The Evolution of Sex Chromosomes and Dosage Compensation in Mammals

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INTRODUCTION

There is a considerable variety of sex determination systems across the animal kingdom. In many alligators, reptiles and turtles it is the temperature at which eggs are incubated what determines the sex of an embryo. In contrast, in mammals, birds and snakes this is controlled genetically, with either an XX female/XY male or ZZ male/ZW female sex chromosome system. Even between closely related subclasses of mammals, remarkable differences exist in size, gene order and content of sex chromosomes. Therefore, it seems very interesting to explore how such different sex chromosome systems have originated and evolved throughout the time. First of all, this essay will describe the main types of mammalian sex chromosomes and discuss their evolutionary relationships using comparative genomics. In addition, the theoretical model of the origin of sex chromosomes will be presented. Finally, Ohno's hypothesis about the origin of dosage compensation will be discussed and new experimental evidence challenging the theory considered.

COMPARATIVE GENOMICS

There are three main groups of existing mammals: eutherians, marsupials and monotremes. Eutherians and marsupials are phylogenetically more closely related because monotreme group diverged first approximately 166 million years ago (Fig. 1). Around 18 million years later eutherian and marsupial mammals diverged from each other. Therefore, it seems rather intuitive that XX/XY sex chromosomes in human and tammar wallaby appear more similar than in platypus, which has ten unpaired sex chromosomes: five X and five Y in males and ten X in females.

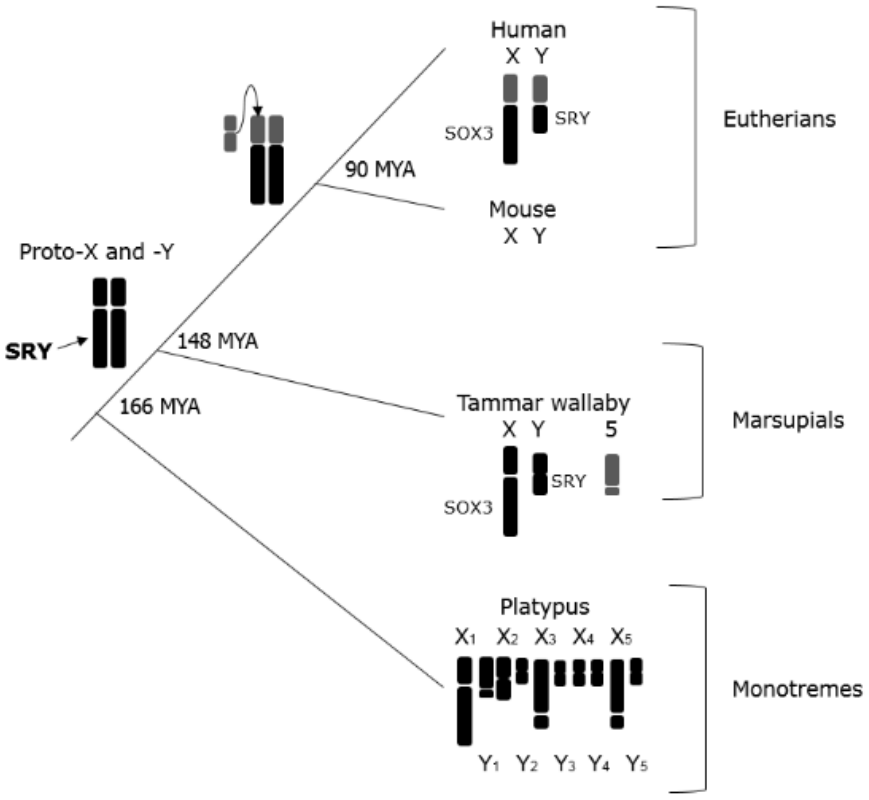

Fig. 1: Main mammal sex chromosome systems and their evolutionary relationships (own diagram, based on Livernois et al, 2012). Numbers near branches indicate estimated divergence dates, million years ago (MYA). SRY is a sex-determining gene evolved from SOX3 region on the X chromosome. Grey colour indicates homologies between eutherian XY chromosomes and the marsupial autosome.

In eutherian mammals, the X chromosome is well conserved between species. It is relatively big (155 Mb in humans) and encodes a wide range of genes involved in housekeeping, intelligence, sex and reproduction-related functions (Livernois et al, 2012). In contrast, the eutherian Y is small, gene poor and varies largely between species in respect to structure and gene content. The model marsupial X and Y are smaller than respective eutherian chromosomes, possibly because the remaining homologous region is located on the autosome 5 (Fig. 1). It is believed that this added region resulted from a single fusion of sex chromosomes with an ancestral autosome after the marsupial/eutherian split. In eutherians and marsupials SRY is identified as the sex determining gene, whereas its analogue in monotremes remains unknown. Most surprisingly, none of X chromosomes in monotremes share sequence homology with therian X chromosomes. Instead, certain regions of autosomes 6, 15 and 18 were found to be homologous to eutherian sex chromosomes. This suggests that the ancient precursors of current sex chromosomes were chosen independently in different lineages, possibly from a pool of predisposed autosomes.

EVOLUTION OF SEX CHROMOSOMES

The finding that the sex chromosomes in one mammal lineage share homologous sequences with autosomes in another has led to the development of a current model of the sex chromosome evolution. Despite the fact that different sex chromosome pairs have evolved independently across lineages, they have all originated by a common evolutionary pathway (Fig. 2).

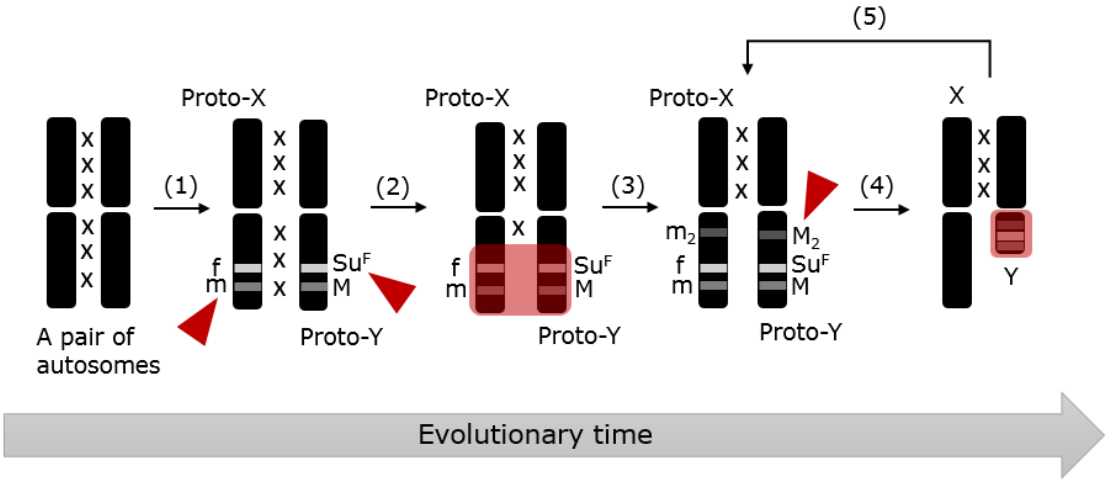

Fig. 2: Theoretical model of the origin of sex chromosomes from a pair of homologous autosomes (own diagram, based on Orr and Kim, 1998). Stage 1: An initial locus M undergoes the change to m, where m is a recessive allele that leads to the appearance of females. M in the Proto-Y remains unchanged. The second locus in the Proto-Y acquires a mutation from f to Su^F, where Su^F is a female suppressing factor. Stage 2: Recombination (represented by crosses) in this region is supressed in order to keep the sex-specific genes together. Stage 3: New sex specific alleles aggregate close to this sex determining region and recombination is further reduced. Stage 4: The loss of recombination ultimately results in Y degeneration. Stage 5: The process of sex-specific allele aggregation and Y chromosome degeneration continue gradually over the time.

The initial step in this process is an acquisition of two sex determining genes by a chromosome pair. For instance, a recessive mutation can occur in the Proto-X chromosome, resulting in a male sterility allele which causes the appearance of females. The other chromosome maintains the initial allele unchanged. If the second chromosome now undergoes a mutation to a dominant female-suppressing allele that leads to individuals developing as males, recombination in this region will be suppressed in order to ensure successful reproductive functions. This will ultimately result in a small sex determining region on an otherwise ordinary chromosome where replication is restricted. It is commonly believed that an acquisition of a single sex determining factor is sufficient to trigger the process (Livernois et al, 2012, Carvalho, 2002). However, it is important to highlight that a selection pressure for reduced recombination in the region will only be induced in the presence of at least two separate sex-determining mutations (as crossing over between these could reverse to hermaphrodism or result in sterility and therefore reduces the fitness).

As new male beneficial genes start to aggregate close to this region increasing fitness of individuals, recombination with the Proto-X is further restricted. Finally, in the absence of genetic recombination, the Proto-Y will degrade. However, specific mechanisms that drive this process are largely debatable. A number of different theories has been proposed, including Muller's ratchet, background selection and genetic hitchhiking. It is important to note that most of these models are usually based on calculations that rely on a number of theoretical parameters, which are hard to estimate accurately. With the lack of direct experimental evidence and limitations in empirical hypothesis testing, it is challenging to conclude which theory should be accepted.

DOSAGE COMPENSATION

As the Proto-Y chromosome gradually degraded, corresponding genes remained as a single copy in the heterogametic XY sex. However, this presented with a challenge because there was now unequal expression of genes on sex chromosomes between sexes. In addition, it did not match expression from the autosomes in males, i.e. $X/AA \neq 1$. It is clinically evident that monosomy, the presence of only one chromosome from a pair, results in developmental abnormalities, and is lethal in most of the cases in humans. Therefore, a novel mechanism had to evolve to compensate for reduced levels of gene expression from the Y chromosome. It was proposed that this involved two critical steps: firstly, the genes on the single X chromosome in XY males were upregulated to match the expression in autosomes (Ohno, 1967). As it was passed on to the next generations, XX females had ultimately two hyper-expressed X chromosomes. Therefore, one of them had to be inactivated to restore the balance between sexes (Fig. 3). It is most likely that the acquisition of such dosage compensation proceeded slowly, as genes that could not tolerate any decreased dosage gradually lost their function.

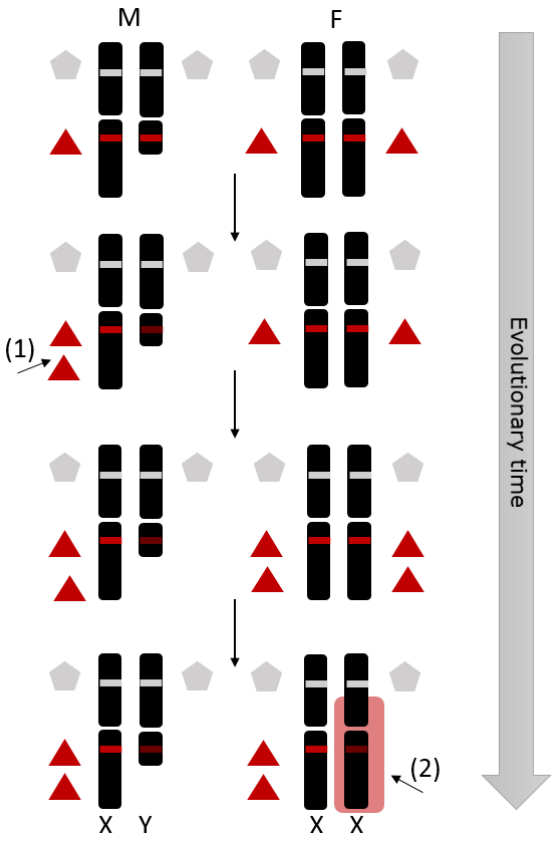

Fig. 3: Theoretical model of the evolution of dosage compensation (own diagram, based on Livernois et al, 2012). Step 1: Upregulation of unpaired region on the male X chromosome to match expression levels in females. Step 2: Inactivation of one of X chromosomes in females to restore the balance between sexes. Triangles and pentagons represent protein products translated from corresponding genes, colour coded.

Experimental Evidence for Dosage Compensation

Traditionally, Ohno's hypothesis was supported by evolutionary arguments but recent technological advances have provided new evidence from microarray expression data. It has been shown that for genes expected to be dosage-sensitive (transcription factors, ribosome proteins) expression from the X chromosome is similar to that from the autosomes, resulting in the X/AA ratio around 1 (Pessia et al, 2011). However, it is important to consider that despite being a powerful genomic tool, microarray technique is known to have limited sensitivity and reproducibility. Therefore, the results of these experiments cannot be taken for granted and should be verified by confirmatory experiments. In fact, RNA sequencing in human and mouse reported a ratio of ~0.5, indicating the absence of X upregulation. (Xiong et al, 2010). Further proteomic analysis also supported this finding at the protein level. If that was actually the case and the initial step in the evolution of dosage compensation had never happened, it would challenge the entire concept of X chromosome inactivation.

There is now an alternative hypothesis proposed, which states that as the Y chromosome gradually degraded and genes lost their function, there was a selective pressure for corresponding hyper-functioning X proteins to evolve. These proteins maintained their original function at half the gene dose and ensured equal activity in respect to the autosomes. Similarly to Ohno's theory, these hyper-functioning proteins were then inactivated on one of X in females to ensure a similar expression ratio between sexes.

Rather surprisingly, there was no evidence found for dosage compensation in birds, insects and reptiles (Xiong et al, 2010). Research on monotremes showed partial dosage compensation, as some X-specific genes were mono-allelically transcribed, but for others both alleles were expressed (Deakin et al, 2008). Although scientifically important, these findings do not really address the timeline of the evolution of dosage compensation. Therefore, it remains unclear whether dosage compensation is a common mechanism which initially applied to all species but was lost in some of the lineages, or it is a recent genetic sophistication which evolved only in eutherian and marsupial mammals.

CONCLUSIONS

Across the animal kingdom, there is a remarkable variety of systems that determine the development of sexual characteristics in an organism. In all mammals sex is determined genetically, with an XX female/XY male sex chromosome system. However, significant differences exist in size, structure and gene content of sex chromosomes even between closely related subclasses of mammals. This essay has described the main types of mammalian sex chromosomes and discussed their phylogenetic relationships. Eutherians and marsupials are evolutionary more closely related because monotreme group diverged first 160 million years ago. Therefore, it seems comprehensible that their XX/XY sex chromosome systems share more similarities compared to monotremes. Based on comparative genomics, the current model of the sex chromosome evolution has been developed. Having originated independently from different regions of an ancestral genome, mammalian sex chromosomes have all evolved in a strikingly similar way. Firstly, the Proto-Y chromosome underwent two acquisitions of sex-determining genes. In order to keep sex-specific alleles together, recombination in this locus was suppressed. In the absence of recombination, the Y chromosome was ultimately degraded. However, specific forces of evolution that played a role in this process remain controversial. Finally, the mechanism of dosage compensation was considered. Evolved to equalise the gene expression between sexes, it involved two critical steps: upregulation of unpaired region on the male X chromosome and later inactivation of one of two X chromosomes in females. However, recent RNA sequencing and proteomics experiments provided new evidence that might challenge the entire theory of dosage compensation. It is a great example of how technological advances can redefine our understanding and suggest new perspectives even to widely accepted theories.

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