Whence Epigenesis? (Extending "The Hierarchical Genome and Differentiation Waves" §1.02)

Presented in the Embryo Physics Course http://www.embryophysics.org
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Status of “Given a Spherical Cow” HG1.01

- Evsikov, S.V., R. Gordon, W.R. Buckley & S.P. McGrew (2010). Is the zygote spherically symmetrical, despite appearances? , in preparation.
- Others are welcome to participate. We’ll dialogue the pros and cons, especially if we disagree on the conclusion.

HGDW §1.02 The Epigenetic Problem

- The original definition of *epigenesis* referred to the observation, dating back to Aristotle, that you can't see the adult animal when you look at its early embryo
- Therefore the parts somehow appear during embryogenesis, rather than being preformed
- HGDW = Gordon, R. (1999). *The Hierarchical Genome and Differentiation Waves: Novel Unification of Development, Genetics and Evolution*. Singapore & London, World Scientific & Imperial College Press http://www.worldscibooks.com/lifesci/2755.html.

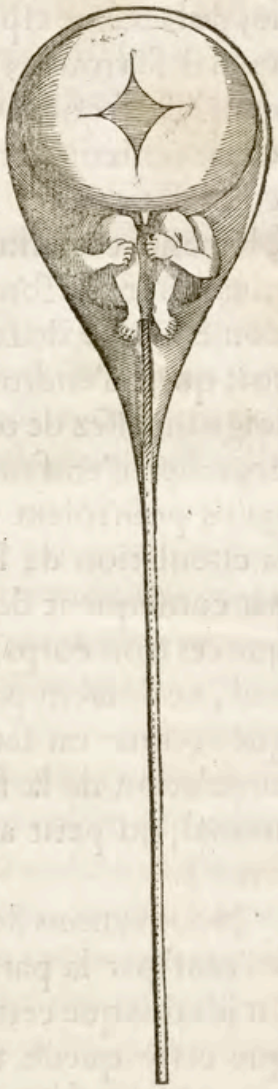
- http://www.funpic.hu/

The Alternative: Embryos as Blow-Up Dolls

que la tête ſeroit peut-être plus grande à proportion du reſte du corps, qu'on ne l'a deſſinée icy.

ART. XC. Ce que c'eſt que l'œuf de la femme, & comment un enfant vient ordinairement au monde.

Au reſte, l'œuf n'eſt à proprement parler que ce qu'on appelle *placenta*, dont l'enfant, aprés y avoir demeuré un certain temps tout courbé & comme en peloton, briſe en s'étendant & en s'allongeant le plus qu'il peut, les membranes qui le couvroient, & poſant ſes pieds contre le *placenta*, qui reſte attaché au fond de la matrice, ſe pouſſe ainſi avec la tête hors de ſa priſon; en quoi il eſt aidé par la mere, qui agitée par la douleur qu'elle en ſent, pouſſe le fond de la matrice en bas, & donne par conſequent d'autant plus d'occaſion à cet enfant de ſe pouſſer dehors & de venir ainſi au monde.



L'experience nous apprend que beaucoup d'animaux ſortent à peu prés de cette maniere des œufs qui les renferment.

ART. XCI. Que l'on peut pouſſer bien plus loin cette nouvelle penſée de la generation, & comment.

L'on peut pouſſer bien plus loin cette nouvelle penſée de la generation, & dire que chacun de ces animaux mâles, renferme lui-même une infinité d'autres

Homunculus in Human Sperm

- Hartsoeker, N. (1694). *Essay de dioptrique, Jean Anisson*
- www.hps.cam.ac.uk/visibleembryos/s1/1_4_2_Hartsoeker.jpg

Preformation vs Epigenesis

- **"Mechanical philosophers in the 1600s built on Aristotelian epigenesis to explain generation entirely in terms of moving particles and attractive forces, but because this risked charges of atheism for dispensing with God, a new theory of pre-existence soon became dominant.**
- **"The theory of pre-existence had it that all adult structures were already present in the egg, only much smaller**. God had generated every germ at the Creation, one within the other like a Russian doll. The related doctrine of preformationism argued that the body of the new being was complete in the parent seed so that during gestation the embryo only increased in size. 'Ovists' placed the germ in the egg, 'animalculists' in the sperm."
- Buklijas, T. & N. Hopwood. (2008). Making Visible Embryos. http://www.hps.cam.ac.uk/visibleembryos/index.html

The Regress of Homunculi back to Adam or Eve

- http://commons.wikimedia.org/wiki/File:Russian.dolls.hugeset.arp.jpg

The fundamental question of developmental biology

- How do cells differentiate
- in the right place,
- at the right time,
- into the right kinds?

Why is this Epigenesis?

- Because of the evidence that for almost all multicellular organisms the DNA is the same in each cell?
- Thus something has to make the cells differ from one another that is not in the DNA.
- By definition, that is named epigenesis.
- Exceptions: chloroplasts and mitochondria, which can differ in numbers between cells.

“Random” Epigenesis

- A clone is a group of cells derived from a single cell.
- All animals are generally clones, since they come from a zygote (single fertilized egg cell)
- Events can occur at random in a cell that do not change the nuclear DNA sequence, yet are “heritable”, in the sense that the subclone of cells derived from that cell retain the change

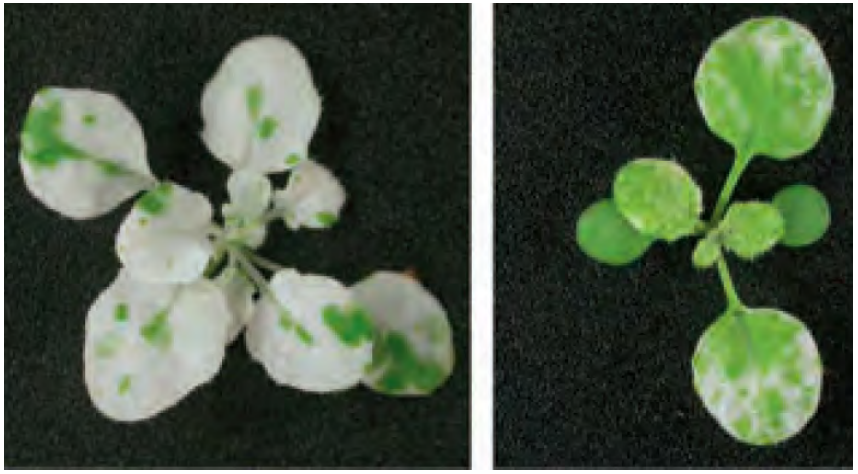
Three Ways to Get Random Epigenesis

1. “Spontaneous” changes in chloroplasts in plants
2. X chromosome inactivation
3. Somatic mutants

Cancer is often presumed to be a somatic mutant.

Variegated Plants

Arabidopsis



With Altered Chloroplasts

- Yu, F., A. Fu, M. Aluru, S. Park, Y. Xu, H. Liu, X. Liu, A. Foudree, M. Nambogga & S. Rodermel (2007). Variegation mutants and mechanisms of chloroplast biogenesis. *Plant Cell Environ* ***30(3), 350-365.***

Variegated Plant: Note Stochastic Effects within Constant overall Form

http://www.victoria-dove.com/Pics/Tropicals/Variegated%20Peperomia.jpg

Variegated Banana Leaf

http://www.tytyga.com/product/image4/600/Variegated_Banana_Tree0008.jpg

Variegated *Agapanthus*
Note lack of stochastic effects

http://
www.eclecticplants.co.uk/dbimages/agtin.jpg

Variegation in Mammals by X Chromosome Inactivation

- Normal (XX) females have two X chromosomes
- In each cell, at the 4 to 20 cell stage of embryogenesis, all but one of the X chromosomes is “inactivated” (Note: online tutorials claim 1000 cell stage: they’re wrong)
- This means that most of the genes on the inactivated X chromosomes are shut down, perhaps by methylation of the DNA and coating of it with certain proteins

Variegation in Mammals by X Chromosome Inactivation

- For this reason, an XXX, XXXX, or XXXXXX person is a phenotypically normal female, with all but one of the X chromosomes inactivated
- Generally, which X chromosome remains active is random
- Note: how could this happen, to leave one chromosome active?

Calico Cat

Pigmentation genes are different on the two
X chromosomes

http://media.photobucket.com/image/calico%20cat/dawnleaf/mystra_cat_white_orange_black.jpg

Tortoiseshell Cat

- Less or no white
- Much smaller patches

- http://www.catsarewonderful.com/calicocats.php

Fun Research Problem

- It has been claimed (with no justification that I can find) that X inactivation occurs at the 64 cell stage*
- So how many spots are there on calico cats, and can the tortoiseshell cat be explained as being the same # of spots "blended/swirled together"?
- Method: photograph cats at cat shows and map onto a 3D rendering, then quantitate spots

- *Nowack, R. (1993). Curious X-inactivation facts about calico cats. *J. NIH Res.* ***5, 60-65.***
- *Osgood, M.P. (1994). X-chromosome inactivation: the case of the calico cat. *Am. J. Pharm. Educ.* ***58(2), 204-205.***

Vitiligo: Visible Human Variegation?

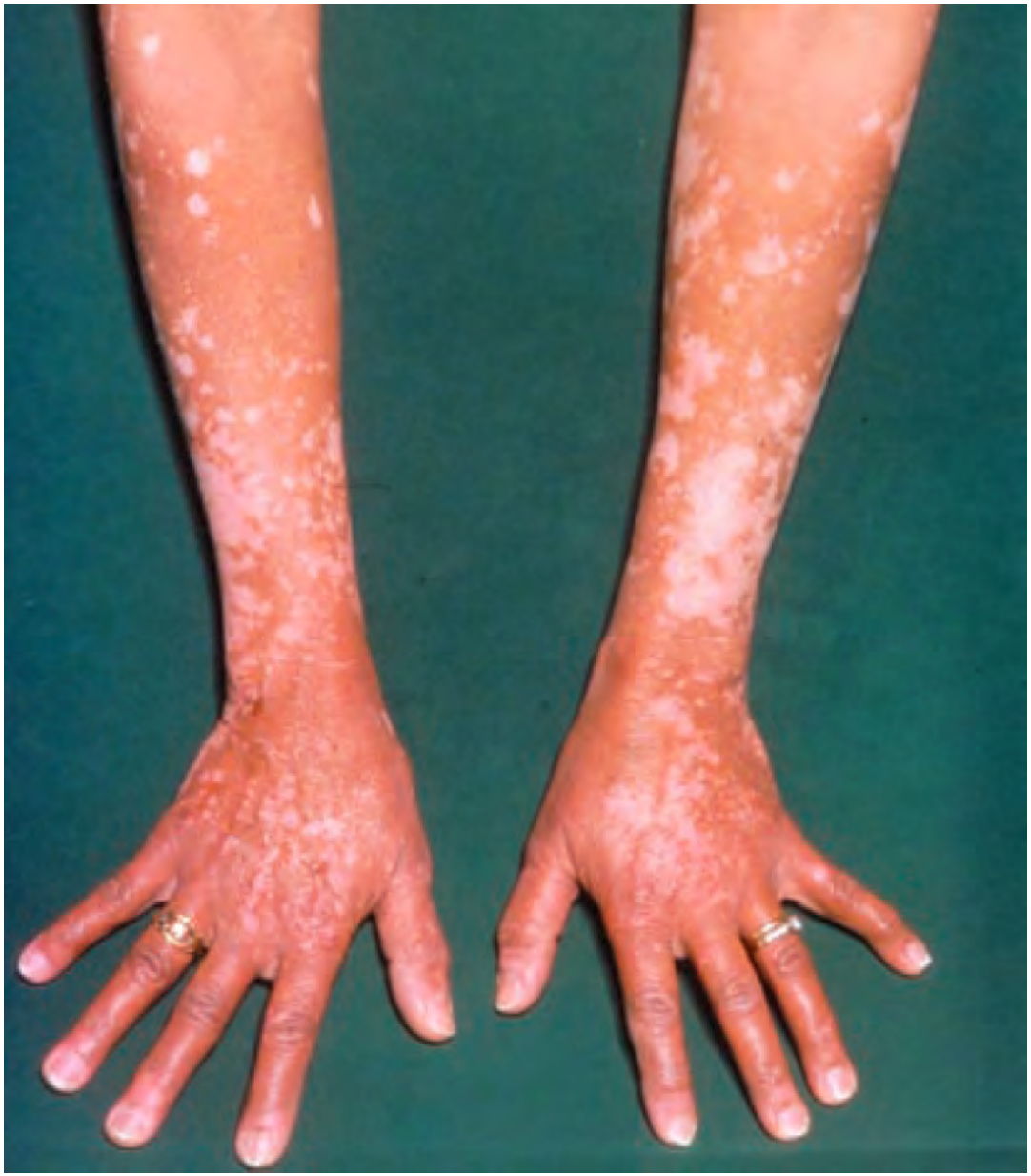
"The most prevailing theories for the pathogenesis of vitiligo are:

- genetic hypothesis
- autoimmune hypothesis
- neural hypothesis
- self-destruction hypothesis
- growth factor defect hypothesis and
- convergence theory."

Njoo, M.D. & W. Westerhof (2001). Vitiligo. Pathogenesis and treatment. *Am J Clin Dermatol 2(3), 167-181*.

Vitiligo

Gawkrodger, D.J. (2009). Vitiligo: what general physicians need to know. *Clin Med* ***9(5), 408-409.***

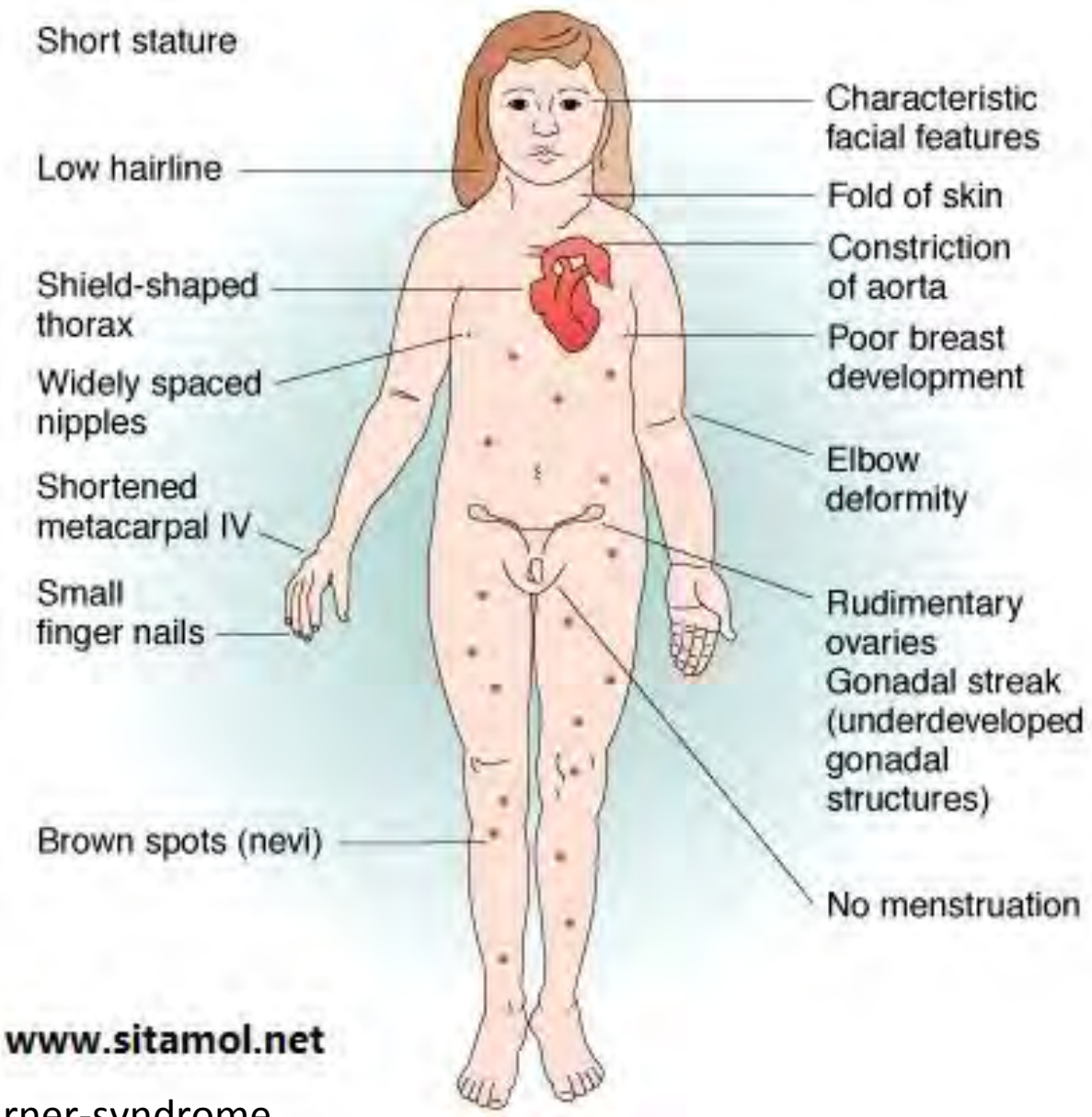


What about X0?

- Predictions?

What about X0?

"The prevalence of Turner syndrome is widely reported as being approximately one per 2,000 live female births although researchers have reported prevalence rates that range from one in 3,125 to one in 5,000 live female births. About 1% to 2% of all female conceptions have a missing X-chromosome. Of these, the majority (99%) spontaneously abort, usually during the first trimester of pregnancy."



http://www.answers.com/topic/turner-syndrome

The Value of Rereading the Pioneers Murray L. Barr: Barr Bodies = Inactivated X Chromosomes

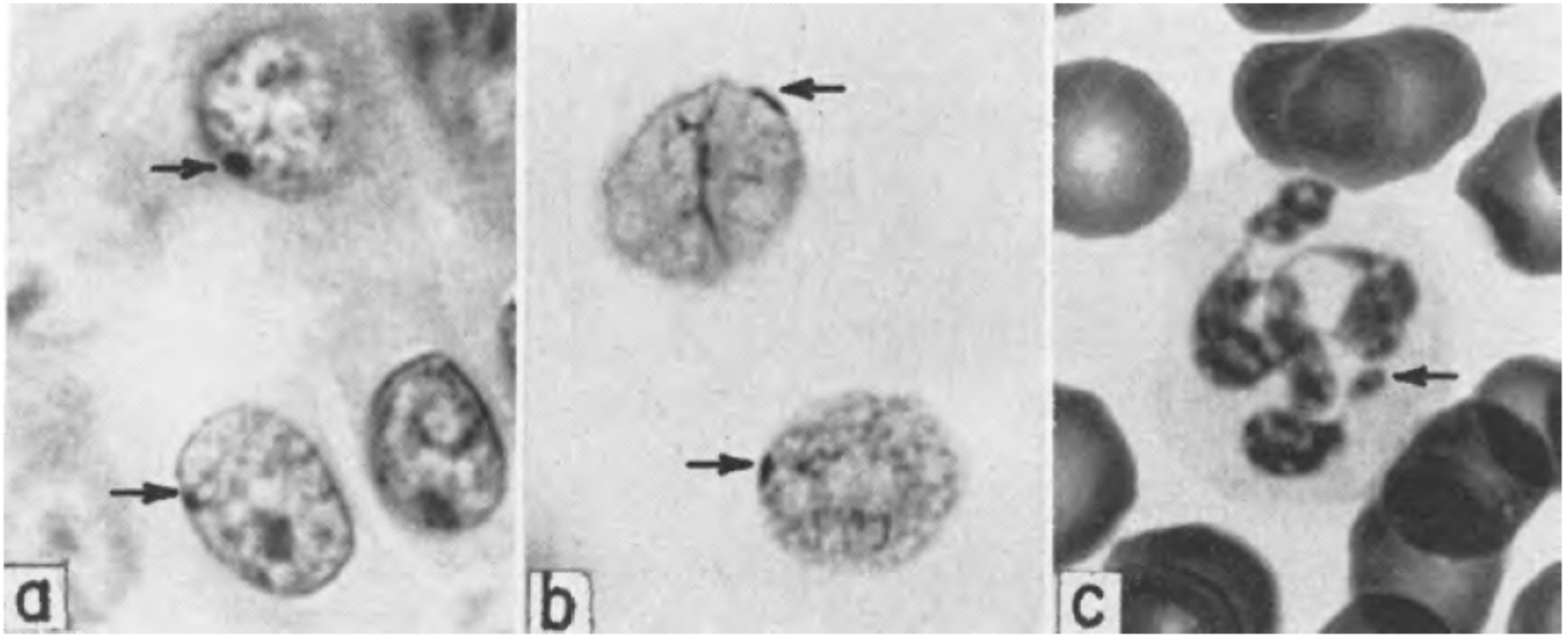


FIG. 1. a. Nuclei in the stratum spinosum of a skin biopsy specimen from a normal female. Hematoxylin and eosin stain. b. Nuclei in an oral smear preparation from a normal female. Thionin stain. c. Neutrophil leucocyte in a blood film from a normal female. Giemsa stain. 1800 ×.

Barr, M.L. (1960). Sexual dimorphism in interphase nuclei. *Am J Hum Genet* ***12, 118-127.***

Barr, M.L. (1959). Sex chromatin and phenotype in man. *Science* ***130, 679-685.***

- **“the XO sex chromosome arrangement results in a fertile female in the mouse and an infertile female in man“**
- **Of course, by “man” he meant women, but those were still sexist days of the English language**

Variegation in Mammals by X Chromosome Inactivation

- Generally, which X chromosome remains active is random
- However, skewed X inactivation up to 90:10 instead of 50:50 in XX females has been observed

Three hypotheses for skewed X inactivation

- 1: that skewed X inactivation is due to an X-linked allele that confers a proliferative advantage to cells
- supported by the finding that the level of skewed X inactivation as measured in lymphocyte DNA increases with age, particularly after age 60 years, which suggests that it confers a proliferative advantage to a subset of peripheral lymphocytes
- Lymphocyte - Any of the nearly colorless cells found in the blood, lymph, and lymphoid tissues, constituting approximately 25 percent of white blood cells and including B cells, which function in humoral immunity, and T cells, which function in cellular immunity

Three hypotheses for skewed X inactivation

- 2: that skewed X inactivation is due to a genetic predisposition
- comes from heritability studies that have estimated that about one-third of the variance in the X inactivation phenotype is due to genetic factors
- from a study that showed that skewed X inactivation in identical twins typically favors the same X chromosome
- and from several studies that reported linkage of skewed X inactivation to specific loci on the X chromosome.

Three hypotheses for skewed X inactivation

- 3: that acquisition of skewed X inactivation is a protective mechanism to reduce expression of detrimental X-linked alleles
- there is considerable evidence that skewed X inactivation can protect females from disease when the preferentially inactivated X chromosome carries a detrimental allele

Three hypotheses for skewed X inactivation

- Reference:
- Lose, F., D.L. Duffy, G.F. Kay, M.A. Kedda & A.B. Spurdle (2008). Skewed X chromosome inactivation and breast and ovarian cancer status: evidence for X-linked modifiers of *BRCA1. J Natl Cancer Inst* ***100(21), 1519-1529.***

Xist = X inactive specific transcript

- This is a large, noncoding RNA
- The *Xist gene is located on the X* chromosome
- upregulated on the future inactive X (Xi) chromosome when random X chromosome inactivation (XCI) initiates
- Once upregulated, *Xist coats the entire Xi chromosome*
- Well, not quite: some genes in common with Y chromosomes are somehow not coated

Tsix. A non-coding RNA

- ***Its gene is antisense to, and overlaps with,*** the *Xist gene*
- *Tsix transcription across Xist is* thought to repress *Xist upregulation and XCI initiation.*
- *The future Xi* chromosome downregulates *Tsix and upregulates Xist.*
- *The future* active X chromosome downregulates *Xist and sustains Tsix* transcription.

Xite. A non-coding RNA

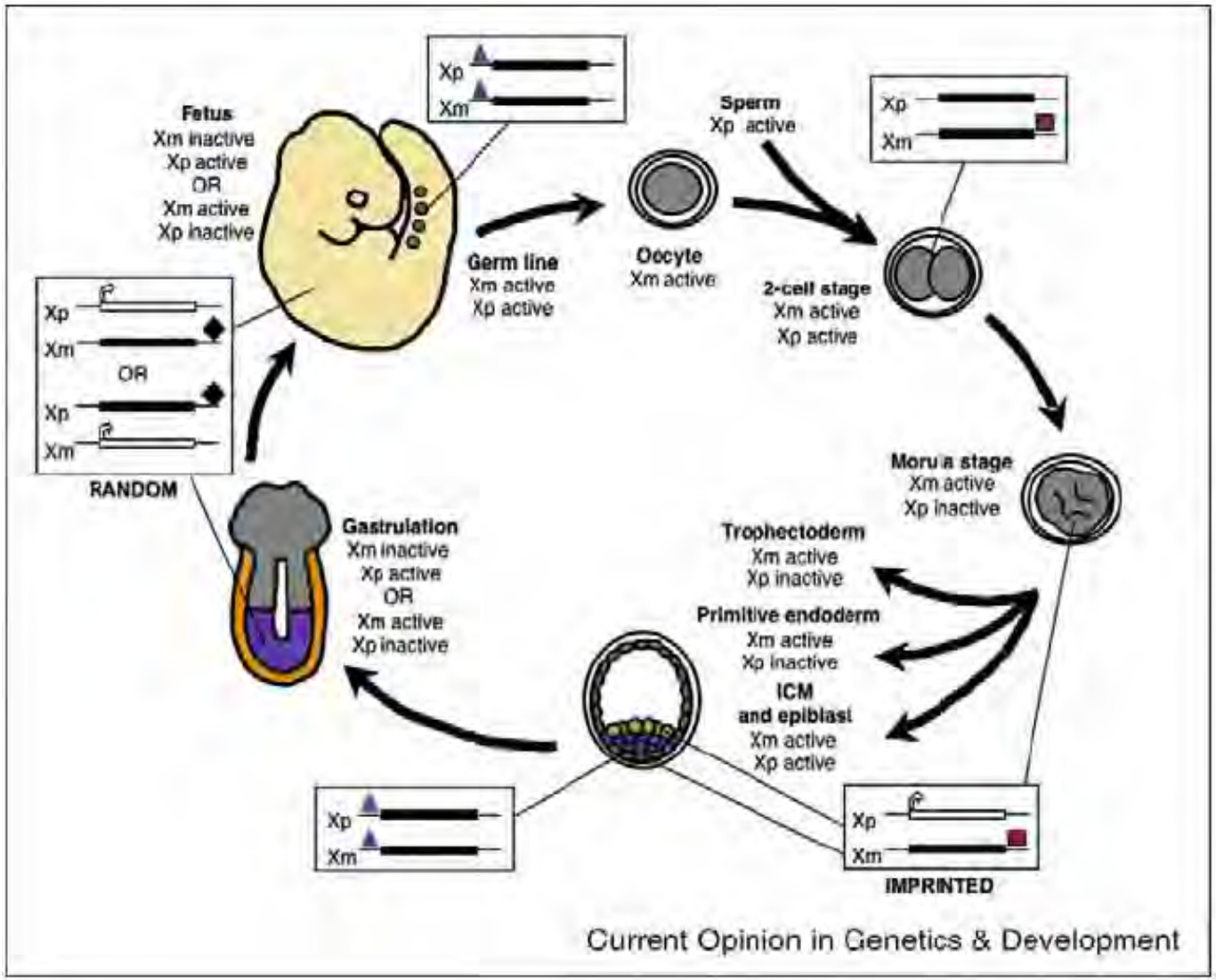
- ***Its gene contains an enhancer for Tsix that***

sustains its expression during differentiation

Xpr

- ***Xpr. The X chromosome-pairing region. An X-linked region that lies*** 200-kb upstream from *Xist and is involved in X chromosome pairing* prior to XCI

X inactivation: A Model for Epigenetic Control of Gene Expression?

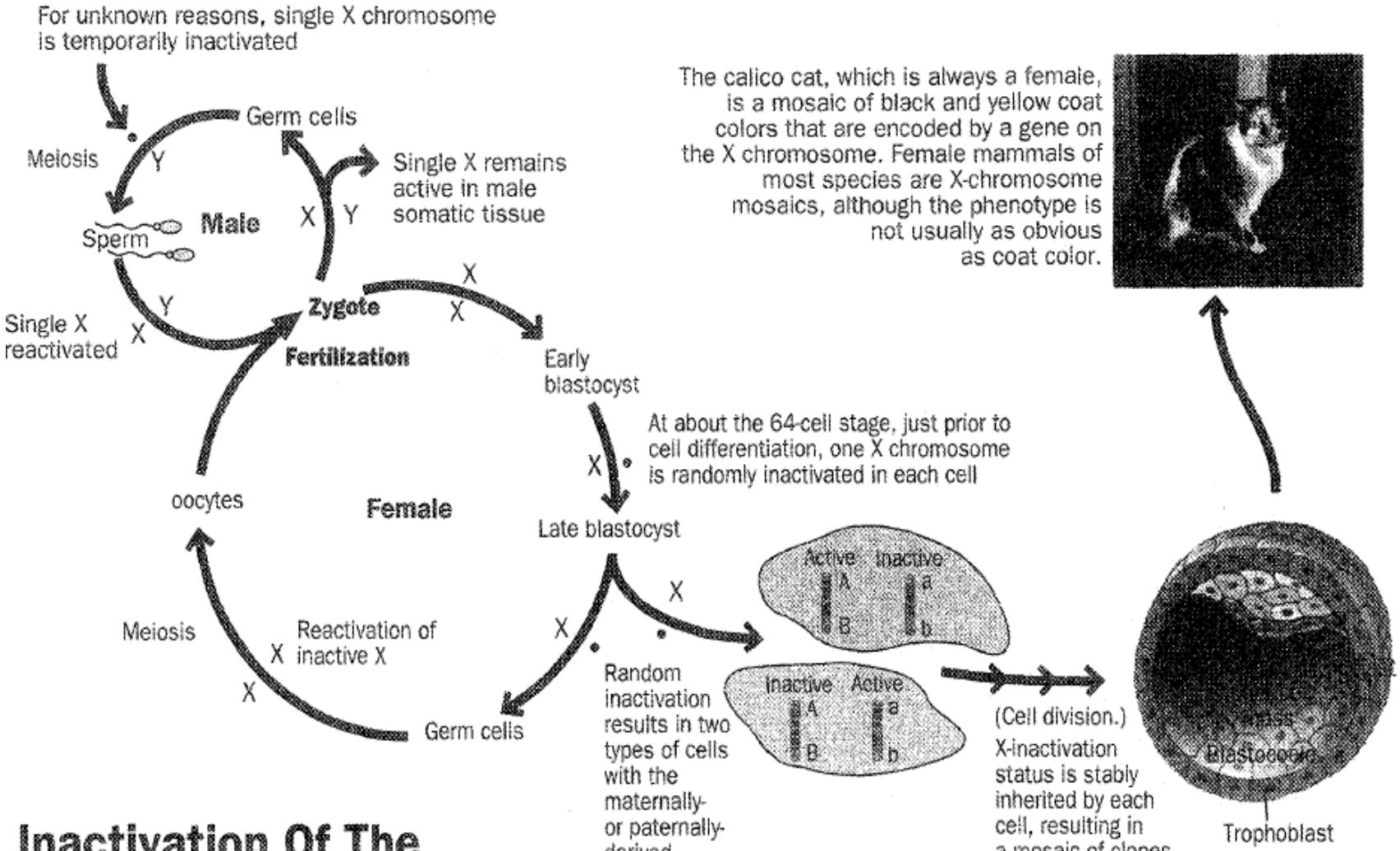


Xm = maternal X chromosome
Xp = paternal X chromosome

Senner, C.E. & N. Brockdorff (2009). *Xist* gene regulation at the onset of X inactivation. *Curr Opin Genet Dev* ***19(2), 122-126.***

Life Cycle of X Inactivation

Nowack, R. (1993). Curious X-inactivation facts about calico cats. *J. NIH Res.* ***5, 60-65.***

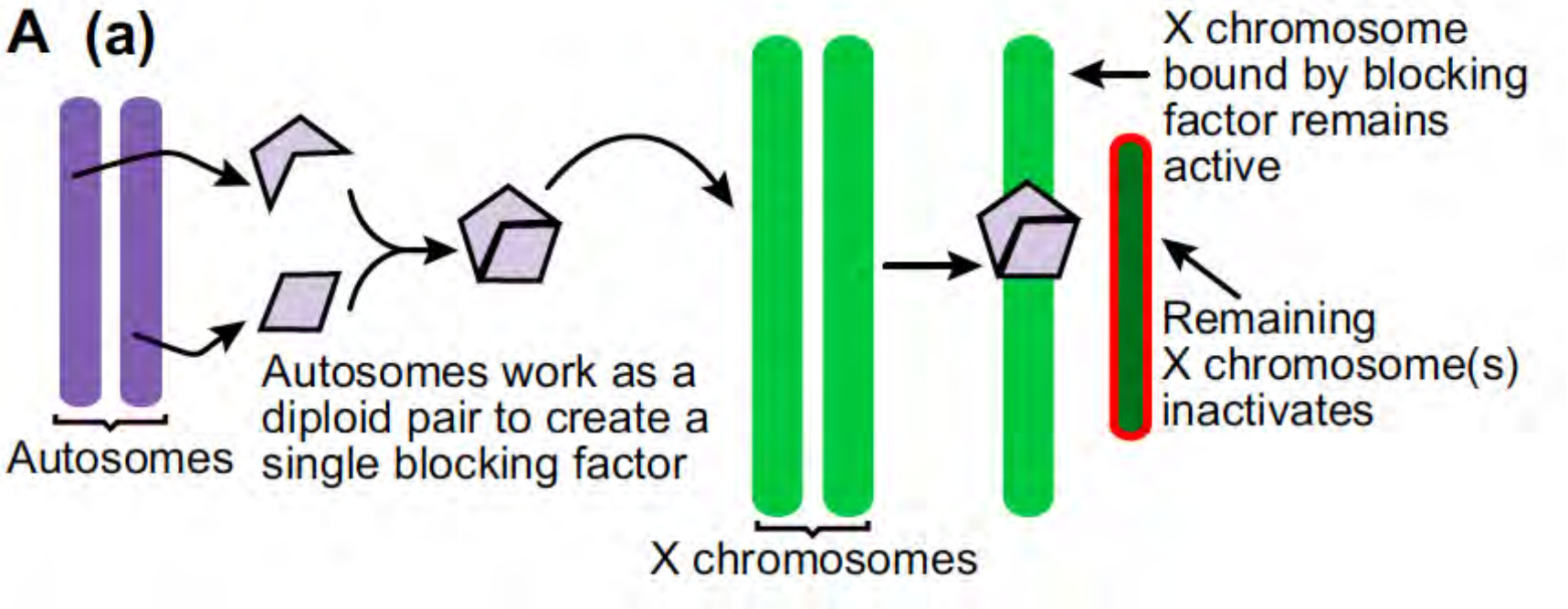


Inactivation Of The X Chromosome In Gametogenesis

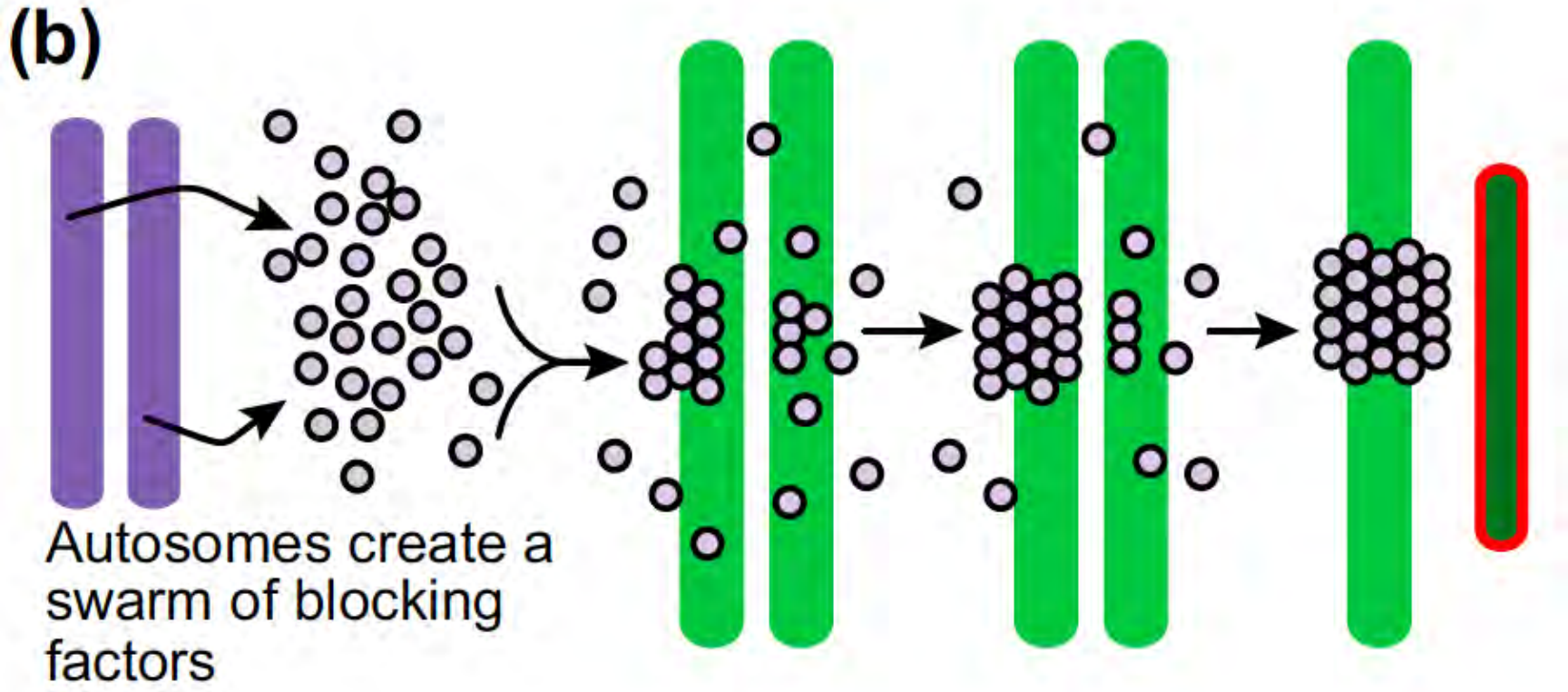
State of the Art

- “The mechanisms for determining both how many X chromosomes are present and which to inactivate are unknown.“
- Starmer, J. & T. Magnuson (2009). A new model for random X chromosome inactivation. *Development* ***136(1), 1-10.***

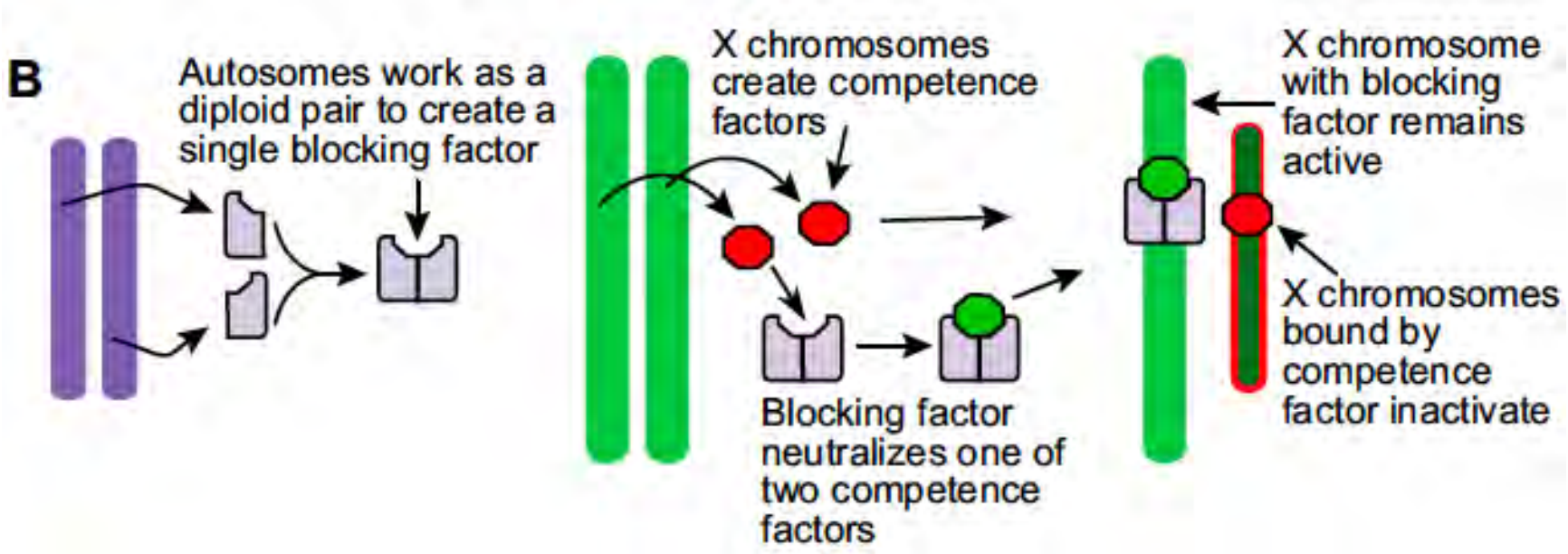
Model Aa


A (a)
Autosomes
Autosomes work as a diploid pair to create a single blocking factor
X chromosomes
X chromosome bound by blocking factor remains active
Remaining X chromosome(s) inactivates

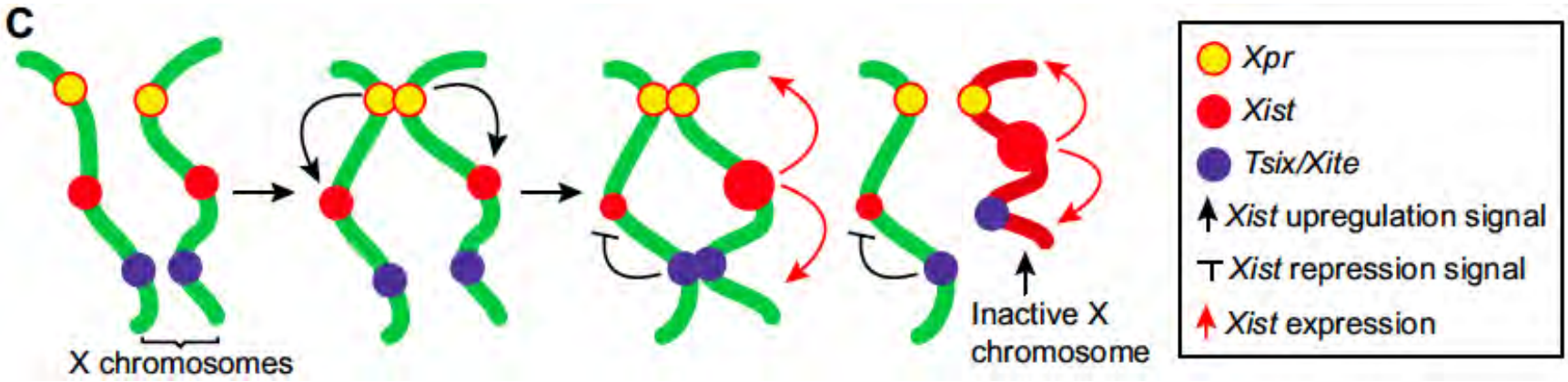
Model Ab



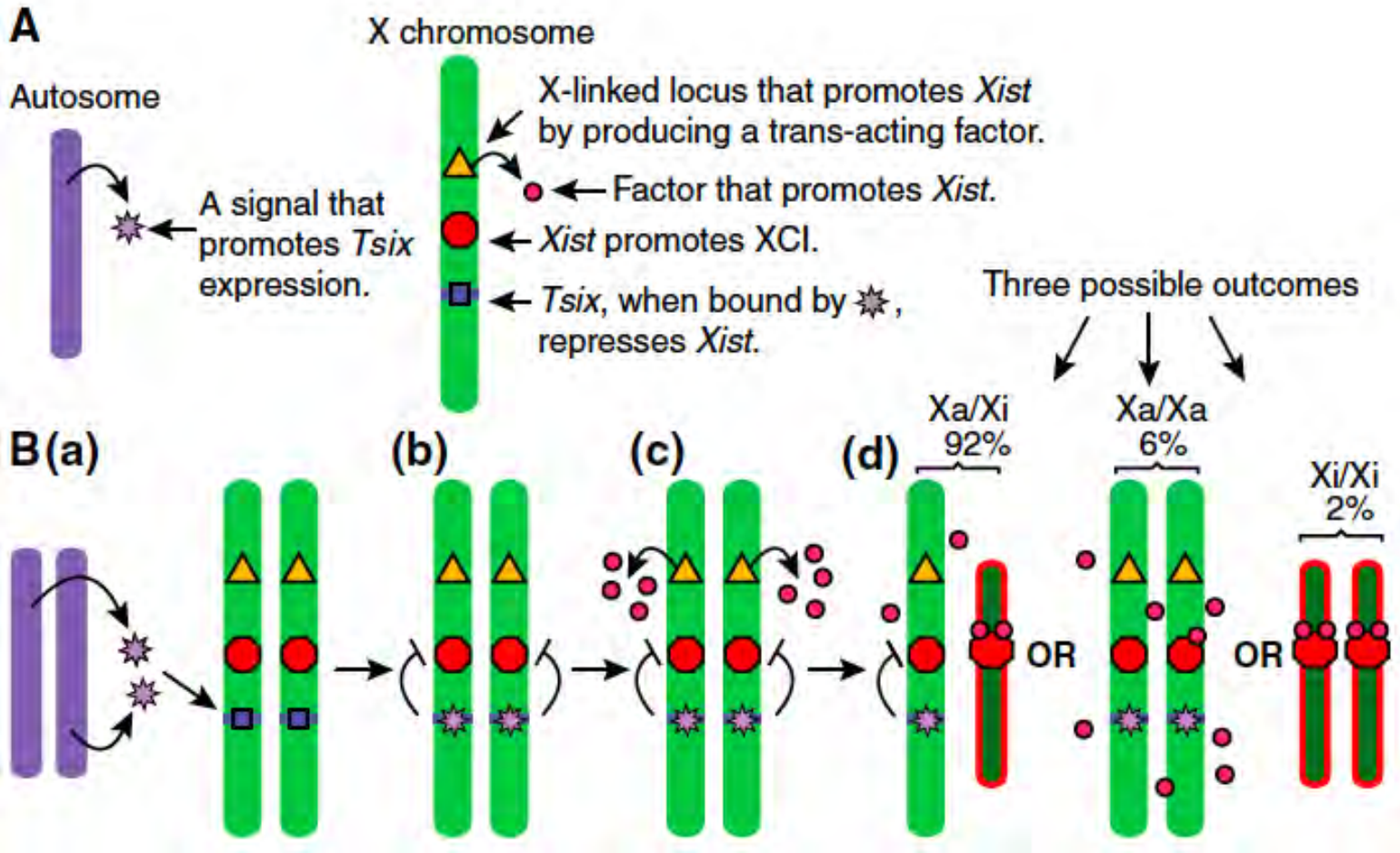
Model B


B
Autosomes work as a diploid pair to create a single blocking factor
X chromosomes create competence factors
Blocking factor neutralizes one of two competence factors
X chromosome with blocking factor remains active
X chromosomes bound by competence factor inactivate

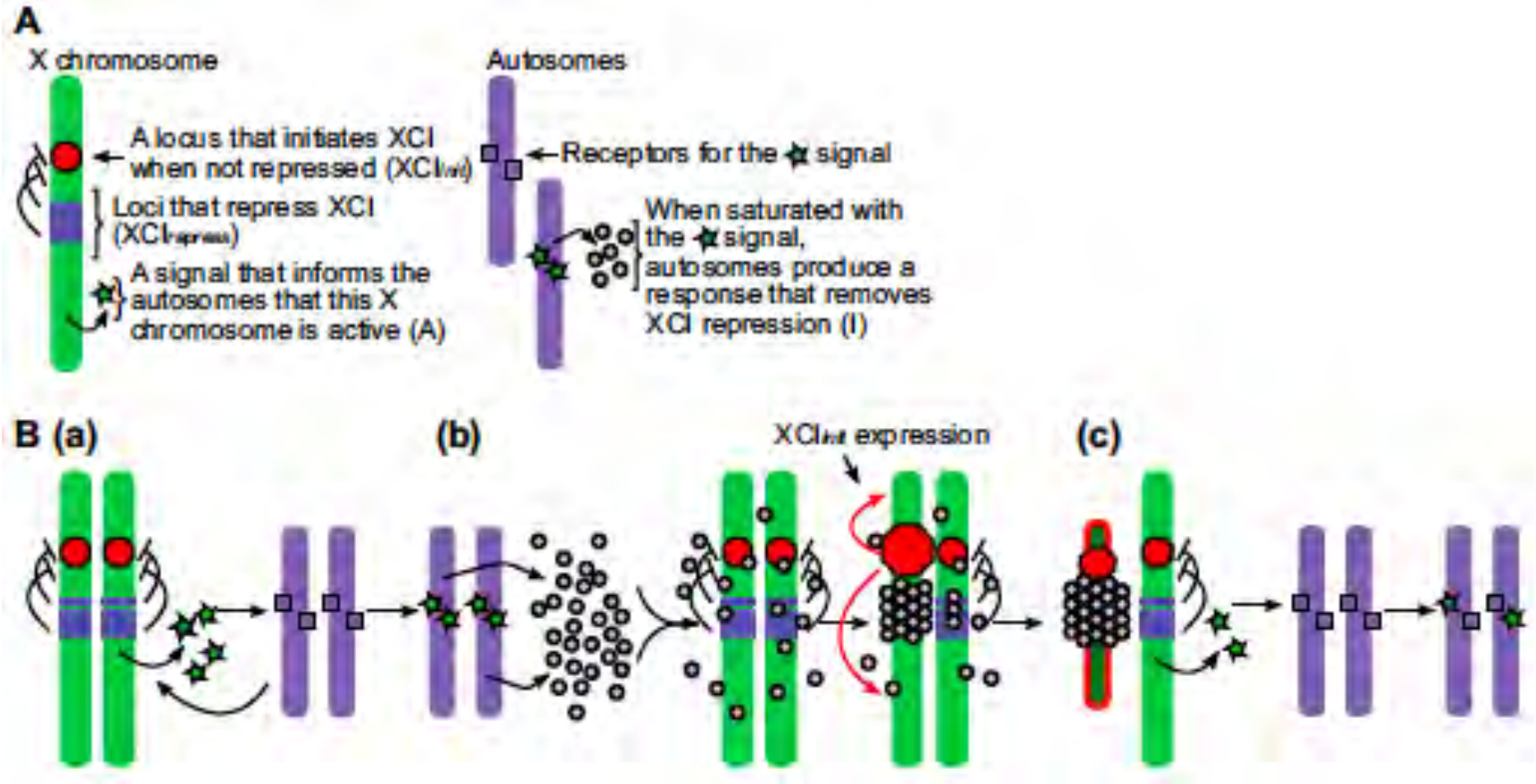
Model C


C
X chromosomes
Inactive X chromosome
Xpr
Xist
Tsix/Xite
Xist upregulation signal
Xist repression signal
Xist expression

Stochastic Model



Feedback Model



Feedback Model

- (**A) A** description of the components required for the model.
- (**B,a) The active X** chromosomes produce a trans-acting signal, A, that saturates specific sites on the autosomes.
- (**b) Once saturated, the** autosomes produce a swarm of inactivation signals, I. These signals bind to each other and to XCI inhibitors on the X chromosomes. Once all of the XCI inhibitors on an X chromosome are sufficiently bound by I, the XCI initiator induces inactivation.
- (**c) With only one** active X chromosome producing A, the autosomes are no longer saturated with A and stop producing I.

X Ignorance: The Current Paradigm for Large Scale Control of the Genome

- How one chromosome stays active while others get inactivated
- How some genes on X stay active anyway
- Why those genes screw up human X0 or XXY, but not mice
- Why *Xist* only gums up X chromosomes
- How just one “blocking factor” would be made in a cell