**REVIEW ARTICLE**

# Mary Lyon's X-inactivation studies in the mouse laid the foundation for the field of mammalian dosage compensation

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Mary F. Lyon (1925-2014)

Mary Frances Lyon was born in 1925 in Norwich, England. In 1943 she entered Girton College, a part of Cambridge for women and graduated in 1946. She started graduate school, with R. A. Fisher at Cambridge and then moved to the Institute of Animal Genetics at Edinburgh where C. H. Waddington had developed good facilities for mouse work. Lyon earned her Ph.D. in 1950 and remained at Edinburgh to work on the mutagenic effects of radiation. The project moved to Harwell in England because of the need for more space. Lyon moved with the project and became head of the genetics division in 1962. There she remained for the rest of her career. She received a number of honours for her work, including being made a Fellow of the Royal Society in 1973; election to the U.S. National Academy of Sciences in 1979; receiving the Royal medal of the Royal Society in 1984 and the Wolf Prize in Medicine in 1997. She died on Christmas Day, 2014.

In the first 10 years of her postdoctoral work she published several papers on mouse and radiation genetics and one in 1960 on the murine sex-linked mottled gene. This paper, entitled, 'A further mutation of the mottled type in the house mouse' was published in the *Journal of Heredity* (Lyon 1960). Though rarely quoted, is highly significant. In it, she concludes 'This prompts the suggestion that in heterozygotes some event occurs at an early embryonic stage which determines whether the mutant or normal gene shall be active in each cell present at that time, and that the subsequent pattern depends on movement of the descendant cells. It is not possible to suggest what is the nature of this early event'. This 'early event' was specified a year later in a much quoted one page paper in *Nature* (1961). Lyon proposed that the mosaic phenotype of female mice heterozygous for some sex-linked mutants, including those in females heterozygous for coat colour mutants translocated to the X chromosome, was due to random embryonic and somatically permanent X chromosome inactivation. The model became known as the single active X hypothesis, was widely and quickly accepted.

One of the few criticisms of the idea came from a group of human cytogeneticists who pointed out that in individuals heterozygous for a normal X chromosome and a long-arm isochromosome-X, only the iso-X was late replicating and inactive (Muldal *et al.* 1963). This observation appeared contradictory to the Lyon hypothesis of random inactivation. It was soon pointed out that in such individuals, cells containing an active long-arm iso-X and an inactive normal X would be homozygous deficient for the short arm of the X chromosome. These cells would not be expected to survive and so the observed nonrandom pattern of X inactivation was, in fact, predictable on the Lyon hypothesis (Gartler and Sparkes 1963).

A major critic of Lyon's work was Hans Gruneberg, a prominent mammalian geneticist at University College, London. He thought that the observed patterns of X-linked skin markers in mice did not support Lyon's ideas, and he published lengthy criticisms of her work (Gruneberg, 1966a, 1966b, 1967). She responded to some of his criticisms (Lyon 1966). He did not understand how these complicated variegated patterns could develop. Lyon was not certain either and stated in her 1960 paper 'that the subsequent pattern depends on the movement of the descendant cells'. In my opinion, Gruneberg was blinded by the detail and failed to see the forest for the trees. Fortunately, Lyon was not deterred by the criticism. At the same time Mintz (1967) published her experiments with allophenic mice that showed how the X-linked variegated patterns could form.

Mary Lyon was not the only worker fascinated by X-linked variegation. Another mouse geneticist, Liane Russell, working at the same time as Lyon, also observed that variegation in coat character expression is associated with X-linked

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genes and with autosomal genes translocated to the X chromosome. She published a major eight page paper in *Science* and a longer one in *Genetics* the same year (1961) as Mary Lyon's one page paper in *Nature*. Some of the observations were very similar. Russell focussed on the X-linked associated variegation in X:autosomal translocations. She stressed that two X chromosomes were necessary for this phenomena and emphasized the possible relationship between mammalian X chromosome variegation and the V type position effect in *Drosophila* (Russell and Bangham 1961). In her 1961 paper in *Genetics* she suggests that even the X-linked variegating traits may all be V type effects (Russell 1961).

One of the most surprising aspects of the early work of Mary Lyon, and apparently all of Liane Russell's work on murine X chromosome variegation, was the lack of reference to dosage compensation. X-chromosome inactivation explains mammalian dosage compensation, a major achievement. Lyon referred to dosage compensation in the summary of her third paper on mammalian X chromosome inactivation (Lyon 1962). It was not mentioned in the 1960 or 1961 papers.

On the other hand, Beutler *et al.* (1962) independently described the single active X hypothesis on the basis of their observations on the expression of the human X-linked glucose-6-phosphate dehydrogenase (*G6PD*) gene. Males and females with one and two X chromosomes had the same levels of G6PD activity, and since their colleagues at the City of Hope (S. Ohno *et al.* 1959, 1960, 1961) had shown that sex chromatin represented a single inactive X chromosome rather then the joined two X chromosomes as originally thought (Barr and Bertram 1949), they concluded that the similarity of G6PD levels in males and females was due to the presence of single active X chromosomes in males and females, the other X chromosome in females being inactivated. Most importantly, they were able to demonstrate in heterozygotes the existence of two populations of red blood cells with respect to G6PD phenotypes.

It is interesting to note the different ways in which Lyon, Russell and Beutler looked at their material and came to their conclusions. Lyon knew from the genetic evidence that only one of a pair of X-linked alleles was being expressed and the problem for her was the underlying embryology that explained the variegation. She collaborated with Richard Gardner a mammalian embryologist and published some work with him (Gardner and Lyon 1971). I doubt if Lyon's apparent lack of interest in dosage compensation had any effect on the nature of her early work on X inactivation. Russells work might have benefited from an appreciation of dosage compensation. She might then have seen that the *Drosophila* and mammalian systems of dosage compensation are quite different and she might not have put so much emphasis on the possible relationship of position effect in *Drosophila* and mammalian X chromosome variegation effects. Beutler recognized that the single active X observation of Ohno *et al.* (1959, 1960, 1961) explained mammalian dosage compensation. This allowed him to concentrate on a straight forward analysis of his data.

Although, Mary Lyon will always be best known for her ideas and work on X-chromosome inactivation, she continued to work throughout her career on other aspects of murine genetics. She published nearly 200 papers, the majority of which were unrelated to X-chromosome inactivation.

She continued to work on radiation genetics throughout her career and had a strong interest in mapping the mouse genome. For example, in 2003 and 2004, she was first and senior author for two papers dealing with murine cataract formation to illustrate the extent of her interest and work aside from the X chromosome.

An important question that arose soon after the 1961 *Nature* paper and Russell's 1961 *Science* article was the nature of the centre from which inactivation spread. She and Russell were early thinkers about this question (Lyon *et al.* 1964; Russell 1964).

In 1990, Arthur Riggs suggested the existence of way stations or booster elements along the X chromosome to account for the spread of the X-inactivation signal. Lyon was taken with this idea and suggested that LINE elements, which are frequent on the X chromosome, could be such boosters and published several papers on the subject (Lyon 1998, 2000, 2003, 2006).

In concluding this article about Mary Lyon's scientific life, it is worth considering research at the City of Hope Medical Center that interacted with her work. Susumu Ohno was a member of the City of Hope Medical Center and in 1959 showed that the sex chromatin body was an inactive X chromosome (Ohno *et al.* 1959). This finding turned out to be a critical building block in Lyon's single active X hypothesis. A coauthor on the Ohno paper was W. D. Kaplan, an American drosophila geneticist who had carried out postdoctoral work with Mary Lyon on murine radiation genetics shortly after they both received their doctoral degrees (Kaplan and Lyon 1953a,b). Kaplan returned to USA where he joined Ohno at the City of Hope Medical Center. Bruce Cattanach, a close colleague of Lyon's also spent time at the City of Hope Medical Center working with Ohno (Ohno and Cattanach 1962) and returned to England to carry out important work on the mapping of the X inactivation centre through the use of X:autosomal translocations. Ernie Beutler, who independently developed the single active X hypothesis of dosage compensation was also at the City of Hope during this period and interacted with Ohno. In 1964, H. J. Muller, who started the field of dosage compensation (1914) spent a year at the City of Hope working with W. D. Kaplan, the same Kaplan who had worked with M. Lyon on radiation genetics in 1953. Muller and Kaplan published two papers on their work at the City of Hope (Muller and Kaplan 1964, 1966) and their prime message was that it was possible that both *Drosophila* and mammals achieved dosage compensation through depression of X chromosome activity in the female. This view proved incorrect, which seems strange, since Muller had predicted the correct evolutionary outcome for *Drosophila* over 30 years earlier.

As far as I can tell from Mary Lyon's papers, she was not interested in the evolutionary aspects of dosage compensation, but it would have been fascinating to have ease- dropped on a conversation between Ohno, Beutler, Muller and Lyon on the subject.

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