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Review

Sex Determinants in the Genome – Lessons from the Animal Kingdom

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ABSTRACT

The immense value of sex differentiation as a means of enriching and evolving the genome has been proven by the vast variety of sex determining mechanisms to which organisms of all kinds resort. From single gene switching pathways found in lower level organisms to haplodiploid reproduction in hymenoptera, temperature-determined sex in reptiles and sex chromosomes in mammals and avians, nature and evolution have designated an impressive amount of effort to ensure that sex-specific variations remain under well-regulated control. Therefore enhancing our efforts to study some of the strategies recruited for the above may also lead to a better understanding of the inherent complexity of sexual dimorphism in general.

Key words: *differentiation, sex determining mechanisms, single gene switching pathways, temperature-determined sex, sex chromosomes, sexual dimorphism*

Introduction

Scientists throughout the ages have always been fascinated by the vast variety and ingenuity of reproduction mechanisms employed by various species. But even so, up to this day, we have yet to string the exact course of evolution that has led to the current wealth of sex determination mechanisms, a key element to the biological wonder also known as life.

However, despite the lack of a concise theory of how these mechanisms have come about, an organized system of studying them has been established. Sex determination mechanisms as a whole can be divided into chromosomal and non-chromosomal. Accordingly, non-chromosomal mechanisms can furthermore be distinguished into gene-related and environmental, with the latter generally considered as the evolutionally more ancient (Figure 1).

Environmental Sex Determination

Environmental sex determination, and especially when temperature related, is considered the forerunner of most contemporary mechanisms (Figure 2). It is mostly employed in several species of turtles, crocodiles, tuatara giant lizards and selected fish specimens.

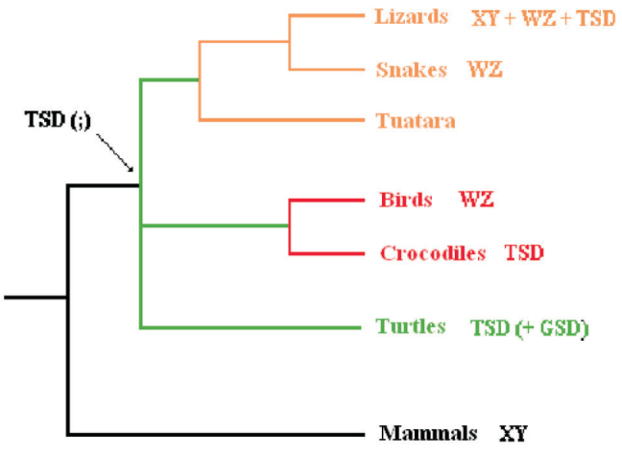

Fig. 1. A scheme that roughly indicates the evolutional pattern of sex determination mechanisms. XY – XY chromosomal sex determination, WZ – WZ chromosomal sex determination, TSD – temperature-dependent sex determination, GSD – gene-dependent sex determination.

For this assortment of mechanisms, the effect of several environmental factors, such as temperature, during

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Fig. 2. The basic principle of environmental sex determination is that environmental effects cause the affinity of several molecules for their receptors to increase or deteriorate.

fertilization and the primary stages of embryogenesis can alter the pattern of certain crucial proteins that effectively control the development of sex traits. For example, many species of reptiles, including most turtles and almost all crocodilians, have no discernible sex chromosomes, nor is their sex determined by the presence or absence of specific genes. In these organisms, it is the temperature of the environment in a specific period of incubation that can determine whether the animal in question will turn into a male or a female. This particular period, which usually coincides with the middle third of development, is also known as the thermosensitive period (TSP), and it has been mainly linked to the efficiency of aromatase, the key enzyme for the conversion of androgens into estrogens.

More specifically, in reptiles, while steroidogenesis begins very early, prior even to the thermosensitive period, aromatase activity tends to remain universally low. With the onset of the thermosensitive period however, aromatase activity seems to increase in certain temperatures, which vary for each species. For example, in marine and freshwater turtles, higher temperatures cause an exponential increase of aromatase activity, whereas in lower temperatures aromatase activity remains low. The different levels of aromatase activity then guide the differentiation of the indifferent gonad into an ovary or testis. Once the thermosensitive period is over and the fate of the gonad has been established, further changes in temperature seem to have no effects[1].

As mentioned above, the thermosensitivity of the gonads has also been demonstrated in several fish and some amphibians. These however tend to combine gene-dependent or chromosomal sex determination with the mechanism demonstrated here. As a result, the effects of temperature may go against the genotypic directions, allowing the existence of animals in genotypic and phenotypic sex discordance, a phenomenon known as sex reversal[2] (Table 1).

Gene Dependent Sex Determination

The next step in the evolutionary scale takes us to gene dependent sex determination. With these mechanisms, sex can be determined by focusing on a single gene.

Gene dependent sex determination can be differentiated by chromosomal from the fact that there is no specialized set of chromosomes (sex chromosomes), while the gene itself can often be found in various locations within the genome, due to transpositions. For example, in the *Megaselia scalaris* species the sex determining gene is actually a transposone that regularly alternates its home among the chromosomes.

For example, the haplodiploid genetic system we encounter in the insect order of Hymenoptera allows the laying of both unfertilized eggs that typically develop into uniparental haploid males and fertilized eggs that can give us biparental diploid females. The best understood strategy for this seems to be single-locus complementary sex determination (sl-CSD), in which sex is determined by multiple alleles at a single locus. Heterozygotes at that sex locus develop as females whereas hemizygotes and homozygous diploids develop as males, thus providing us with the pattern presented above[3].

Moving along the same lines, a single gene is also considered responsible for determining sex in *Drosophila melanogaster*, and more specifically the activation of the *sxl* gene (sex-lethal) in females during the early stages of development in response to the ratio of X chromosomes to autosomes (X:A ratio). The latter is communicated early in development through the delicate balance between the dose-sensitive X chromosome numerator elements, which include genes such as *sis-a*, *sis-b*, *runt* and less so *sis-c*, and the autosomal denominators, such as *dpn*, in conjunction with the maternally derived products of the *da* gene and the more recently studied *emc*, *groucho, her* and *snf*. An early form of the SXL protein is then produced that allows the correct reassembling of the later gene transcripts through sex-specific splicing of its mRNAs, unlike in males, where the delayed gene activation leads to the production of an inactive protein. Once the SXL active state has been established, it then goes on to regulate a series of other proteins that control female development, once again through the process of alternative splicing, leading finally to the two alternative products of the *doublesex* gene (*dsx*), DSX^{F} and DSX^{M} [4–6].

Similarly, the active state of the *xol-1* gene in males of the *Caenorhabditis elegans* species acts like a switch for

TABLE 1
SEX REVERSAL: THE COMBINATION OF GENOTYPIC AND TEMPERATURE-DEPENDENT SEX DETERMINATION ALLOWS A PHENOMENON KNOWN AS SEX REVERSAL, WHERE THE PHENOTYPIC SEX DOES NOT ALWAYS AGREE WITH THE GENOTYPIC DIRECTIONS

	Female-producing temperatures	Male-producing temperatures
XX (ZW)	Female (in accordance with genotype)	Female OR male (in discordance with genotype)
XY (ZZ)	Male OR female (in discordance with genotype)	Female (in accordance with genotype)

✗	✓	✗	✓	✗	✓
xol-1 —	sdc-1 sdc-2 — sdc-3	her-1 —	tra-2 — tra-3	fem-1 fem-2 — fem-3	tra-1
✓	✗	✓	✗	✓	✗

Fig. 3. Activating xol-1 acts like a switch in the sex determination pathway.

the pathway of genes that determine sex (Figure 3) and that, through a pathway of inhibitory genes, finally lead to the active or otherwise state of the TRA-1 protein, which acts as a transcription factor. As before, the X:A ratio is communicated with the help of several »X-signal elements«, such as the SEX-1 protein that acts on the level of transcription and the FOX-1 protein that acts post-trancrisptionally. These two, among others that have yet to be deciphered, manage to suppress the levels of the XOL-1 (XO lethal) key protein, setting off the mechanism that determines sex[7–9].

However, *C.elegans* worms are special in that the choice lies between males with one X chromosome and hermaphrodites with two. Indeed, the *C.elegans* hermaphrodites pose an interesting issue. These are specialized females which in the fourth and final larval stage (L4) produce around 300 sperm, to use for self-fertilization when there are no males available. This requires a careful regulation of switching between male and female differentiation of the same germ cells without the benefit of the usual sex determination gene pathway, since the »male« genes that normally regulate spermatogenesis are inherently inactive in hermaphrodites. Instead, a specific series of genes take over in a specific stage of development and act in place of the HER-1 protein to inhibit *tra-2* and allow spermatogenesis to take place for a specific period of time. Once this is over, a new series of genes take their place, *tra-2* is once again active, and the adult hermaphrodite is free to continue with oogenesis for the rest of its life[9,10].

Still, one should bear in mind that in both of these species the genes in question are permanently localized in a specialized set of chromosomes, despite the rest of the key factors in the sex determining pathways being scattered among the rest of the chromosomes. So it would be possible to consider these two an intermediate step between gene dependent and chromosomal sex determination.

Chromosomal Sex Determination

The next and final step in the evolutionary scale is what is commonly known as chromosomal sex determination. Along the passage of time, genes that were related to determining sex began to gather in specific chromosomes, which are now labeled as sex chromosomes since their presence or absence in an organism heralds the establishment of a particular sex.

Most species that adopt a chromosomal strategy of determining sex seem to follow a common pattern:

- There are two distinct sex chromosomes that differentiate in both size and content
- One sex requires a pair of sex chromosomes of one kind, while the other sex requires a pair of sex chromosomes of both kinds

Although the various sets of sex chromosomes that belong to contemporary organisms display a wide scale of differences and similarities, most of which can be traced to the existence of a common ancestral chromosome, we distinguish between two major varieties of sex determination mechanisms, depending on the sex that requires a unanimous set of said sex chromosomes:

Z/W sex determination

It can be attributed to species that require two sex chromosomes of the same kind for males (ZZ) and of two different kinds for females (ZW), such as birds, snakes, lizards and several fish.

The avian Z and W in particular seem to have no relation to the mammalian X and Y, but to have evolved from different pairs of autosomes. And this is part of the reason we are not yet certain which of the two carries the genetic trigger for sex determination. To this day, there are two major theories under investigation. Sex may depend on Z chromosome dosage, according to the example of *Drosophila melanogaster* and *C.elegans*. One candidate gene for this theory is the *DMRT1*, which is located on Z chromosomes, escapes dosage compensation and is expressed specifically in the gonads, and is thus capable of linking the number of Z chromosomes with gonadal differentiation. On the other hand, sex may be determined by the feminizing presence of the W chromosome, following the example of Y in eutherian mammals. There are two different mechanisms that are being studied and can support this theory. One includes the *FET1* gene, which is located on W, does not have a Z homologue and is expressed almost exclusively in the female urogenital system. The other includes the *ASW* gene, also known as *WPKCI*, and its Z homologue *ZPKCI*, since it has been proposed that the products of those two genes are capable of dimerisation, with a ZPKCI homodimer acting as a testis factor and a WPKCI/ZPKCI heterodimer preventing this effect.

One way to discern between the two theories would be to look into different combinations of Z and W chromosomes. Indeed, scientists have studied ZW aneuploidy in an effort to better understand how things work. It turns out that ZZZ animals develop testes but are infertile, ZWW animals die early in embryonic development, but ZZW combinations manifest as intersexual: the animals appear female on hatching, but slowly turn into males at sexual maturity. It is still possible, thus, that a combination of the above is in fact applied[11,12].

X/Y sex determination

It can be attributed to species that require two sex chromosomes of the same kind for females (XX) and of two different kinds for males (XY), such as mammals and several species of plants and insects.

One interesting prospect studied in the marsupial X and Y chromosomes is that they need not exclusively control all aspects of sex. The basic marsupial Y chromosome is the smallest of any mammal but retains its ability to turn the undifferentiated gonads into testes. However, the formation of the mammary glands and scrotum develops before gonadal differentiation takes place and is independent of gonadal hormones. In fact, it appears to be under the control of genes located on the X chromosome. So it happens that XXY animals have testes, but a pouch with mammary glands has replaced their scrotum, whereas XO animals have no testes, but an empty scrotum in place of a pouch[13].

Conclusion

It becomes apparent that the mechanisms devised to determine sex exhibit an extraordinary variety and ingenuity when examined as a whole. The purpose however is both to comprehend the molecular strategies employed by other organisms and to find ways of applying that knowledge as regards sex determination and differentiation on the species that interests us most, man[14].

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SPOLNE DETERMINANTE U GENOMU – LEKCIJE IZ ŽIVOTINJSKOG CARSTVA

SAŽETAK

Golema vrijednost spolne diferencijacije, kao sredstvo za obogaćivanje i razvoj genoma, dokazala se kao nepregledno šarenilo spolno određenih mehanizama kojima pribjegavaju sve vrste organizama. Od promjenjivih putova jednoga gena nađenog u organizmima nižih vrsta do haplodiploidne reprodukcije kod opnokrilaca, temperaturno određenog spola kod reptila te spolnih kromosoma kod sisavaca i ptica, priroda i evolucija uložile su impresivan trud kako bi osigurale da spolno specifične promjene ostanu pod dobro reguliranom kontrolom. Zbog toga, pojačavanje naših napora za proučavanje nekih strategija potrebnih za gore navedeno, može također voditi boljem razumijevanju prirođene kompleksnosti spolnog dimorfizma uopće.