

# X-Chromosome Inactivation: The Case of the Calico Cat<sup>1</sup>

Marcy P. Osgood<sup>2,3</sup>

*Albany College of Pharmacy, Union University, 106 New Scotland Avenue, Albany NY 12208*

## PROLOGUE

This lecture addresses the topic of the first level of control of eucaryotic gene expression, that of inactivation of a whole chromosome. The coat color pattern seen in calico cats is used to illustrate the phenomenon. In these cats, always female, mosaic patches of cells express two different hair colors, yellow or black, depending on the inactivation of one of the X-chromosomes in each patch of fur. Recent studies have indicated that a new type of RNA, transcribed only from the inactive X, is responsible for the loss of function of the silent X-chromosome. Though mechanistic studies have not been completed, there is excitement at the unraveling of one part of the puzzle of the control of gene expression.

## AN INTRODUCTION TO THE TOPIC

Descriptions of the pertinent chemistry (DNA, proteins) and the architecture (histones, nucleosomes, 30 nm filaments) of the chromosomes in eucaryotes are vital in understanding the function of the genetic structures. However, such descriptions do not address the central question of how the genes on the chromosomes actually code for proteins, and when those proteins are expressed. The when is especially interesting, because it addresses the key question of development of an organism from fertilized egg to mature individual. If each cell in an organism contains the same exact set of chromosomal instructions, how is it possible for a skin cell to differ so drastically from a neuron, and when were those decisions made in the history of the individual cell's development?

The control of eucaryotic gene expression can be considered at different levels of organization, as well as temporally. The first is at the scale of activation or inactivation of the whole chromosome. The second tier of control includes activation/inactivation of different parts of the chromosome, that is discussed to some degree in a study of heterochromatin and euchromatin. The third, and most important, level of control is generated by selective transcription. More on this topic later.

I would like to focus on the first level of control of eucaryotic gene expression, which is accomplished by the actual turning on or off of a complete chromosome. This occurs in organisms, such as mammals, which utilize the convenient system of X and Y chromosomes to determine the sex of the organism: two X chromosomes create a female, and one X and one Y create a male. A problem is inherent in this system in that female organisms, with two X-chromosomes, have in effect a double shot of all the genes

that are carried on the X, while those that are male have just a single dose. Such an imbalance of genes is confusing at best, and may even be lethal. The solution is to silence, or inactivate, one of the X-chromosomes for most of the lifetime of each somatic female cell(1-3).

## THE CASE OF THE CALICO CAT

This is a cat with the coat color commonly referred to as calico. (A colored overhead, slide, or best of all, a living, purring cat in the classroom, is shown at this point.) Those of you who have had such a cat know one crucial fact about them; they must be spayed, or you will end up with lots of pretty calico kittens. In other words, all calico cats are female. The coat color is a result of the sex of the cat, and not the other way around.

Recall that there are two X-chromosomes in each cell in this cat, one maternal and one paternal, except for the germ cells, the eggs, which are haploid. On each X-chromosome, there is a gene that can code for either yellow or black coat color. During the development of the cat embryo, at the stage of 64 cells, one of the two X-chromosomes in each cell is inactivated, leaving only one X in working order. This X-silencing is random within the embryo, with the choice of which X is inactivated in a particular cell unaffected by the choice of that cell's neighbors. From that point on in the development of the cat embryo, all the daughter cells of each of the original 64 will continue to have that same X-chromosome inactivated, and so the same active gene for coat color, either black or yellow, depending on that initial inactivation. In general, these "sister" cells tend to remain close to each other during later stages of embryonic development. The graphic result of such a patchwork of clones of cells, expressing either black or yellow coat color, is this strikingly marked animal.

Only female cats have two X-chromosomes; only female cats can demonstrate the calico coloring. The white coat color often seen in calico cats in addition to the yellow and black patches is a result of an autosomal influence on the final coat markings(1-4).

## THE Xist/XIST GENE AND RNA

The story of how this selective silencing occurs is fascinating, and as yet unfinished. Most of the following information is derived from recent papers (cited in reference 4), and was the result of 30 years of focus on the question. The original proposition that the "extra" X was inactivated was put forward by Mary Lyon of the Medical Research Council Radiobiology Research Unit in Didcot, England in 1961(5). In a satisfying turn of events, it was one of Dr. Lyon's former students, Sohaila Rastan, who was head of the English group that elucidated part of the story with their work on the mouse genome. More information was added to the tale by

<sup>1</sup> Lecture from a fourth-year molecular biology course.

<sup>2</sup> Assistant Professor of Medicinal Chemistry.

<sup>3</sup> Present address: Department of Biology, The University of Michigan, Ann Arbor MI 48109-1048

a group from the United States working in the human genome, led by Carolyn Brown and Huntington Willard of Case Western Reserve University in Cleveland.

The key player in the story is a gene on the X-chromosome designated the "X-inactive-specific-transcript" gene, or *Xist*, as it is called in the mouse genome (pronounced "exist".) In humans, this gene is located on the inside third of the longer arm of the X-chromosome and is designated *XIST*, with the same pronunciation. As its name indicates, *Xist/XIST* is present on both X chromosomes, but this gene is specifically expressed only on the inactive X-chromosome; it is not expressed on the active X, a situation which certainly seems backward.

The *Xist/XIST* gene does not code for a protein; it is transcribed into RNA, but not an RNA like any other. This mammoth transcript (17 kb in humans) contains a large number of STOP codons, such that the longest open reading frame, which could potentially code for a protein, is only 400 base pairs long. This would be a remarkably short (and wasteful) message, if the *Xist/XIST* transcript was mRNA. To further cloud the issue, the open reading frames in the *Xist* and *XIST* genes are entirely different. If you remember from our discussion about the sequences that code for histone proteins, there is extremely strong evolutionary pressure to conserve the sequences of crucial proteins across species. The lack of such similarity between mouse and human *Xist/XIST* genes argues further that the *Xist/XIST*-RNA is not a messenger RNA. The third piece of evidence was gathered through the use of fluorescence *in situ* hybridization, which allows determination of the location of the RNA within the cell. This technique showed that almost all of the *Xist/XIST* transcript remains in the nucleus; therefore, the *Xist/XIST*-RNA is not carrying a message to the ribosomes in the cytoplasm for translation into protein.

Other studies confirmed that the unusual RNA coded for by the *Xist/XIST* gene was also not transfer RNA, ribosomal RNA, or even small nuclear RNA. At this point the story seems bogged down in negatives: the wrong chromosome, coding for an RNA that does not conform to any rules, or perform any of the normal functions. The *Xist/XIST* RNA does have a job, however, and the exact nature of this function and its mechanism are the next part of this as yet unfinished story.

The function of this perplexing RNA is to congregate in the nucleus around the inactive X-chromosome, which is also called the Barr body. This structure is formed of tightly condensed heterochromatin. The fluorescence *in situ* hybridization studies showed the concentration of the transcript around the Barr body, which is associated with the nuclear membrane. Exactly why the *Xist/XIST* RNA does this is still unknown, though the researchers involved are full of possible interpretations of their data, all of them, not surprisingly, providing an explanation for the mechanism of X-inactivation. Some of their suggestions include:

1. The *Xist/XIST* RNA could act as an enclosure around the X-chromosome, preventing the binding of RNA polymerases, which are necessary for normal transcription.
2. The *Xist/XIST* RNA could work, alone or in conjunction with some as-yet unspecified nuclear factors, to cause a conformational change in the chromosome, which somehow silences it.

3. The mysterious transcript perhaps causes the X-chromosome to bind to a specific site on the nuclear membrane, which then causes the chromosome to become nonfunctional.
4. The act of transcription of the *Xist/XIST* RNA is itself the switch that causes the turning off of the X-chromosome.

Any of these possible explanations, if proven, will also have to allow for recovery of an X-chromosome fully capable of replication (during mitosis) and reactivation (if it ends up in a germ cell.) No permanent change can have occurred to the chromosome's DNA. Finally, the question of how the *Xist/XIST* gene is itself turned on must be answered; at the moment, the speculation is that somehow it is autosomally regulated(4,5).

## THE BIG PICTURE

Obviously, the calico cat's coloring is not the only trait that is expressed in such a mosaic fashion. All female mammals are a melange of their active and inactive X-chromosomes, being expressed in clonally-derived patches of cells. The case of the calico cat is, however, the most vivid and graphic illustration of the inactivation of a complete chromosome in a commonly known animal.

Another example of the silencing of whole chromosomes can be observed in a less familiar organism. The mealy bug has its entire set of paternally-inherited chromosomes condensed into genetically inert heterochromatin. Though this is not a fact of major interest to many, studies on the mechanism of mealy bug chromosomal inactivation may provide mechanistic and structural information of value to human geneticists.

The X-inactivation story has already provided insights into an area that is compelling to most: the development of tumors. Analyses to determine which X-chromosome is silenced in each of the cells in a cancer demonstrate that, in the majority of cases, the same X is inactive in all the rogue cells; this is strong evidence that the tumor is monoclonal in origin.

Continuing studies on the mechanism of X-inactivation and the *Xist/XIST* genes will add to the understanding of the control of gene expression at this level. But this is just the simplest part of the fascinating puzzle of how and when the genetic information is expressed in organisms. In our next class, we will begin a discussion on the regulation and choices involved in selective transcription, and the jigsaw will begin to take on color and form. Do not expect a clear picture to emerge, though. The filling in of all the pieces may take a while.

*Am. J. Pharm. Educ.*, **58**, 204-205(1994); received 2/1/94, accepted 3/18/94.

## References

- (1) Micklos, D.A. and Freyer, G.A., *DNA Science*, Cold Spring Harbor Laboratory Press, Burlington NC (1990) pp. 87-110.
- (2) Alberts, B., Bray, D., Lewis J., Raff, M., Roberts, K. and Watson, J.D., *Molecular Biology of the Cell*, 2nd ed., Garland Publishing, Inc., New York NY (1989) pp. 577-578, and 1190-1191.
- (3) Wolfe, S.L., *Molecular and Cellular Biology*, Wadsworth Publishing Company, Belmont CA (1993) pp. 553-554.
- (4) Nowack, R., "Curious X-inactivation facts about calico cats," *J. NIH Res.*, **5**, 6065(1993).
- (5) Lyon, M., "Gene action in the X-chromosome of the mouse (*Mus musculus*)," *Nature* **190**, 372(1961).