From the editor's desk

In Remembrance: Mary Frances Lyon

(15 May 1925 – 25 December 2014)

I start this piece from that day in 1969 when I first heard of the Lyon's hypothesis in the final semester of my master degree course in zoology at Banaras Hindu University in Varanasi. A lecture on evolution of sex chromosomes introduced us to the concept of inactivation of the X-chromosome in mammalian females, a mechanism that compensates for the numerical disparity of the X-chromosome between the sexes (female, XX; male, XY). The concept of a whole chromosome getting inactivated all at once intrigued me instantly, and to get a better insight of this I read almost through that night Ohno's *Sex chromosomes and sex-linked genes*, the only book which gave a reasonable treatment to vertebrate sex chromosomes at that time. Later that year, I joined as a Ph.D. student in the Cytogenetics Lab. I was still struggling with getting good chromosome preparations when we were visited by the renowned mouse geneticist, Hans Grüneberg, FRS, from Galton Laboratory, University of London. As is common in most labs, research students in the lab explained their work to Prof. Grüneberg. When my turn came I told him that I wanted to work on X-chromosome inactivation. He asked me a few questions, one of which was 'Is the entire X-chromosome inactivated?' to which my answer was 'Yes' and I cited a paper of Charles Ockey, published in the journal *Cytogenetics* in 1967, which showed lack of incorporation of ^{3}H-uridine in the sex chromatin. Grüneberg continued, 'Have you tested his finding?', and my answer was a reluctant 'No'. He persisted, 'And you believed it?'. I was silent. Our conversation ended with his comment, 'You cannot give hypotheses only, you have to prove them also; you cannot write cheques only, you have to cash them also'. Only a little later I realized that Prof. Grüneberg was a critic of various tenets of Lyon's hypothesis (see Gartler, special section in this issue), but this rhyming 'homily' from him taught me to evaluate published evidence critically. I did not pursue my Ph.D. research on X-inactivation, but my interest and curiosity persisted. Later my group did carry out a small piece of work on inactivation of X in males during spermatogenesis. My appetite for it was largely whetted by teaching dosage compensation to various groups of students, and in lab seminars. Only recently, a relatively large group of research students asked me to talk to them about Lyon's hypothesis and its ramifications. Obviously, just as I, as a naïve M.Sc. student got excited in 1969 by this disarmingly simple but acutely far-reaching hypothesis, there are many students even in 2015 who get equally excited and curious about it.

The 1961 paper of Mary Lyon (see paper appended with editorial) is a watershed in mammalian genetics, not only because it helped understand certain cytological and genetic observations that had bothered geneticists for a long time, but also because it liberated their thought process from the then prevailing drosophilist dogma on sex determination and dosage compensation. It is also important to appreciate that Lyon based her hypothesis on simple X-linked coat pattern traits, the normal female phenotype of the single-X mouse, and the sex chromatin in mammalian females. Her postulate, that inactivation occurs during early development randomly in any of the two Xs in a cell, and that once inactivated the pattern is followed irreversibly in the descendent cells, is a statement of remarkable ingenuity. While teaching this topic, I have often first placed before students the evidence that was available to Mary Lyon and asked them to come up with an explanation of the quandary. Initially they are nonplussed, but when Lyon's hypothesis is unfolded, the majority find it logically the most satisfying explanation, and some even wonder why it did not occur to them in the first place!

A huge mass of data from genetic, chromosomal and biochemical studies accumulated within a couple of years in support of the hypothesis, but there were also some that apparently did not match its tenets. Most of the latter were generally clarified without much difficulty, for example nonrandom inactivation of the normal X in X–autosome translocations. However, it would be simplistic to think that Lyon's hypothesis of X-inactivation has remained unaltered. Several caveats have been added to all the tenets of the hypothesis. We now know that randomness of X-inactivation is confined only to eutherian mammals, it is irreversible only in somatic cells, and that not the entire, but 'almost' the entire X-chromosome is inactivated (see Graves, and Disteche, special section in this issue). That notwithstanding, Lyon's own work and those of many others have gone on to show the robustness of her conjectures. However, functional monosomy of the X-chromosome as a mechanism of dosage compensation raises important questions regarding its selective value, particularly when monosomy of the autosomes in mammals is generally lethal. Mary Lyon came up with the idea of duplication of X-linked genes either on the X itself, or on autosomes, as a mechanism to restore disomy of the X-linked genes, examples of which are many. Gene duplication

does account for a proportion of the genes but quite clearly a large number of X-chromosomal genes are not duplicated. The most remarkable findings have appeared more recently which demonstrate upregulation of the nonduplicated X-chromosomal genes in both males and females (see Disteche, special section in this issue), a mechanism analogous to the situation in *Drosophila* where the single X-chromosomal genes in males are hyperactivated to compensate for the double dose of the X-chromosome in females.

It would be a very narrow view of her work if we restricted it within the confines of dosage compensation. Lyon's hypothesis brought in its wake several concepts that either did not exist or had been relegated to archival importance, at that time but today are important pillars of functional genomics, of the human genome in particular. The concept of an Xce/XIC (X-chromosome controlling element) as the regulator of X-inactivation, and the later discovery of XIST, a noncoding RNA, as the *cis*-acting regulator of X-inactivation were in fact among the first and the most formidable demonstrations of the importance of RNA as a functional gene product. Today, a wide variety of long, short, mini and sno RNAs form an integral part of transcriptome of any genome; mRNA is no longer the only vehicle of gene function (see Lakhotia, special section in this issue). The concepts of genomic imprinting and epigenetics that were almost forgotten found their best manifestation in irreversibility of X-inactivation. The first time DNA methylation was proposed as a mechanism of memorizing molecular ancestry in eukaryotic cells it was to explain the irreversibility component of Lyon's hypothesis. In the rapidly growing field of medical genetics, X-chromosome inactivation adds a new dimension, especially in explaining X-linked disorders, in women, developmental sex disorders, and sex determination (see Greenfield; special section in this issue).

Dr Mary Frances Lyon spent almost her entire professional career at MRC Radiobiological Research Unit in Harwell in England (see Greenfield, special section in this issue) where she joined in 1954 after her Ph.D. (1951) under James Falconer at Edinburgh, after initial training with R. Fisher at Cambridge. She headed the Genetics Department of the unit from 1964. Though she retired in 1990, she continued to work in the lab until 2012. It might raise a few eyebrows among today's youth that in 1947 as a graduate student of zoology in Cambridge, Lyon was awarded only a 'titular' degree because women were not eligible for formal education at premier institutions.

X-chromosome inactivation was but one of her contributions to mammalian genetics. Her work on T-mutants as well as on Tfm mice set new paradigms in mouse genetics. She was elected Fellow of the Royal Society (1973) as well as Foreign Member of the US National Academy of Sciences (1979). In 2004, the MRC Lab at Harwell established a 'Mary Lyon Centre' of mice stocks in her honour. The metaphorical use of the term 'Lyonisation' for dosage compensation by X-inactivation is a natural tribute to her contribution.

When H. Sharat Chandra succeeded in convincing Indian Academy of Sciences in 1984 to revive *Journal of Genetics* after years of neglect, he requested Lyon to join the Advisory Board of the journal, and she continued to be a member until 2013, when it was felt that a separate advisory board was no longer needed. Mary Lyon passed away a year ago on 25 December 2014. This issue of the journal, includes a special section dedicated to her memory with five articles that review the status of sex chromosomal function in the current scenario. I am personally grateful to the authors of these articles for agreeing to our request and writing them in a relatively short time. I am particularly grateful to Jenny Graves, Christine Disteche, Andy Greenfield and MRC Radiobiological Laboratory for sparing some of her memorable pictures, and for the permission to publish them in this issue. They reveal the livelier side of Lyon. I am confident that these articles will not only provide readers the history and the state of the art of Lyonisation but will stimulate some of them to enter this ever-growing and exciting field. I put on record my appreciation of my fellow editors of the journal who actively supported and encouraged me in this endeavour, and to the publications team of the Indian Academy of Sciences for smooth processing of this work.

Rajiva Raman

Editor-in-Chief

Mary Lyon in pictures. (a) Mary Lyon in Kyoto, Japan, 1991. (b) During her Ph.D. days in Institute of Animal Genetics, Edinburgh: with her mice, 1950. (c) Mary Lyon relaxing. (d) Birthday celebrations at the X-inactivation meeting, Oxford: with Noui Takagi, 2011. (e) Birthday celebrations at the X-inactivation meeting, Oxford: with Christine Disteche and Jenny Graves, 2011. (f) At Edinburgh, Ph.D. days, 1950. (g) At MRC, Harwell, UK, 1963. (h) Celebrations at her 65th birthday at MRC, Harwell: with Bruce Cattanach, 1989. (i) At inauguration of the Mary Lyon Centre, MRC Harwell, 30 September 2004. (j) Meeting on the 'Condensed chromatin and X-inactivation' at IISc., Bengaluru, India: with Ed Southern, 1981. (k) At a lunch in Oxford with Steve Martin and Jenny Graves.

We reprint here the historic paper of Mary Lyon published in *Nature* (1961) which enunciated basic tenets of the 'Lyon's hypothesis' that envisioned inactivation of the X-chromosome as a mechanism of dosage compensation in mammals.

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GENETICS

Gene Action in the *X*-chromosome of the Mouse (*Mus musculus* L.)

Ohno and Hauschka[1] showed that in female mice one chromosome of mammary carcinoma cells and of normal diploid cells of the ovary, mammary gland and liver was heteropyknotic. They interpreted this chromosome as an *X*-chromosome and suggested that the so-called sex chromatin was composed of one heteropyknotic *X*-chromosome. They left open the question whether the heteropyknosis was shown by the paternal *X*-chromosome only, or the chromosome from either parent indifferently.

The present communication suggests that the evidence of mouse genetics indicates: (1) that the heteropyknotic *X*-chromosome can be either paternal or maternal in origin, in different cells of the same animal; (2) that it is genetically inactivated.

The evidence has two main parts. First, the normal phenotype of *XO* females in the mouse[2] shows that only one active *X*-chromosome is necessary for normal development, including sexual development. The second piece of evidence concerns the mosaic phenotype of female mice heterozygous for some sex-linked mutants. All sex-linked mutants so far known affecting coat colour cause a 'mottled' or 'dappled' phenotype, with patches of normal and mutant colour, in females heterozygous for them. At least six mutations to genes of this type have been reported, under the names mottled[3,4], brindled[3], tortoiseshell[5], dappled[6], and 26*K* [2]. They have been thought to be allelic with one another, but since no fertile males can be obtained from any except, in rare cases, brindled, direct tests of allelism have usually not been possible. In addition, a similar phenotype, described as 'variegated', is seen in females heterozygous for coat colour mutants translocated on to the *X*-chromosome[7,8].

It is here suggested that this mosaic phenotype is due to the inactivation of one or other *X*-chromosome early in embryonic development. If this is true, pigment cells descended from cells in which the chromosome carrying the mutant gene was inactivated will give rise to a normal-coloured patch and those in which the chromosome carrying the normal gene was inactivated will give rise to a mutant-coloured patch. There may be patches of intermediate colour due to cell-mingling in development. The stripes of the coat of female mice heterozygous for the gene tabby, *Ta*, which affects hair structure, would have a similar type of origin. Falconer[9] reported that the black regions of the coat of heterozygotes had a hair structure resembling that of the *Ta* hemizygotes and homozygotes, while the agouti regions had a normal structure.

Thus this hypothesis predicts that for all sex-linked genes of the mouse in which the phenotype is due to localized gene action the heterozygote will have a mosaic appearance, and that there will be a similar effect when autosomal genes are translocated to the *X*-chromosome. When the phenotype is not due to localized gene action various types of result are possible. Unless the gene action is restricted to the descendants of a very small number of cells at the time of inactivation, these original cells will, except in very rare instances, include both types. Therefore, the phenotype may be intermediate between the normal and hemizygote types, or the presence of any normal cells may be enough to ensure a normal phenotype, or the observed expression may vary as the proportion of normal and mutant cells varies, leading to incomplete penetrance in heterozygotes. The gene bent-tail, *Bn* [10], may fit into this category, having 95 per cent penetrance and variable expression in heterozygotes. Jimpy, *jp*, is recessive, suggesting that the presence of some normal cells is enough to ensure a normal phenotype, but Phillips[11] reported one anomalous female which showed the jimpy phenotype. Since it showed the heterozygous phenotype for *Ta* this animal cannot be interpreted as an *XO* female ; it is possible that it represents an example of the rare instance when by chance all the cells responsible for the jimpy phenotype had the normal gene inactivated.

The genetic evidence does not indicate at what stage of embryonic development the inactivation of one *X*-chromosome occurs. In embryos of the cat, monkey and man sex-chromatin is first found in nuclei of the late blastocyst stage[12,13]. Inactivation of one *X* at a similar stage of the mouse embryo would be compatible with the observations. Since an *XO* female is normally fertile it is not necessary to postulate that both *X*-chromosomes remain functional until the formation of the gonads.

The sex-chromatin is thought to be formed from one *X*-chromosome also in the rat, *Rattus norvegicus*[14], and in the opossum, *Didelphis virginiana*[15]. If this should prove to be the case in all mammals, then all female mammals heterozygous for sex-linked mutant genes would be expected to show the same phenomena as those in the mouse. The coat of the tortoiseshell cat, being a mosaic of the black and yellow colours of the two homozygous types, fulfils this expectation.

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