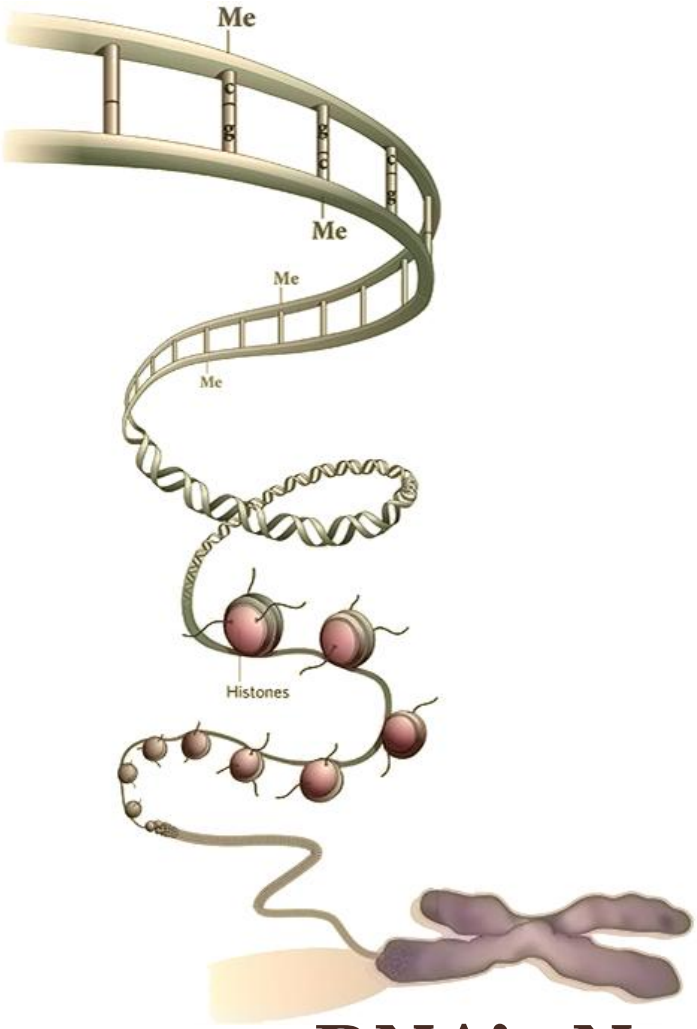


The X as Model for RNA's Niche in Epigenomic Regulation

KGL XII/MMX

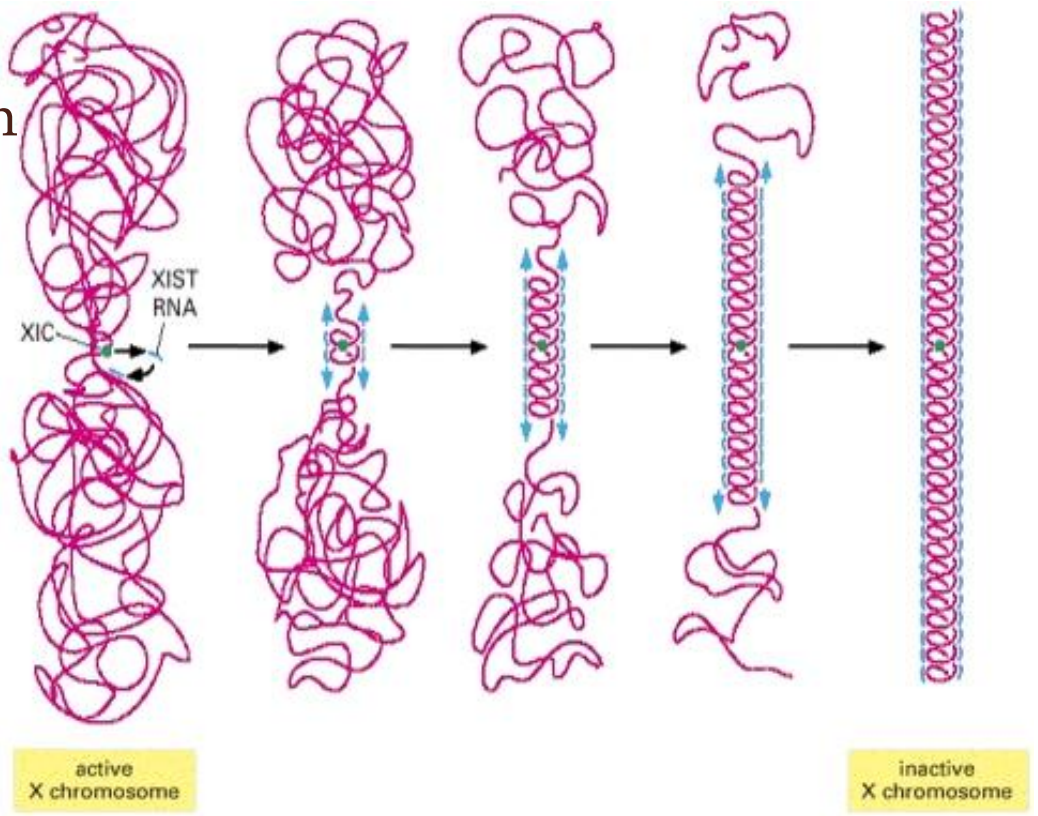
Jeannie T. Lee, Cold Spring Harb Perspect Biol 2010;2:a003749

Only facts and nothing else

- only 1%-2% of the mammalian genome carries protein-coding information;
- 70%-90% of our nucleotides are transcribed;
- 80,000-180,000 RNAs, the majority of which lack conserved open reading frames, formerly thought to be "junk";
- 50 nt to 100 kb "macroRNA," it can be antisense, promoter-associated, or intergenic.

X-CHROMOSOME INACTIVATION (XCI)

- The mechanism of dosage compensation in mammals – a domain phenomenon that extends to include the entire X-chromosome.
- ~1000 protein-coding genes on one of two X-chromosomes become transcriptionally inactivated *in cis* by a single control region known as the "X-inactivation center" or Xic.

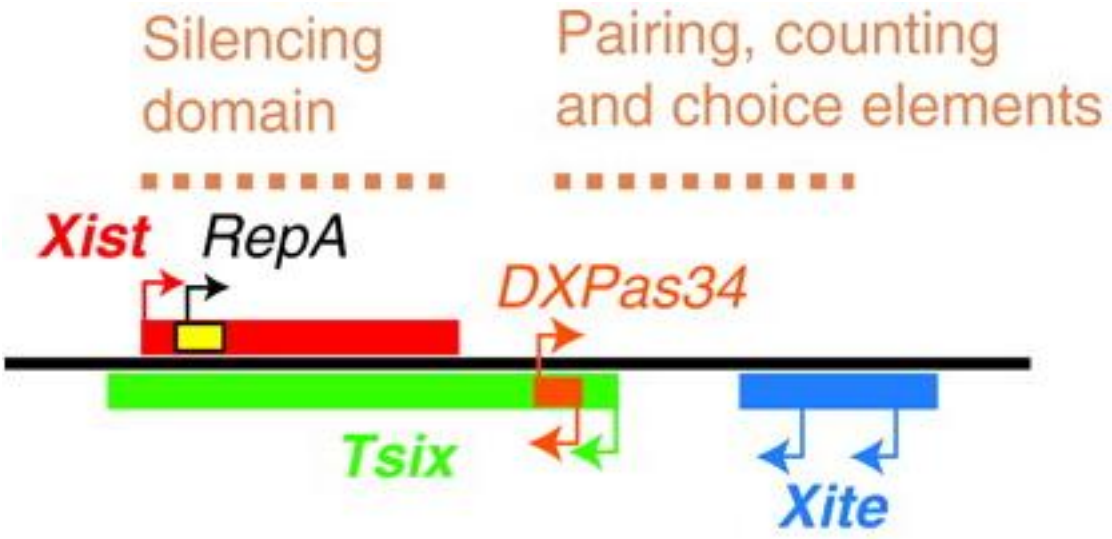


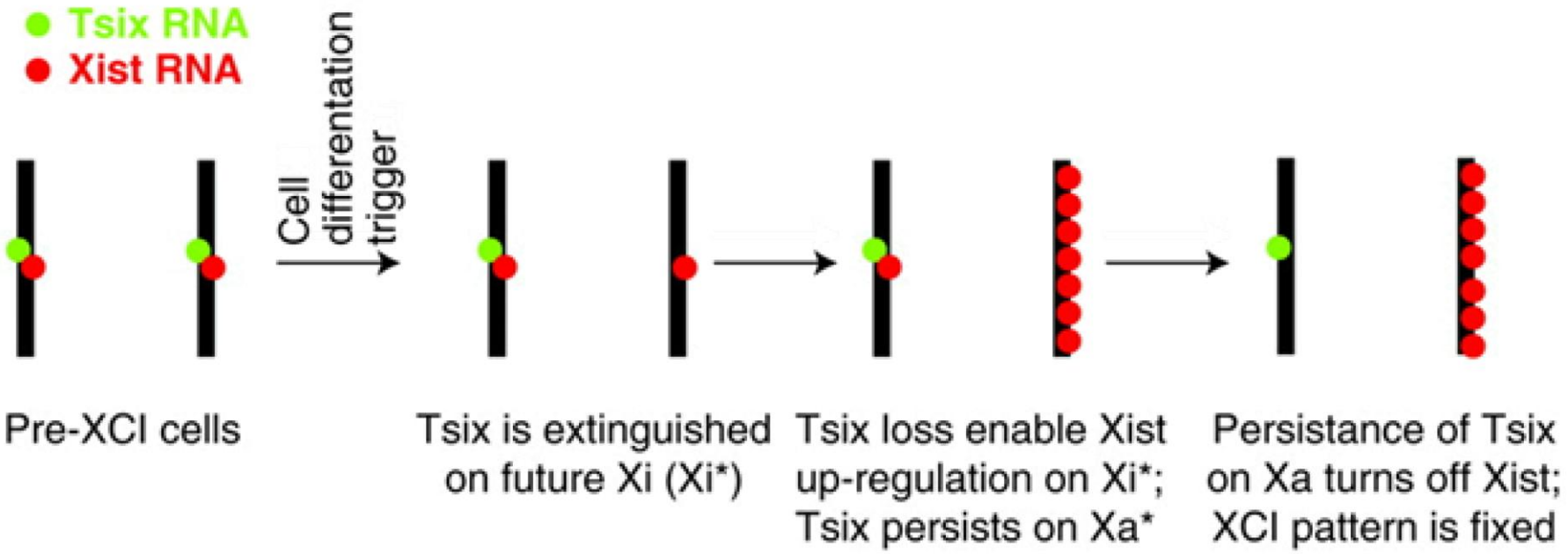
XCI, A NUMBER OF INTERESTING CHALLENGES

- two X-chromosomes in one nucleus are treated in opposite ways during early development – 1 (XY) or 2 Xs (XX) – yes or no for XCI;
- the problem of X-chromosome choice – random selection mechanism that chooses the active X (Xa) and the inactive X (Xi) in a mutually exclusive manner: no lethal XaXa or XiXi;
- silencing factors must be recruited to the future Xi in a colinear fashion, spreading along the chromosome in a strictly *cis*-limited manner – no trans effects on homologous loci of the future Xa.

XCI's repertoire of ncRNA regulators

- The X-inactive specific transcript (*Xist*) gene – large non-coding RNA – specific silencing of the X chromosome from which it is transcribed;
- Embedded within the 50 end of the *Xist* locus is a repeated motif known as "Repeat A" – encodes a separate transcription unit, named "*RepA*."
- The actions of RepA and Xist RNAs are controlled by Tsix, a 40-kb ncRNA that is antisense to both RNAs.

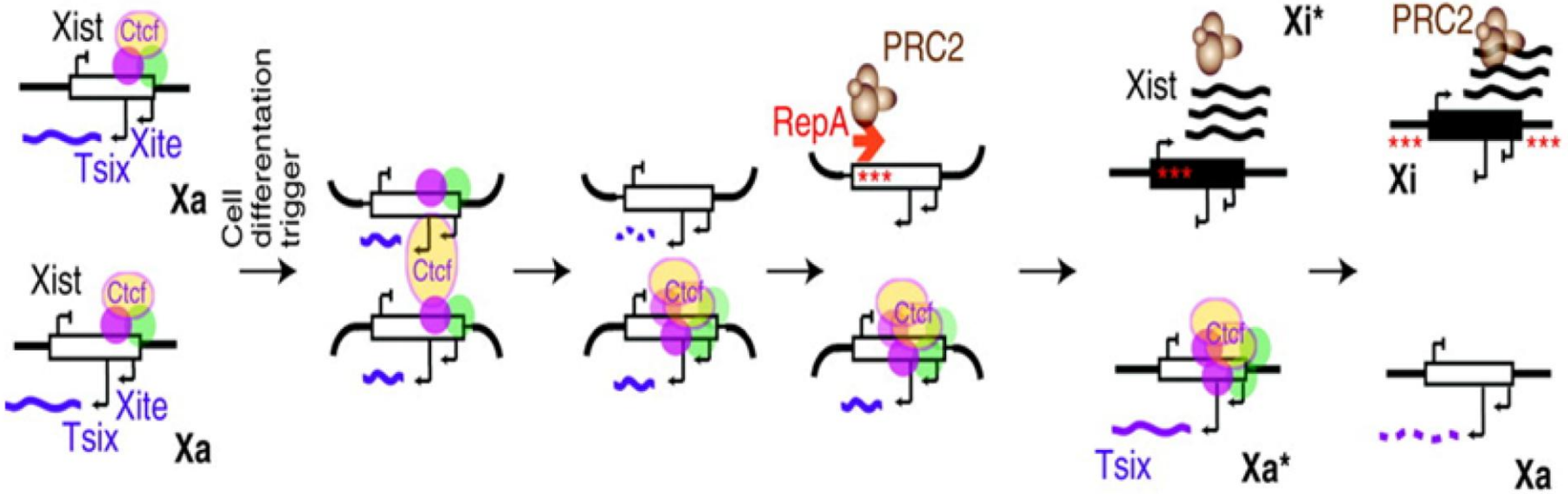




- In undifferentiated cells (pre-XCI), Tsix is expressed from both Xs, Xist RNA is expressed at low basal levels.
- Whether Tsix RNAwill persist during cell differentiation is controlled by the upstream locus, *Xite*, that enchanses expression of *Tsix*.
- At the onset of cell differentiation, Tsix persists only on the chromosome selected to become Xa.
- No Tsix on Xi creates a permissive state for *Xist* transactivation and spread of the RNA along the X to initiate chromosome-wide silencing.
- Tsix is eventually turned off on Xa once the pattern of XCI is fixed.

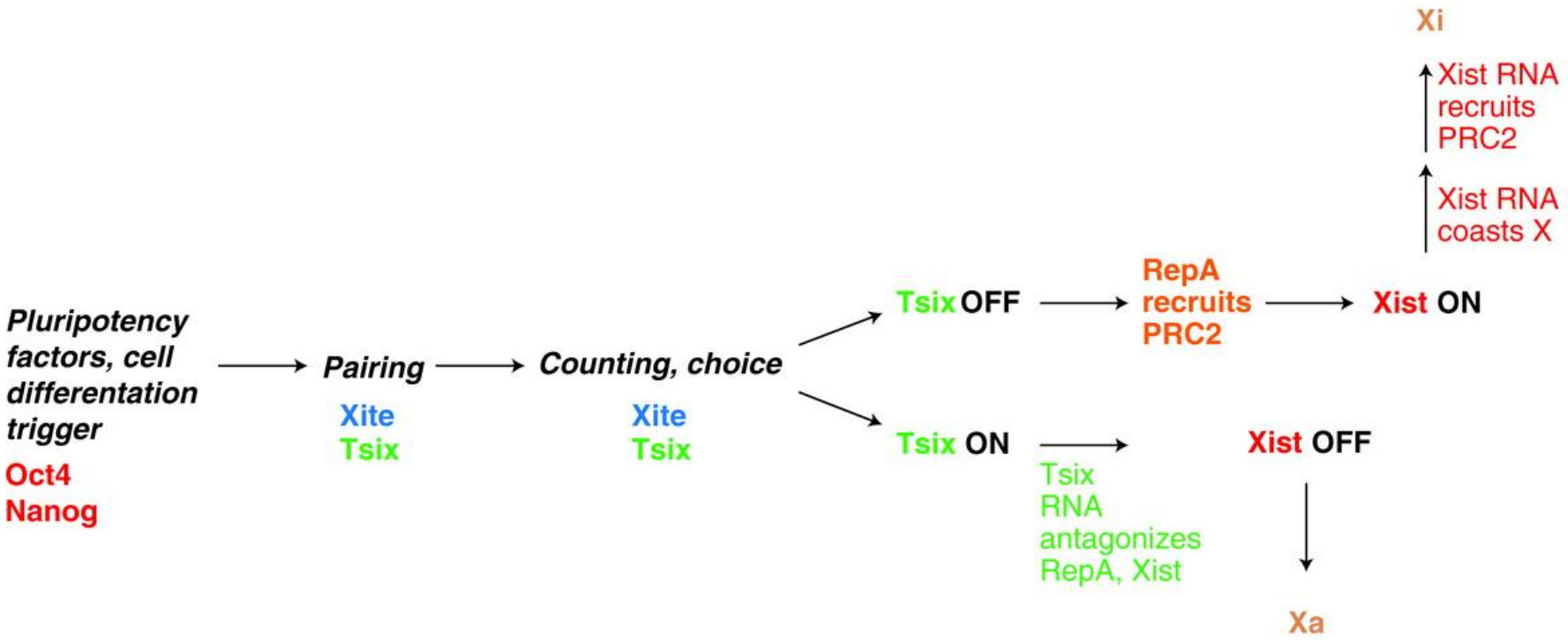
RNA IN PAIRING, COUNTING, AND CHOICE

- Xs make brief contact at the *Xic*.
- Ctcf protein, an 11-Zn finger protein-binding sites within *Tsix* and *Xite* loci and is thought to function as a transcriptional activator of both genes.
- Ctcf interacts with Oct4, a pluripotency factor thought to trigger counting as Oct4 levels fall during cell differentiation.
- Nearly all pairing competent fragments show Pol-II promoter activity – new transcription is necessary for X-chromosome coupling.



- In the pre-XCI state – both expressing Tsix, no Xist. Falling levels of Oct4 during cell differentiation triggers X-X pairing.
- Mediated by Ctcf, Tsix, and Xite and enables cross-talk to achieve correct counting and mutually exclusive Xa and Xi fates.
- Transcription factors such as Ctcf, Oct4 (red, green circles) shift to bind one X – maintains Tsix expression and becomes Xa.
- Loss of factor binding on the future Xi enables RepA to target PRC2 and H3-K27me3 to the 5′ end of *Xist* – activation.
- Xist RNA spreads along the future Xi and recruits PRC2 to it to initiate chromosome-wide silencing.

THE SCHEME


Xi
Xist RNA recruits PRC2
Xist RNA coasts X
Pluripotency factors, cell differentation trigger
Oct4
Nanog
Pairing
Xite
Tsix
Counting, choice
Xite
Tsix
Tsix OFF
RepA recruits PRC2
Xist ON
Tsix ON
Tsix RNA antagonizes RepA, Xist
Xist OFF
Xa

Chromatin paradox

- chromatin states of *Tsix* and *Xist* are intimately locked together – when *Tsix* is euchromatic, *Xist* is also; when *Tsix* is heterochromatic, *Xist* is likewise;
- their expression patterns do not also mirror each other;
- *Tsix* and *Xist* thrive in opposite chromatin environments, with*Tsix* being activated by open chromatin, and *Xist* being repressed by it.

Polycomb proteins regulated by RNAs

- After *Tsix* down-regulation Polycomb repressive complex 2 (PRC2) binds to RepA RNA.
- PRC2 has Histone Methyltransferase activity and trimethylates histone H3 on lysine 27.
- In undifferentiated cells, RepA RNA is biallelically expressed, binds PRC2 without catalyzing H3 trimethylation.
- During cell differentiation, the down-regulation of Tsix RNA on the future Xi allows the RepA-PRC2 complex to load onto the *Xist* chromatin and induce H3-K27me3, an event that would then lead to activation of the *Xist* promoter, accumulation of Xist RNA, and its spread along the X.

Roles that ncRNAs play

- high levels of Tsix RNA prevent the initiation of XCI by interfering with the RepA-PRC2 complex.
- When Tsix is down-regulated on the future Xi, RepA RNA productively engages PRC2, targets the Polycomb activity to the *Xist* promoter, and triggers the 100-fold up-regulation of Xist RNA by trimethylating H3-K27 to create a patch of heterochromatin at the 5′ end of *Xist*.
- Xist then binds PRC2 and spreads PRC2 and its H3-K27 trimethylase activity along the X to initiate chromosome-wide silencing.

The Advantages of Long ncRNA

- Unlike proteins and small RNAs, large ncRNAs are stably tethered to the site of transcription and therefore tag the allele of origin
  - long ncRNA remains bound via RNA polymerase to its parent locus during the act of transcription – tag for the locus.
- Large ncRNAs are drawn from larger sequence space than proteins and can therefore achieve greater sequence specificity
  - The diversity of sequences specified by RNA is a direct function of its length, composition, and nucleotide permutation. But there is virtually no limit as to how long a macroRNA can be.

- A good example of the inactivation of the X chromosome. And the best creature in the world.