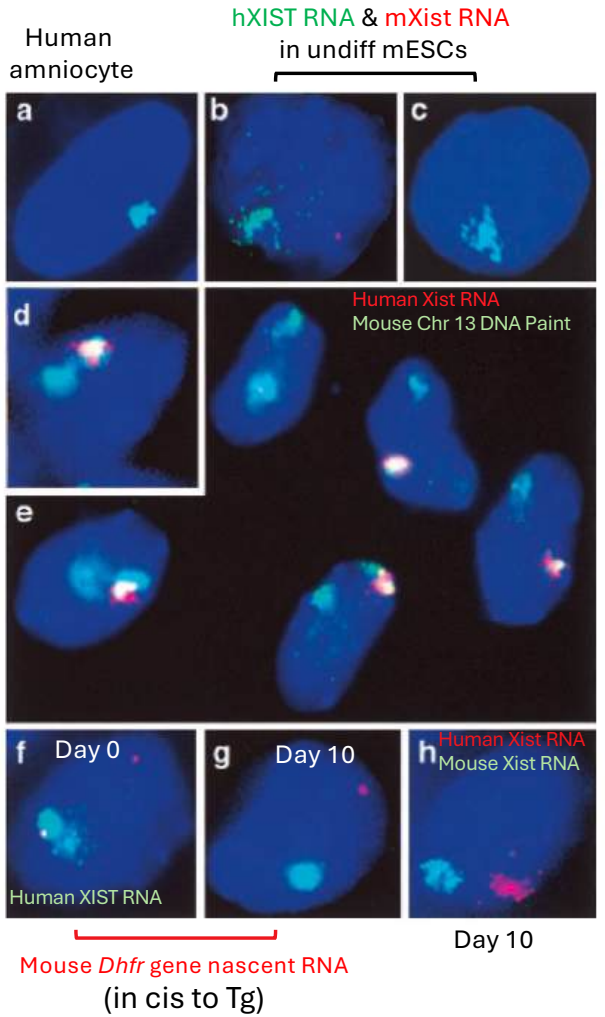



Table 1. *XIST* and *Dhfr/Rep-3* expression patterns in transgenic line hY21

| | | | | | Total no. of cells with *XIST* RNA domains |
|---|---|---|---|---|---|
| Undifferentiated | 44 (20%) | 72 (32%) | 54 (24%) | 54 (24%) | 224 |
| Differentiated | 33 (11%) | 39 (13%) | 131 (45%) | 88 (30%) | 291 |

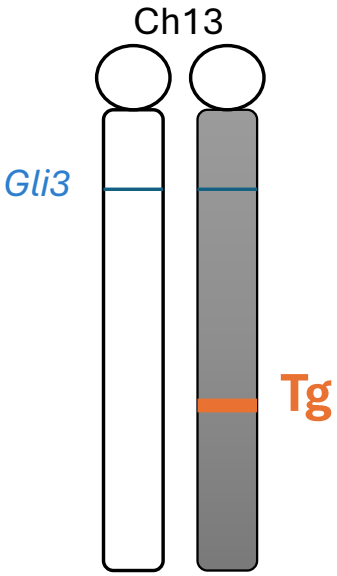
Key: ⊘ *XIST* RNA domain • *Dhfr-Rep3* RNApinpoint
NB Only cells with dense *XIST* RNA domains were considered.

Heard et al, PNAS 1999



# Chimeric mice produced from ES cells with autosomal multicopy h*XIST* YAC transgenes show severe phenotypes and no germ-line transmission

Chromosome 13 multicopy hXIST YAC transgenes –> Polydactyly phenotype
Known semi-dominant autosomal disorder, due to *Gli3* haploinsufficiency




**Gli3 -** Transcription factor in the Hedgehog signaling pathway
•Location: Mouse Chromosome 13 (Chr13qA5)
**Phenotypic Effects in Mice (Haploinsufficiency):**
1.Polydactyly:
2.Mice with heterozygous mutations in Gli3 (e.g., Gli3Xt/+, also known as extra toes) often show extra digits (usually preaxial or postaxial polydactyly).
3. Gli3 mutations can lead to optic nerve hypoplasia, coloboma, and other developmental eye abnormalities.
4.Craniofacial anomalies
5.Abnormal brain development (especially telencephalon)

- No multicopy mouse *Xist* YAC transgenic mice by pronuclear injection – only single copy Tgs
- Single-copy mXist YAC transgene (Tg53) on Chromosome 13 –> no apparent phenotype
- No transgenic Xist expression ; other genes on the YAC expressed normally
- Efficient germ-line transmission

Heard et al, HMG 1996
Heard et al, unpublished

# Defining the minimal region necessary and sufficient to trigger X inactivation

Transgenes can help define the minimal region required to recapitulate Xic functions, as well as revealing how *Xist* is regulated: during differentiation , only in female cells, monoallelically?


Clone and purify large fragments of DNA as yeast artifical chromosomes
Use these to make transgenes in mice and in ES cells

**Only single copy *Xist* YAC Tg mice obtained, with no sign of Tg cis-silencing. In ES cells, even the largest transgenes (500kb) did not function as single copy Xics to induce random XCI in cis or of the X (in male and female ESCs)**

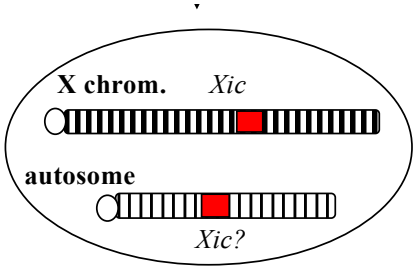
**WHY ?**

**Missing Sequences?**

Enhancers? Nuclear localisation? Chromatin state?

Germ line transmission?

Single copy *Xist* Tgs during imprinted XCI
Okamoto et al, Nature 2005
(Next Week!)




**Single copy Xist Tg**

**No** *Xist* accumulation from Tg &/or X

=> Does not behave like the Xic in X;A translocations

**Multicopy Xist Tg**

*Xist* expression from Tg; rarely from endogenous X

=> Does not behave like the Xic in X;A translocations

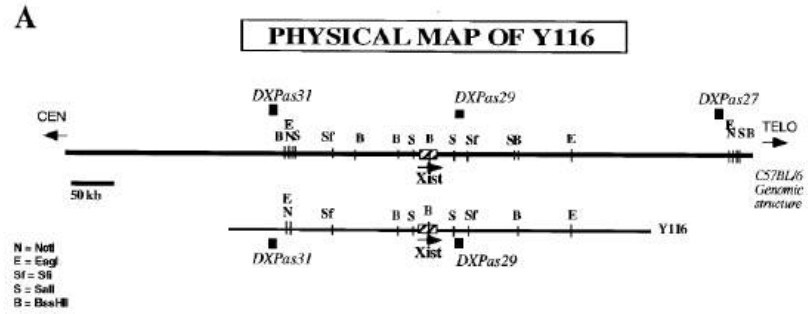
# Multicopy *Xist* transgenes express Xist in differentiating ESCs can trigger XCI in cis

Multicopy arrays are not proof that *Xist* alone is sufficient either in cis, or for Xic function
There must be missing sequences to enable full Xic function as single copies

Cell, Vol. 86, 83–94, July 12, 1996, Copyright ©1996 by Cell Press

## A 450 kb Transgene Displays Properties of the Mammalian X-Inactivation Center

Jeannie T. Lee,*† William M. Strauss,‡
Jessica A. Dausman,* and Rudolf Jaenisch*



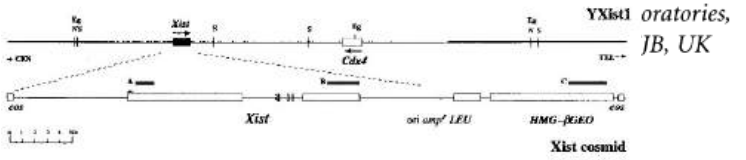

## Long-range *cis* effects of ectopic X-inactivation centres on a mouse autosome

Jeannie T. Lee*† & Rudolf Jaenisch* Nature 1997

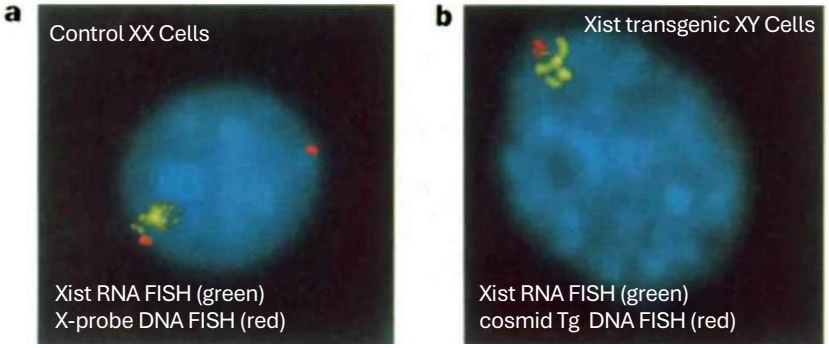
## *Xist* has properties of the X-chromosome inactivation centre

Nature 1997

Laura B. K. Herzing, Justyna T. Romer,
Jacqueline M. Horn & Alan Ashworth




40kb Xist cosmid
Transfected into male ESCs

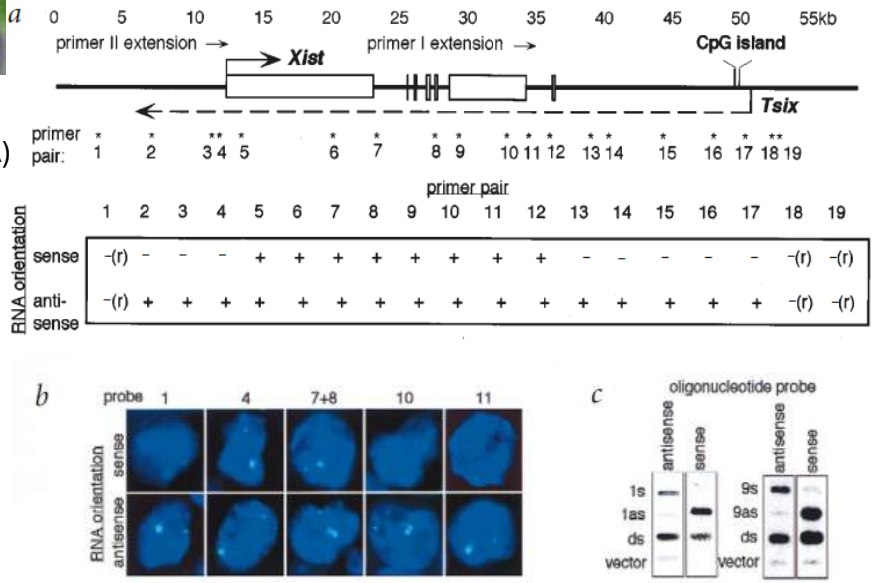


# 3’ antisense transcription across the mouse *Xist* gene (*Tsix*)

Jeannie Lee
(Harvard , USA)

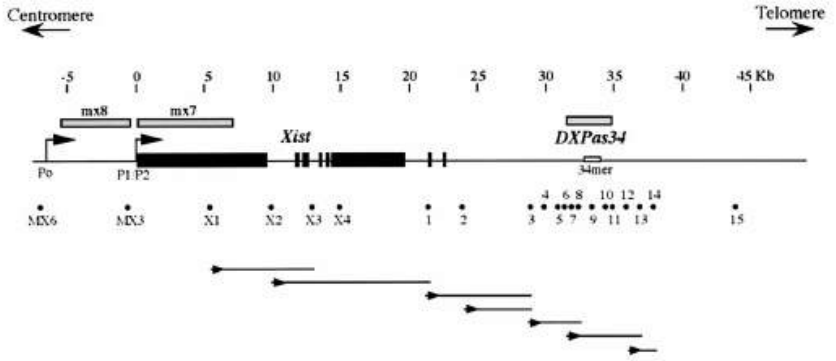
**Tsix, a gene antisense to Xist at the X-inactivation centre**

Jeannie T. Lee, Lance S. Davidow & David Warshawsky




Functional Analysis of the *DXPas34* Locus, a 3′ Regulator of *Xist* Expression

E. DEBRAND, C. CHUREAU, D. ARNAUD, P. AVNER, AND E. HEARD*




- Deletion of *DXPas34 / Tsix* promoter leads to systematic *Xist* up-regulation on the deleted X (ie fully skewed, non-random XCI)
- *Tsix* is a cis-repressor of *Xist*
- *Tsix* impacts choice (not counting) and is not the *Xce*

**Random primed RT-PCR analysis**

| | PCR rounds | 5′ *Xist* MX 6 | 5′ *Xist* MX 3 | *Xist* X1 | *Xist* X2 | *Xist* X3 | *Xist* X4 | *Xist* 1 | 3′ *Xist* 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Undifferentiated XX ES cells | One | - | + | nt | + | nt | nt | nt | + | + | + | + | + | + | + | nt | + | - | - | - | - | - |
| | Two | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Differentiated XX ES cells | One | nt | - | nt | + | nt | nt | nt | + | - | - | - | - | - | - | nt | - | - | - | - | - | - |
| | Two | nt | + | + | + | + | + | + | + | - | + | + | + | + | + | + | +- | - | - | - | - | - |
| Female somatic tissue | One | nt | - | nt | + | nt | nt | nt | + | - | - | - | - | +- | +- | nt | +- | - | - | - | - | - |
| | Two | nt | - | + | + | + | + | + | + | - | + | + | + | + | + | - | + | - | - | - | - | - |



For references see Furlan and Galupa, Cells 2022 and Augui et al, NRG 2011

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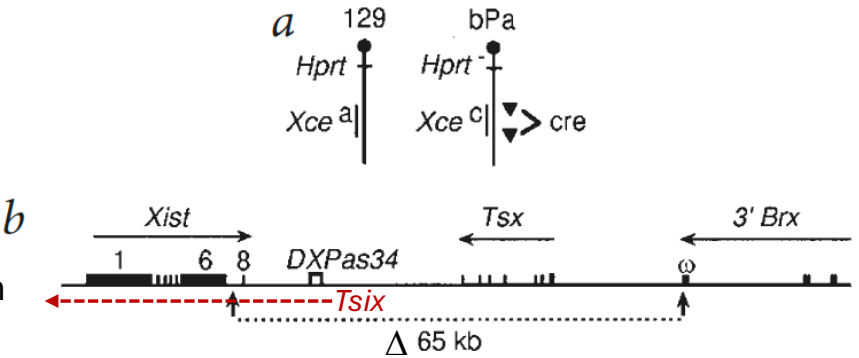
# A 65 kb deletion 3' to *Xist* disrupts counting

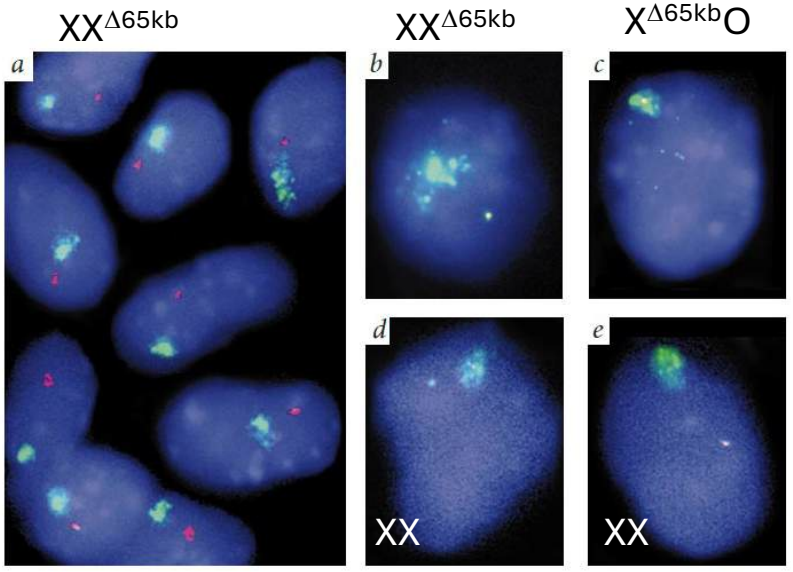
**Role of the region 3´ to *Xist* exon 6 in the counting process of X-chromosome inactivation**

Philippe Clerc & Philip Avner

- The X inactivation centre (Xic) is initially 'sensed and 'counted' : at least two copies of Xic must be present per diploid genome in order for inactivation to occur.
- 65-kb cre/loxP deletion 3´to Xist exon 6 which includes *DXPas34* and the *Tsix* promoter
- In differentiating XX ES cells containing one deleted X chromosome, X inactivation still occurs but is never initiated from the intact X chromosome.
- In differentiating XO cells, the mutated Xic is capable of initiating X inactivation, even in the absence of another Xic.
- => role for this region 3´ to Xist exon 6 in counting process (unlike *Tsix*)
- Counting ensures that one X chromosome remains active in diploid cells.
- Counting is mediated by a repressive mechanism which prevents inactivation of a single X chromosome in diploid cells.

E. Heard, May 19th, 2025




- Deletion of 65 kb region 3' to *Xist* leads to systematic *Xist* up-regulation on the deleted X whether or not a second X is present!
- *Region is important for counting*

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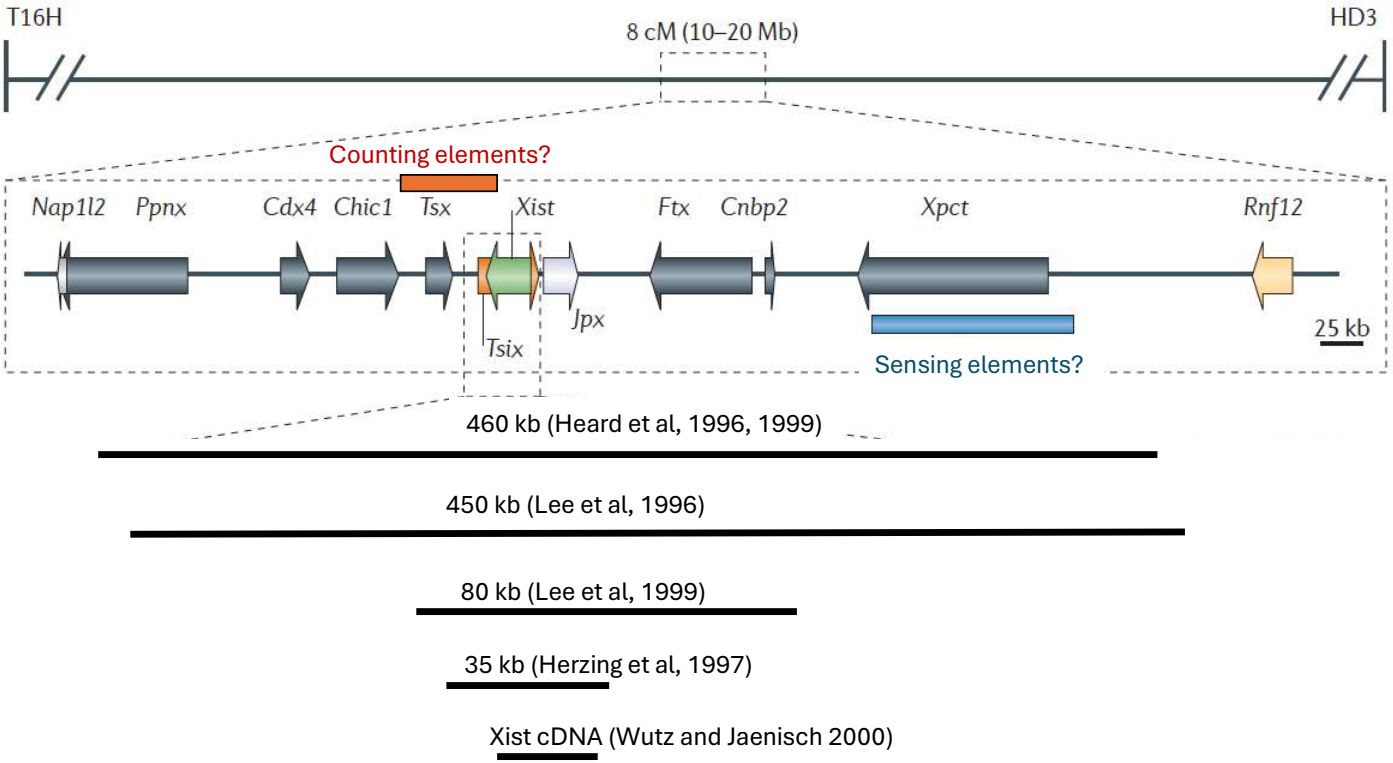
# The Xic is a complex locus including multiple regulators in addition to *Xist*

Numerous deletions in the region around *Xist*. Almost all produce some effect on *Xist* expression patterns.

See Furlan and Galupa 2022
Augui et al 2011




- Is Xist RNA the sole trigger of X inactivation in cis?
- What are the missing elements that can recapitulate
  (i) accurate Xist expression (ii) sensing, counting and choice

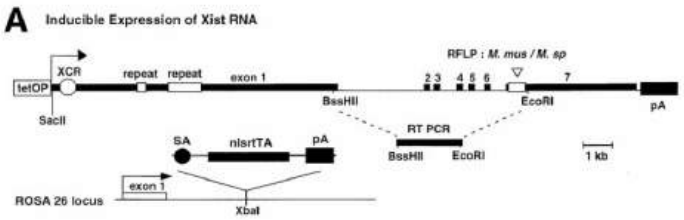
# Inducible single-copy Xist cDNA Tgs: Xist RNA is sufficient to trigger for cis-inactivation

Molecular Cell, Vol. 5, 695–705, April, 2000, Copyright ©2000 by Cell Press

**A Shift from Reversible to Irreversible X Inactivation Is Triggered during ES Cell Differentiation**

Anton Wutz and Rudolf Jaenisch*

function of the *Xic* c




Inducible Xist cDNA transgenes on autosomes in male ES cells

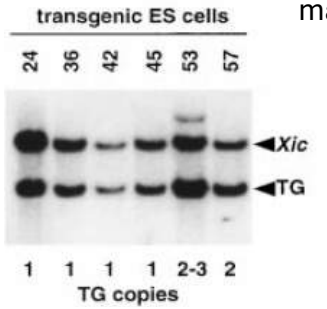



Table 1. Summary of Transgene Integrations

| ES Clone | TG Copies | Chromosomal Location | RNA Paints Dif | H4 Hypoacetylation Dif | Late Replication Dif | Repression of Genes ES | Repression of Genes Dif | Cell Death ES | Cell Death Dif |
|---|---|---|---|---|---|---|---|---|---|
| 24 | 1 | X | ND | ND | ND | ND | ND | + | + |
| 36 | 1 | 11 | + | + | + | + | + | – | – |
| 42 | 1 | 11 | + | + | ND | + | ND | – | + |
| 45 | 1 | 18 | + | ND | ND | ND | ND | – | – |
| 53 | 2–3 | 12 | + | + | + | ND | ND | – | – |
| 57 | 2 | 1 | + | + | ND | ND | ND | – | – |
| 65 | 1 | X | + | – | ND | + | ND | + | + |

Define the time window during which Xist RNA can silence in cis

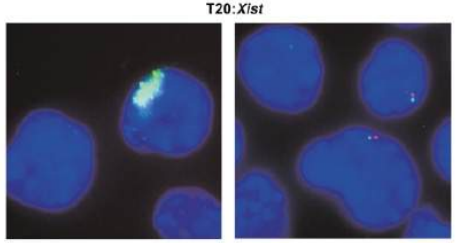
Define the functional domains of Xist RNA using systematic deletion approach

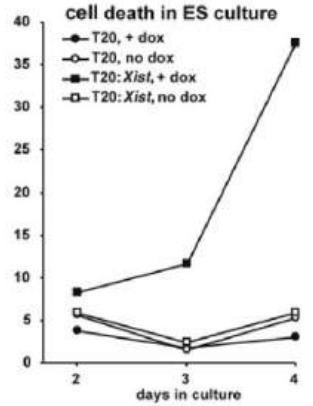
Anton Wutz Rudoph Jaenisch
Whitehead Inst, USA

**Chromosomal silencing and localization are mediated by different domains of *Xist* RNA**

Anton Wutz, Theodore P. Rasmussen & Rudolf Jaenisch

Inducible Xist cDNA on the single X chromosome in male ES cells

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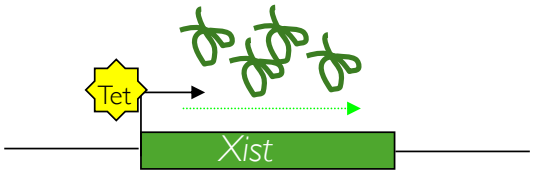
Wutz et al Cell 2001, Nat. Genet. 2002

# Inducible single-copy Xist cDNA Tgs: Xist RNA is sufficient to trigger for cis-inactivation

(but NO sensing, counting, choice)

Inducible *Xist* cDNA transgenes on Ch11 :




**Cellular Memory?**
**Epigenetic Marks?**

Xist-dependent → Xist-independent ?

Days of *in vitro* differentiation (ES cells)

**INITIATION** **IRREVERSIBLE INACTIVATION**

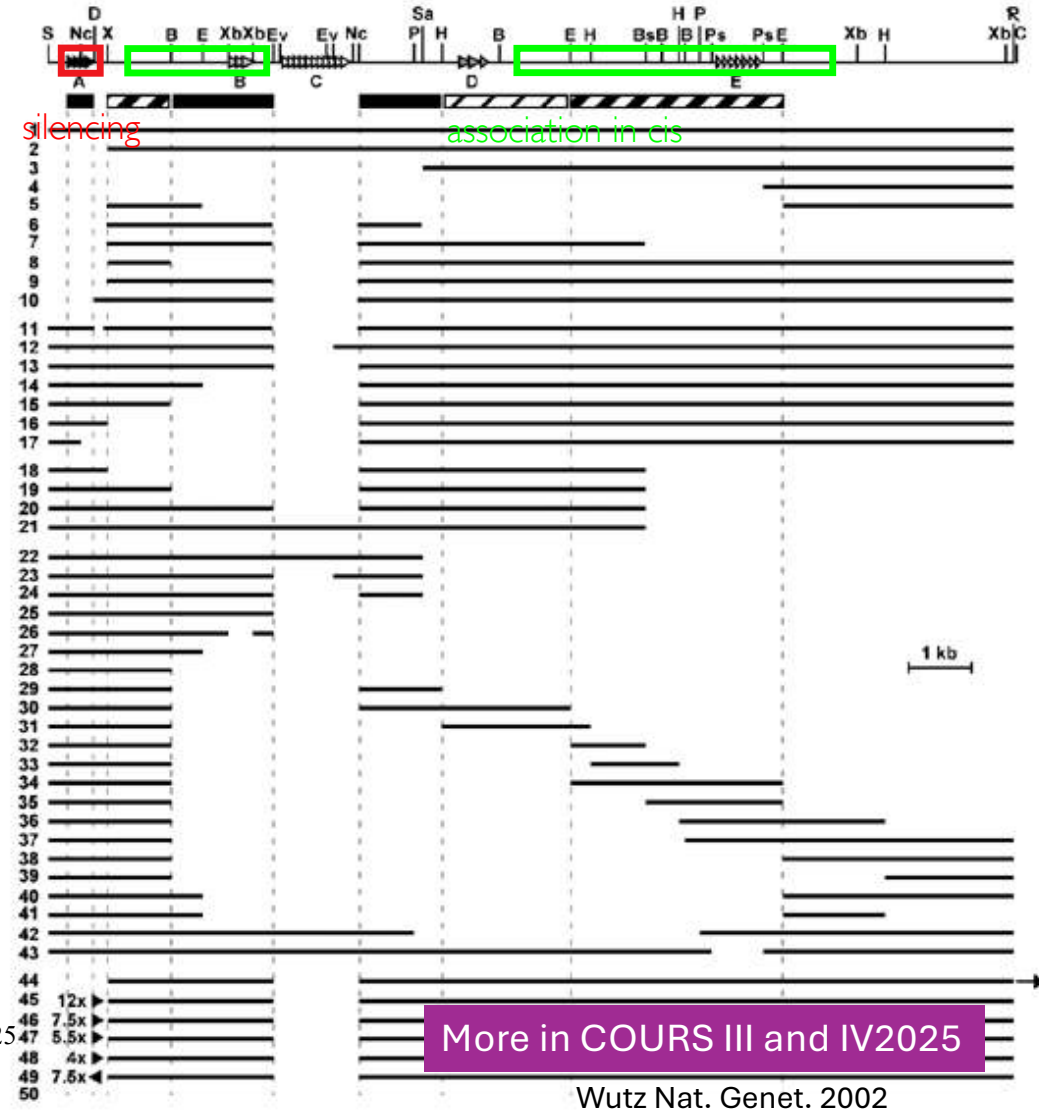
**0 1 2 3 4 5 6 7 8 9**

Xist RNA coating begins in XX cells

Transcriptional silencing begins

(*Northerns, allele-specific RT PCR, RNA FISH*)

# Functional Domains of Xist RNA




Wutz et al, Nature Genetics 2002

| construct | survival % | n | localization clusters | P | MCB |
|---|---|---|---|---|---|
| Xist | 23 ± 8 | 12 | +++++ | + | + |
| ΔSX | 93 ± 4 | 3 | ++++ | + | + |
| ΔSSa | 92 ± 8 | 2 | - | | |
| ΔSPs | 89 ± 8 | 3 | - | | |
| ΔEΔSX | 96 ± 5 | 3 | - | | |
| ΔEvNΔSCΔSX | 81 ± 2 | 2 | - | | |
| ΔEvNΔBCΔSX | 90 ± 1 | 2 | - | | |
| ΔBNΔSX | n.d. | | + | | |
| ΔEvNΔSX | 88 ± 1 | 2 | ++++ | + | |
| ΔEvNΔDX | 103 ± 8 | 3 | ++++ | | |
| ΔEvNΔSD | 26 ± 11 | 3 | +++++ | | |
| ΔEv | 25 ± 14 | 2 | +++++ | | |
| ΔEvN | 21 ± 7 | 2 | +++++ | | |
| ΔEN | 24 | 1 | +++++ | | |
| ΔBN | 25 | 1 | +++++ | | |
| ΔXN | 39 ± 5 | 2 | +++ | | |
| ΔN | 88 ± 6 | 2 | - | | |
| ΔXNΔBsC | 66 ± 5 | 2 | ++ | | |
| ΔBNΔBsC | 26 ± 6 | 4 | ++++ | | |
| ΔEvNΔBsC | 21 ± 0 | 2 | ++++ | | |
| ΔBsC | 33 ± 6 | 8 | ++++ | + | +/- |
| ΔSaC | 34 ± 9 | 9 | ++++ | | - |
| ΔEvΔSaC | 44 | 1 | ++++ | | |
| ΔEvNΔSaC | 37 | 1 | ++++ | | |
| ΔEvC | 41 ± 11 | 11 | +++ | | - |
| ΔEvCΔXb | 39 ± 18 | 2 | +++ | | |
| ΔEC | 64 ± 10 | 8 | + | | |
| ΔBC | 76 ± 8 | 15 | - | | |
| ΔBNΔHC | 34 ± 9 | 4 | n.d. | | |
| ΔBNΔEC | 30 ± 9 | 4 | n.d. | | |
| ΔBC-H2.3 | 52 ± 14 | 4 | n.d. | | |
| ΔBC-EBs1.2 | 58 ± 1 | 2 | n.d. | | |
| ΔBC-H1.2 | 56 ± 0 | 2 | n.d. | | |
| ΔBC-E3.3 | 32 ± 14 | 4 | n.d. | | |
| ΔBC-EBs2K | 77 ± 13 | 2 | n.d. | | |
| ΔBC-H3.3 | 63 | 1 | n.d. | | |
| ΔB | 50 ± 7 | 9 | n.d. | | |
| ΔBC-ES3.7 | 77 ± 2 | 2 | n.d. | | |
| ΔBC-H2.1 | 50 ± 8 | 4 | n.d. | | |
| ΔE | 65 ± 12 | 11 | +++ | | - |
| ΔEΔHC | 60 ± 15 | 3 | +++ | | |
| ΔP | 27 ± 5 | 4 | +++++ | + | |
| ΔPs | 22 | 1 | +++++ | | + |
| ΔSX-3'-SX | 43 ± 3 | 2 | n.d | | |
| ΔEvN:XCR 12 | 17 ± 10 | 2 | n.d | | |
| ΔEvN:XCR 7.5 | 36 ± 13 | 4 | +++++ | | + |
| ΔEvN:XCR 5.5 | 46 ± 16 | 4 | +++ | | |
| ΔEvN:XCR 4 | 80 ± 2 | 2 | +++ | | |
| ΔEvN:XCR 7.5AS | 96 ± 1 | 2 | ++++ | | |
| control T20 cells | 93 ± 7 | 4 | n.a. | | |

More in COURS III and IV2025

Wutz Nat. Genet. 2002



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# What are the missing elements (and factors) that would enable full Xic function?

The fact that large single copy Tgs cannot function as an ectopic Xic and that almost every deletion/mutation in the vicinity of Xist affects initiation of XCI in some way, suggests that there are long range elements  several hundreds of kilobases away from *Xist* that are important for correct Xist regulation and also for full Xic functions: sensing, counting, choice

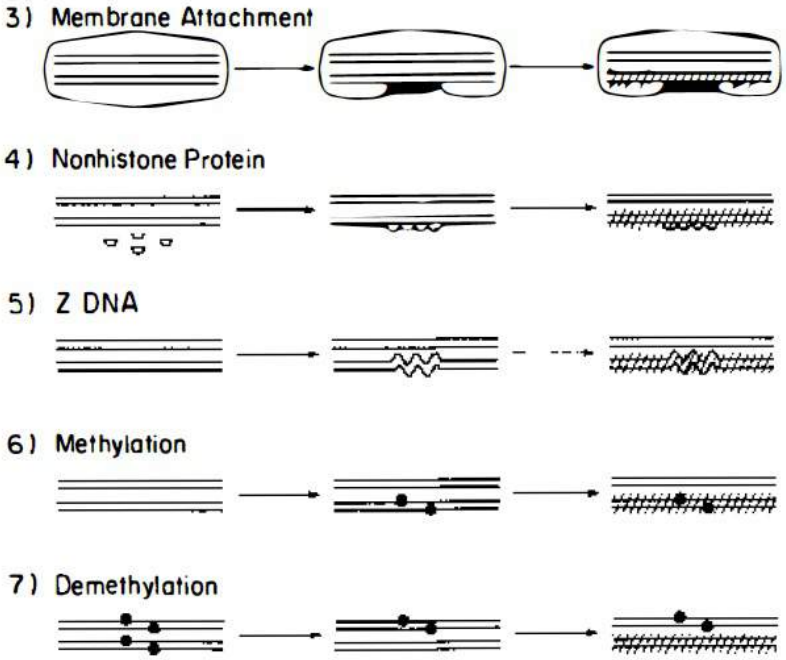


**Fig. 2** A possible model for activation of one *X* chromosome by autosomes. A molecule from the autosomal pair initiates gene activity by the activation centre, Ac, which in turn activates the non-histone protein genes (projections) and also a repressor (Rp) which represses the autosomal genes. For meanings of A, B and C see Fig. 1.

Comings, 1968

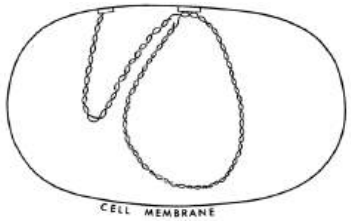


FIG. 1.—The bacterial replicon as proposed by Jacob *et al.* (1963). Before initiation of DNA synthesis, the circular bacterial chromosome is attached at a specific place on the chromosome (replicator) and to a specific location on the bacterial cell membrane (mesosome or mesosome-like structure). DNA synthesis is initiated at this point of attachment to the membrane, and replication always occurs in proximity to the membrane. The segregation of the two daughter strands is controlled by their attachment to the cell membrane (shown here in exaggerated separation).



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# What are the missing loci that would enable full Xic function?

Nuclear positioning

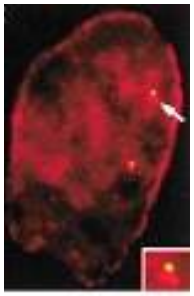
Nuclear compartmentalisation

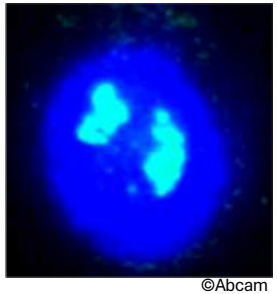
Chromosome organisation

Transcription

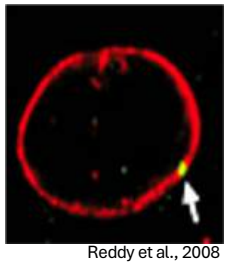
Splicing

Chromatin states



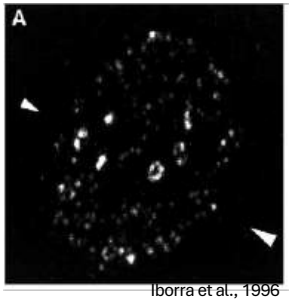


©Abcam

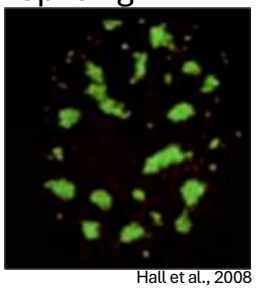


Reddy et al., 2008

Peric-Hupks et al., 2010

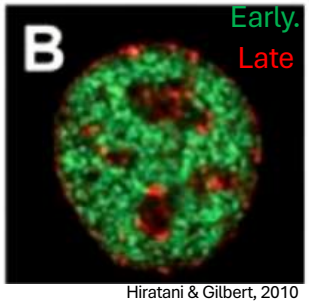



Iborra et al., 1996

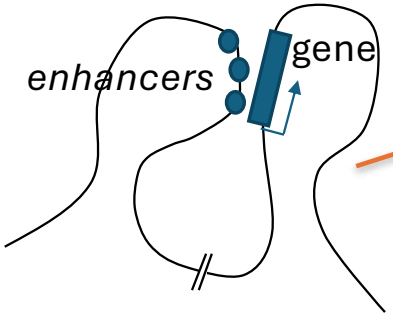


Hall et al., 2008

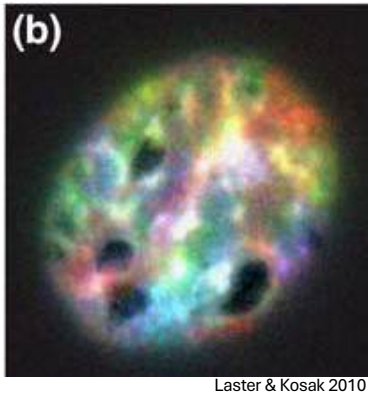
Replication timing




Hiratani & Gilbert, 2010




Long range regulators




Laster & Kosak 2010



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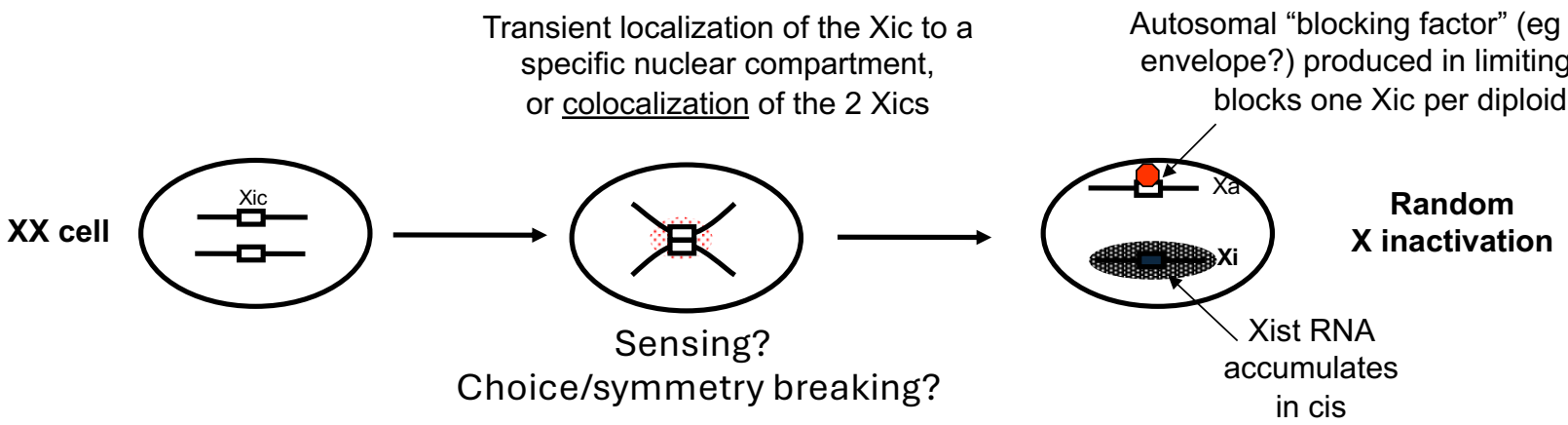
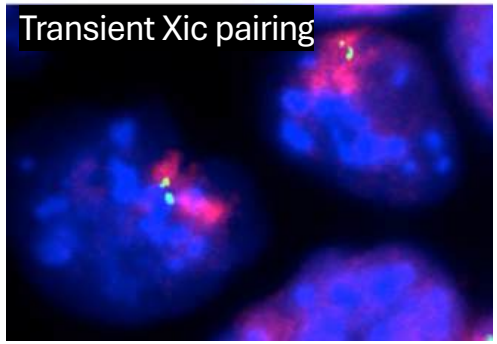
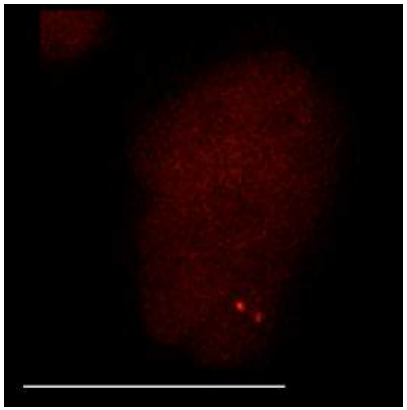
# Regulation of counting and choice by nuclear localization?



**Nuclear positioning and pairing of X-chromosome inactivation centers are not primary determinants during initiation of random X-inactivation**

Tim Pollex[1,2] and Edith Heard[1,*]

Tim Pollex

Bacher et al (2006); Augui et al (2007)

Masui et al (2011) Live cell chromosome dynamics and outcome of X-chromosome pairing events during ES cell differentiation. *Cell* 145: 447-458.



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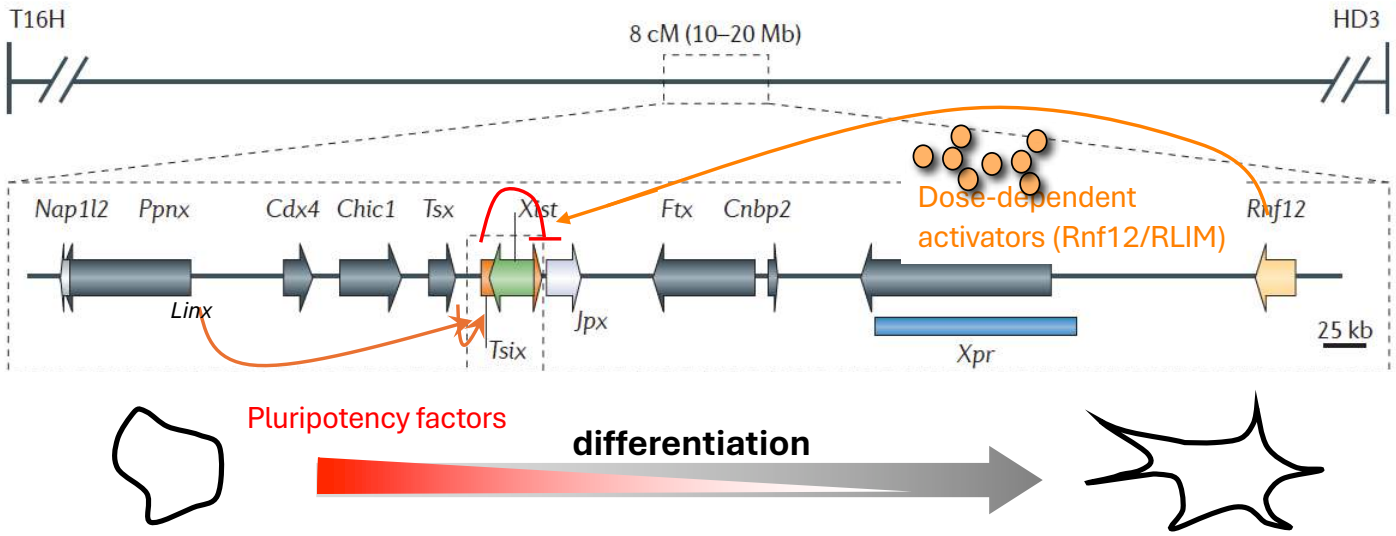
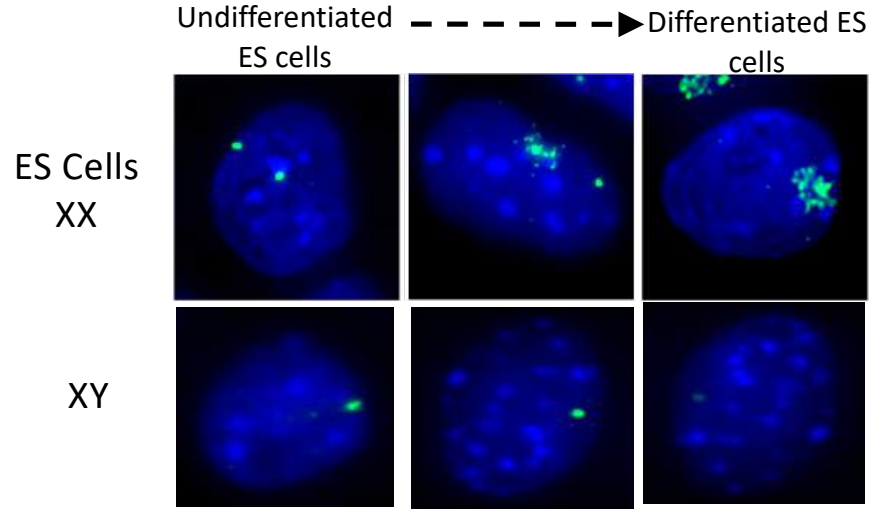
# The Xic is a complex locus including multiple regulators in addition to *Xist*




**How is *Xist* activated:**
**- upon differentiation?**
**- only in XX cells?**
**- only on one X?**

For recent reviews see:
Loda et al, Nat Rev Mol Cell Bio 2022
Furlan and Galupa Cells 2022
Mutzel and Schulz Bioessays 2020

E. Heard, May 19th, 2025

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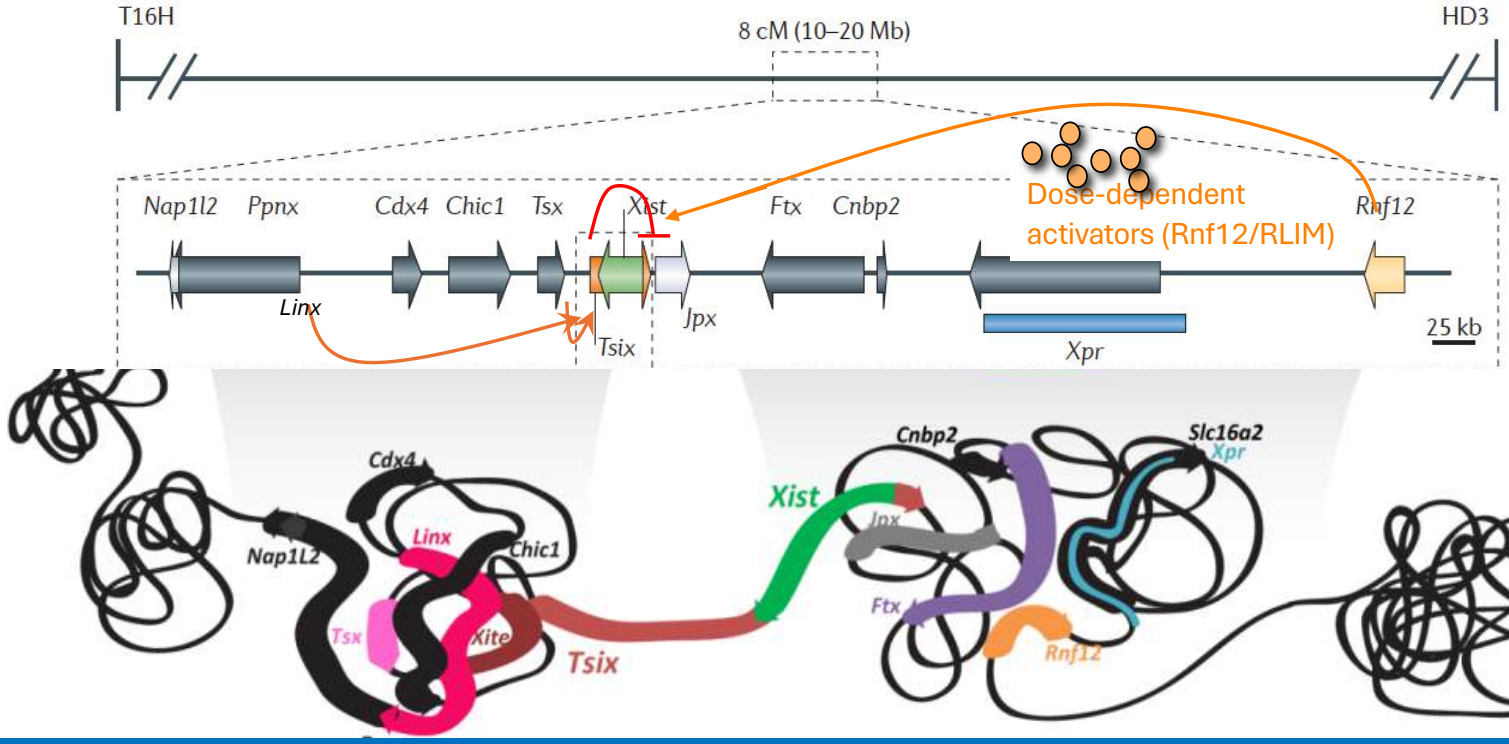
# The Xic is a complex locus including multiple regulators in addition to *Xist*

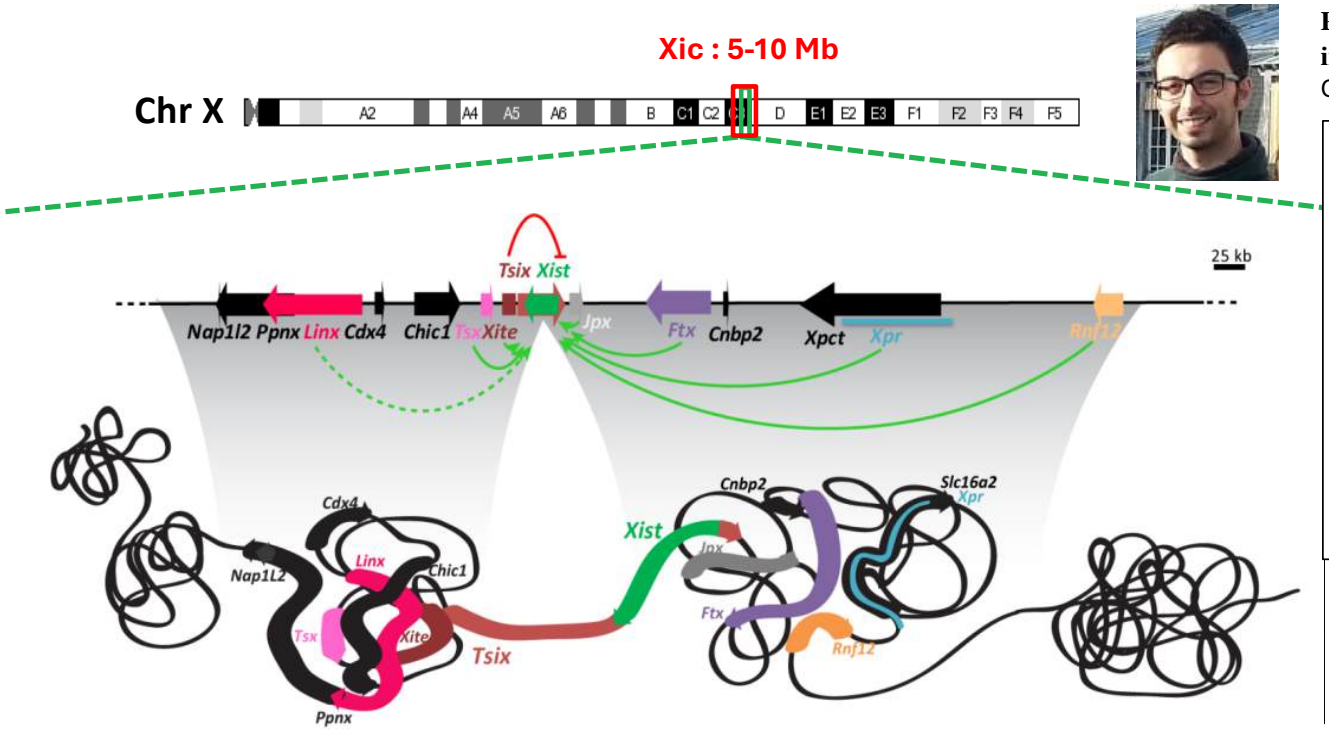
Elphege Nora




Job Dekker
(UMass, USA)

1. The Xic is divided into two topological domains - containing positive and negative regulators of *Xist*, in separate regulatory 'neighbourhoods'
2. Clustering of genes and regulatory elements within TADs enables developmental coordination of expression
3. *None of the long, single copy YAC transgenes covered the full extent of these two TADs (800kb)*

# The Xic is organised into at least two Topologically Associating Domains (TADs)

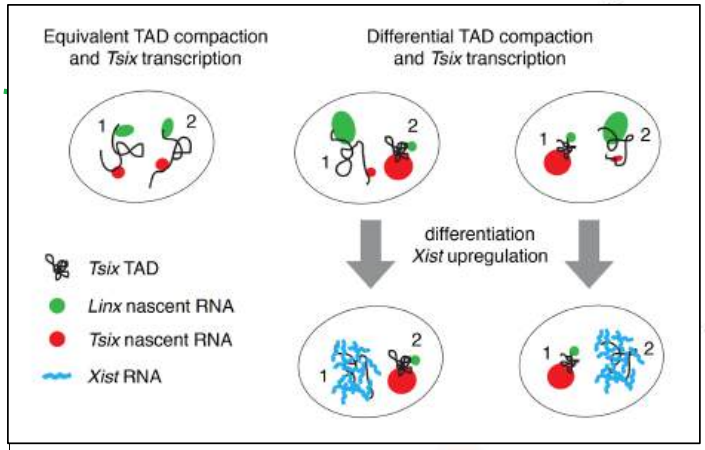


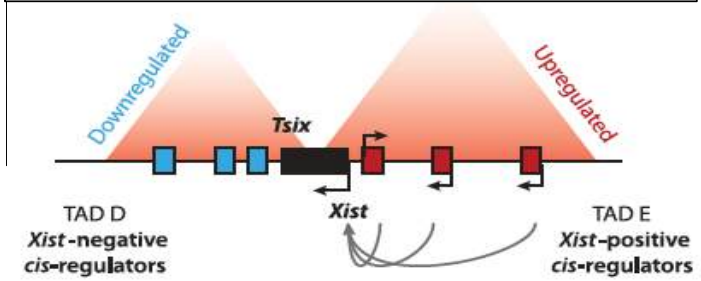

**Spatial partitioning of the regulatory landscape of the X-inactivation centre**

Elphège P. Nora[1,2,3], Bryan R. Lajoie[4]*, Edda G. Schulz[1,2,3]*, Luca Giorgetti[1,2,3]*, Ikuhiro Okamoto[1,2,3], Nicolas Servant[1,5,6], Tristan Piolot[1,2,3], Nynke L. van Berkum[4], Johannes Meisig[7], John Sedat[8], Joost Gribnau[9], Emmanuel Barillot[1,5,6], Nils Blüthgen[7], Job Dekker[4] & Edith Heard[1,2,3]



**Predictive Polymer Modeling Reveals Coupled Fluctuations in Chromosome Conformation and Transcription.**
Giorgetti et al, Cell 2014



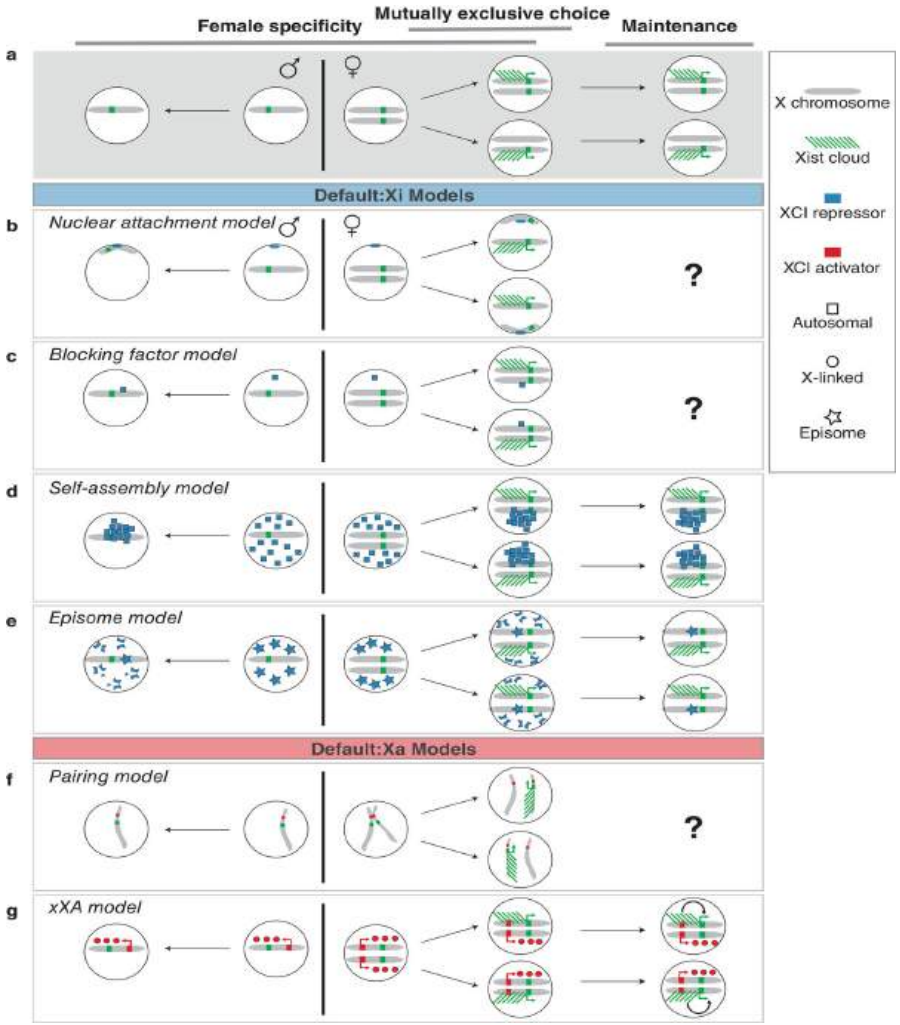




Random monoallelic expression of *Xist* could be due to fluctuations in 3D chromatin organisation of the *Tsix* TAD

Nora et al, Nature 2012
Giorgetti et al, Cell 2014
Galupa et al, Mol Cell 2020
Van Bemmel et al, Nat. Genet. 2019
Galupa et al, Development, 2022

# Today's Models: Dosage Sensing, Threshold Responses, and Epigenetic Memory: A Systems Biology Perspective on Random X-Chromosome Inactivation

Edda Schulz
MPI, Berlin




BioEssays, 2020
DOI: (10.1002/bies.201900163)



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# Next Week (May 26th)

- Genetic control of an epigenetic process: Xist RNA functions and spreading
- Developmental dynamics of XCI , imprinted XCI, evolutionary conservation
- A new era of modern epigenetics – and the epigenetic memory of the Xi
- Other examples of random monoallelic expression

**Steve Quake:** 2nd Lecture – "Understanding the Mysteris of the Cell: How do Mutations arise in our Bodies?
**Lundi 26 mai, 17.00-18.00**

# COURS 2025

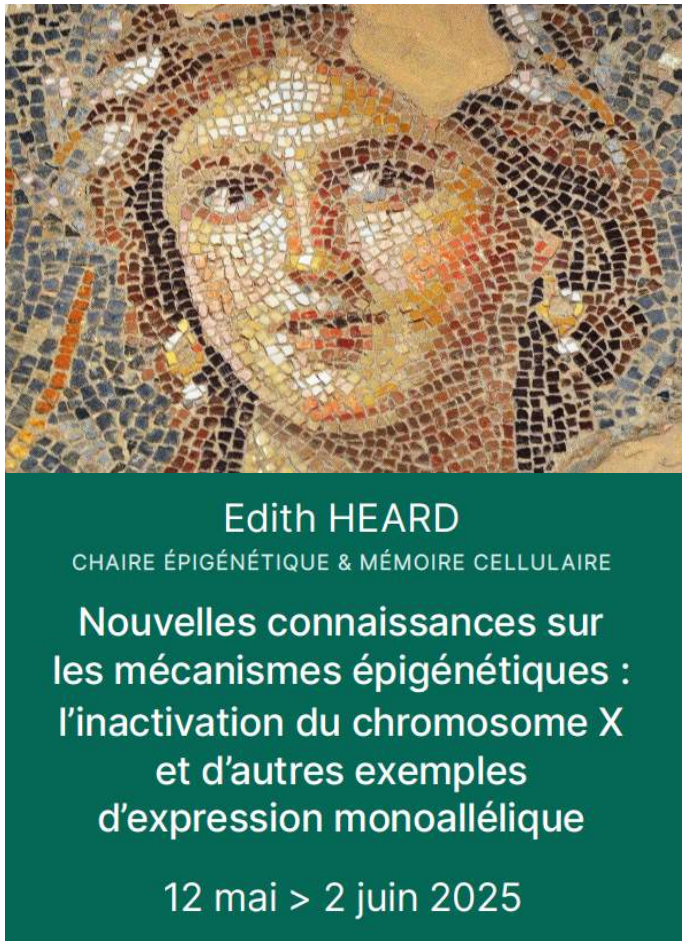
12 mai 2025
Découverte de l’inactivation du chromosome X (lyonisation)

19 mai 2025
La génétique et l’épigénétique de l’inactivation du chromosome X et d’autres exemples d’expression monoallélique

26 mai 2025
Évolution de l’inactivation du chromosome X et dynamique développementale

2 juin 2025
Implications de l’inactivation du chromosome X pour la biologie féminine

**10-11 juin 2025 Colloque**



**CHAIRE D'EPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE**
**Professeur Edith Heard, Année académique 2018-2019**

# Colloque/Symposium: *"X-Chromosome Inactivation"*

Organized by **Edith Heard and Claire Rougeulle**

## June 10th - 11th, 2025

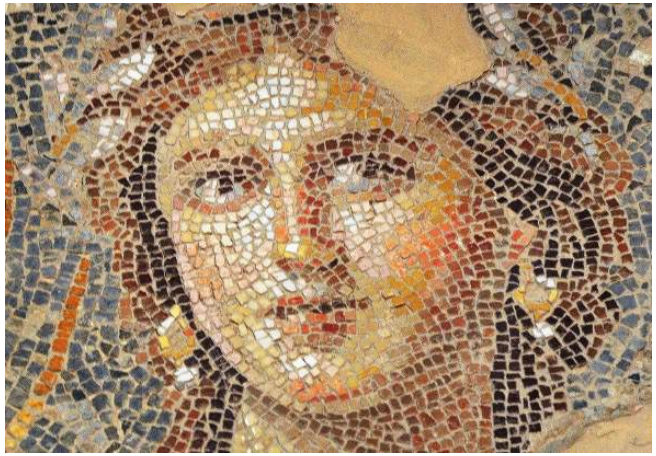
## Salle: Hallwachs

## Collège de France,

11, Place Marcelin Berthelot,
75231 Paris Cedex 05

**SPEAKERS**

Charbel Alfeghaly (France)
Monserrat Anguera (USA) Keynote
Marnie Blewitt (AUS)
Hegias Bontenbal (Netherlands)
Maud Borensztein (France)
Neil Brockdorff (UK) Keynote
Julie Chaumeil (France)
James Cleland (Germany)
Dounia Djeghloul (France)
Rafael Galupa (France)
Jean-Charles Guery (France)
Agnese Loda (Germany)
Osamu Masui (Japan)
Celine Morey (France)
Iku Okamoto (Japan)
Vincent Pasque (Germany)
Edda Schultz (Germany)
James Turner (UK) Keynote



**Free entry, no registration required**