Chapter 1. Features of Sex Determination in Insects

1.1. The Mechanisms of Sex Determination in *Drosophila*

In 1921, one of the founders of modern genetics Calvin Bridges found in *D. melanogaster* several females with a triploid set of chromosomes (3X:3A). After crossing these females with normal males (2X:2A), individuals with an unusual expression of sexual characteristics were found in their offspring along with normal females. All offspring split into classes depending on the ratio of sex chromosomes (X) and autosomes (A):

1. 3X:3A, triploid females.
2. 2X:2A, normal female (ratio X:A is 1).
4. XY:2A, normal females (ratio X:A is 0.5).
5. 2X:3A and (2X + Y):3A, intersexes (according to Bridges terminology) with varying ratio X:A 0.5 to 1. These were individuals with a mixed expression of male and female characteristics. These flies either completely lacked the sectors of the body determined by sex, or during development to a certain point formed organs of one sex, and then organs of the other sex.
6. X:3A, supermales, *i.e.* individuals with hypertrophied male signs, being, however, sterile (ratio X:A is less than 0.5).
7. 3X:2A, superfemales, *i.e.* individuals with an abnormal development of the ovaries and other disorders of sex characters (ratio X:A is greater than 1).

In all the cases when females appear, ratio of number of X chromosomes to autosomes is one. The presence of the male Y chromosome does not affect the normal development of the female.

According to Bridges, the gender in *Drosophila* is determined by the balance of sex chromosomes and a set of autosomes, while the Y chromosome does not play in sex determination any role; hence, the genic balance theory of sex determination. Indeed, in the Y chromosome there are genes of 11 fertility factors influencing the formation of the sperm that do not participate in the formation of the male sexual characteristics. Moreover, it is known that individuals XO in *Drosophila* are males.

There are numerous genes in *Drosophila* that affect the proper sex differentiation including Sxl (sex lethal), da (daughterless), sis (sisterless), tra (transformer), and dsx (double sex). In this regard, genetic interpretations of the genic balance theory of sex determination can be worked out. It was suggested that the ratio of the number of X chromosomes and autosomes might be “detected” by the Sxl gene at the early stages of embryonic development. This gene, in turn, controls simultaneously three aspects of differentiation: 1) the formation of sexual characteristics in somatic cells; 2) the formation of embryonic germ cells; and 3) implementation of dose compensation (*Figure 1.1-1*). According to the Swiss scientist R. Niagara, at the initial stages of sex development in embryos of *Drosophila* the products of the following genes are crucial: sis-a and sis-b (known as XSEs, X Signal Elements, or numerator proteins) located in the X chromosome, and da, located in an autosome (known as ASEs, Autosomal Signal Elements, denominator proteins). The da gene product enters the egg from the mother’s body. Its quantity always corresponds to two doses, as it is translated from the genes localized in the two maternal autosomes. The quantity of products resulted from the genes sis-a and sis-b depends on how many X chromosomes, one or two, an individual possesses. Therefore, the sis/da protein complex is characterized by the ratio of its components 1:2 in males or 2:2 in females.

Observation of haploid and triploid larvae showed that gender in *Drosophila* is not determined by X:A, but rather by the number of X chromosomes. Products of five X chromosome genes were found in *Drosophila* including sisA, sisB (scute), runt, unpaired (sisE), and dm (diminutive) called as numerators as well as several
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Figure 1.1-1. Genetic interpretation of the genic balance theory of sex determination in Dr. melanogaster. The primary signal, depending on the ratio of the number of X chromosomes and autosomes, controls all aspects of sexual differentiation through the action of a key gene Sxl. In females having the X:A ratio equal to one, the Sxl gene is active. In males having the ratio X:A equal to 0.5, the Sxl gene is not active. The activity of the Sxl gene regulates the development of the processes under its control: Dose compensation, and development of sexual characteristics in somatic and germ cells (adopted from Zhimulev I.F. How genes control the development of sex in Drosophila? // Soros Educational Journal. 1997. Vol.12. P.17-22.).

protein cofactors such as product of the autosomal gene deadpan. One of the genes (obviously, da (daughterless)) encodes a protein that functions as autosomal control factor (ASE) and is referred to the denominators. In three hours after fertilization and during the formation of the blastoderm, the products of the genes sisA, scute and runt stimulate the activity of the Sxl early promoter. Its full activation is achieved by using the product of the X chromosome gene unpaired via the Janus kinase. In diploid females (XX:2A), the activity level of UNPAIRED required to activate Sxl promoter is achieved by the 12th division of the blastoderm cells, in haploid females (XO:2A) by the 14th division, and in triploids (i.e. XXX:3A embryos) only in some cells (leading, as a result, to formation of partial gynandromorphs).

The products of the above mentioned genes interact with a key regulatory region of the Sxl gene. The latter contains 8 regions that encode the amino acid sequence (i.e. exons) and are separated with noncoding regions (i.e. introns). It also has two sites (i.e. promoters), “early” and “late” ones, that stimulate the transcription of RNA from this gene. Only in the case when the SIS/DA complex protein contains two doses of SIS, it can activate the beginning of transcription from the early promoter (Figure 1.1-2).

In females the Sxl gene transcript does not contain exon3 with a stop codon. At the blastoderm stage, as a result of translation of that transcript, a complete SXL protein is formed, which activates transcription of tra gene that, further interacting with the protein of the tra2 gene, regulates the production of a specific RNA in females, dsxF (doublesex). The presence of DSX³ in females facilitates involvement in the cascade of the ix gene. Proteins of the dsx³ and ix genes inactivate many genes that are specific to males, and eventually facilitate the development of a female. Under this scheme, the external (somatic) sexual characteristics are formed in females (Figure 1.1-2(b)). In males, activation of the late promoter (Pl) of the Sxl gene leads to the transcription of the third exon in which the stop codon UGA is located. Here the translation stops, resulting in a truncated protein. In the absence of a normally functioning SXL protein, the tra gene forms a short non-functional protein molecule (because the translation is blocked by the UAG codon in the second exon). In males, disruption of the Sxl gene splicing results in inclusion of a specific exon into the transcript that contains the stop codon, and the protein is not synthesized. In the absence of the SXL protein, mRNA of msl2 is not translated and the dose compensation occurs. In addition, in the absence of the normal TRA protein, male-specific genes dsx and fru begin to get transcribed (Figure 1.1-2(a)). The latter is translated into a zinc finger-type transcriptional factor, BTZ, which is responsible for all aspects related to the central nervous system (CNS) in males.

Protein of the tra2 gene is present in both sexes. In the absence of a functional product of the tra gene in males, there is no formation of a normal TRA/TRA2 multienzyme complex. Moreover, in the absence of the normal products of the tra and tra2 genes, the DSXM protein is formed. It represses the development of female sex characteristics.

It was recently shown that the protein Nito (a product of the spenito gene) controls the alternative splicing of Sxl mRNA by interacting with the corresponding SXL protein and pre-RNA and thus engaging Sxl in self-regulation.

Sex differentiation and the appearance of signs associated with sex, particularly behavioral ones, in Drosophila are related to primary sex determination. For example, a complete protein of the Sxl gene leads to the activity of tra and tra2, and they, in turn, control the appearance of transcription regulators of the genes fru (fruitless), dsf (dissatisfaction), dsx, and fit (female-specific independent of transformer). The dsf gene product regulates the sex differentiation outside the nervous system and some aspects of sexual behavior (courtship), whereas fru influences
Figure 1.1-2. A cascade of protein interactions leading to formation of somatic sexual characteristics of the male (a) and female (b). Involved in the cascade are genes Sxl, tra, tra2, and dsx. Rectangles represent coding parts of genes, i.e. exons, with gray portions corresponding to the gene regions directly encoding the amino acids (adopted from Smirnov A.F and Zhi-mulev I.F. Sex regulation 2000. In Encyclopedia modern natural science // General Biology. Vol.2. P.104-117. (Russ)).

the CNS development necessary for courtship, the development of muscles, etc. Other important sex-related genes control a number of characters. For example, the tsx (turn on sex-specificity) gene encodes an odorant-binding protein, sxe1 (sex specificity enzyme), a phospholipase involved in signaling, and sxe2 that determines the cytochrome P450 involved in the metabolism of steroids in different organs. It should be noted that in Drosophila all 46 RNA types are described with different distribution between the sexes. It was also found that the activity of the Sxl gene in Drosophila is regulated by a long non-coding RNA (200 nucleotides). This RNA activates the SxlPe promoter in females (Mulvey et al., 2014). The general scheme of sex determination in Drosophila is presented in Figure 1.1-3.

For Drosophila, a “demasculinization” of the X chromosome is characteristic, which manifests itself in the transfer of some male genes from this chromosome to autosomes. In humans and mice, on the contrary, genes expressed in spermatogonia are especially redundant in the X chromosome. The Y chromosome of Drosophila is believed to have originated from a specialized extra B chromosome, instead of a degenerating autosomal homologue of the X chromosome (Figure 1.1-4). It contains only 16 genes, which together constitute about 0.5% of the total DNA of the Y chromosome. It is inherent in this chromosome that it has almost 11 times greater gain of genes than their loss. This is fundamentally different from the situation in mammals.

In contrast to mammals, where sex hormones of the formed gonad affect the sexual identity of the whole organism, in Drosophila each cell is determined independently in terms of its sex.