Mechanisms of sex determination and Dosage Compensation in *Drosophila*

Genotypic Sex determination:

In genotypic sex determination system,the sex chromosomes play the decisive role in the inheritance and determination of sex,and it may occur in one of the two ways:

1)In the **Y-chromosome mechanism of sex determination**(e.g.,human),the Y chromosome of the heterogametic sex is active in determining the sex of an individual.Individuals carrying the Y-chromosome are genetically male,while individuals lacking the Y-chromosomes are genetically female.

2)In the **X-chromosome autosome balance system**(e.g.,*Drosophila*)the main factor in sex determination is the ratio between the number of X-chromosomes and the number of sets of autosomes.In this system the Y-chromosomes has no effect on sex determination,but is required for male fertility.

Sex Determination in *Drosophila*:

The number of X chromosomes : sets of autosomes (X:A)ratio determines sex in *Drosophila*
First, the X:A ratio is read during development. For wild-type *Drosophila,* the ratio that sets the initial switch for development into females (XX) is 2X : 2 sets of autosomes=1.0 , and the ratio that sets the initial switch for development into males (XY) is 1X : 2 sets of autosomes .
This information is transmitted to the sex determination genes, which make the choice between the alternative female and male developmental pathways, starting with the master regulatory gene *Sex-lethal (Sxl).*
Loss-of-function mutants of Sxl are lethal for female embryo development (meaning that *Sxl needs to be active in females), but they have no* effect on male embryo development (meaning that *Sxl* expression is not necessary for male development).
Alternative splicing of the *Sxl pre-mRNA in* embryos destined to become females or males sets in motion the two different pathways.

Chromosomal constitution in *Drosophila*:Normal male flies have 3 pairs ofautosomes and two sex chromosomes X and Y.Females have 3 pairs of autosomes and 2 sex chromosomes XX.

Bridges Theory : From his experiments, Bridges hold that in *Drosophila*, factors that cause a fly to develop into a male are not localized on the sex chromosomes but are instead found on autosomes. Some female determining factors, however, are localized on the X-chromosome. During primary sex determination, male gametes containing one of each autosome plus a Y-chromosome result in male offspring, not because of the lack of a second X-chromosome and neither for the presence of Y-chromosome. Thus sex is determined by a quantitative balance between X-chromosome and the number of haploid sets of autosomes. This is called **Genic Balance Theory of Bridges**. Bridges proposed that a threshold for maleness is reached when the X : A ratio is 1 : 2 (X : 2A), but that the presence of an additional X (XX : 2A) alters this balance and results in female differentiation.

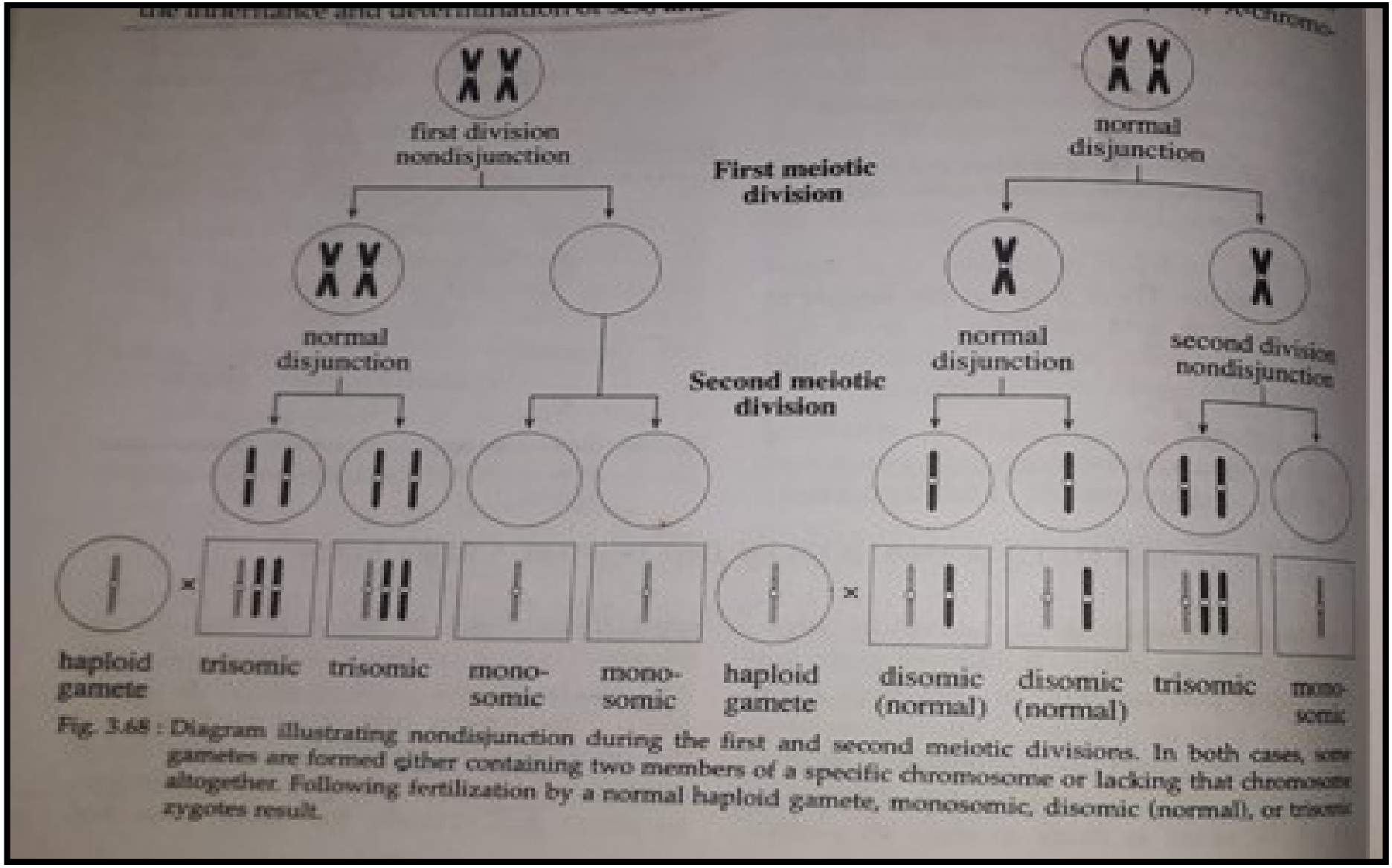


Fig. 3.68 : Diagram illustrating nondisjunction during the first and second meiotic divisions. In both cases, some gametes are formed either containing two members of a specific chromosome or lacking that chromosome altogether. Following fertilization by a normal haploid gamete, monosomic, disomic (normal), or trisomic zygotes result.

Note:Nondisjunction is the failure of paired chromosomes to segregate or separate during anaphase stage of the first or second meiotic divisions.The result is the production of two abnormal gametes,one of which contains extra chromosome (n+1)and the other which lacks a chromosome(n-1)

3A + XXX × 2A + XY

triploid female → normal male →

eggs : (A+X), (2A+XX), (A+2X), (2A+X) sperms : (A+X) (A+Y)

♂ \ ♀	(A+X)	(2A+XX)	(A+2X)	(2A+X)
(A+X)	(2A+2X)	(3A+3X)	(2A+3X)	(3A+2X)
(A+Y)	(2A+XY)	(3A+XXY)	(2A+XXY)	(3A+XY)

Bridges was able to clarify the mode of sex determination in *Drosophila* by studying the progeny of triploid females(3n)fertilized by normal haploid sperm.

TABLE 5.2

Ratio of X Chromosomes to Autosomes and the Corresponding Phenotype in *Drosophila*

X Chromosomes (X) and Sets of Autosomes (A)	X:A Ratio	Phenotype
1X 2A	0.5	Male
2X 2A	1.0	Female
3X 2A	1.5	Metafemale
4X 3A	1.33	Metafemale
4X 4A	1.0	Tetraploid female
3X 3A	1.0	Triploid female
3X 4A	0.75	Intersex
2X 3A	0.67	Intersex
2X 4A	0.5	Tetraploid male
1X 3A	0.33	Metamale

Intersexes and supersexes : Metamales or supermales and metafemales or super-females are sterile and of low viability. Intersexes initially begin to develop like males and organs that develop later become female type. The time of this changeover is called **turning point** which is variable in different flies and with environmental factors. Ultimately intersexes have a mixture of male and female characters and a gonad intermediate between testis and ovary, but intersexes are always sterile.

Mosaics/Gynandromorph : In *Drosophila*, some unusual flies have male characters in some parts and female characters on other

parts of the body. Such flies, called **gynanders** are sterile (unlike hermaphrodites) and can be divided into (i) **bilateral gynander**—male and female characters on two lateral sides, (ii) **antero-posterior gynander**—male characters at anterior and female characters at posterior side or vice versa, (iii) **sex-piebalds**—a predominantly one-sexed fly with patches of tissues of other sex.

When a female zygote loses one of its X-chromosome during the first mitotic division, the two cells would be of XX and XO constitution, respectively. Each of these cells is reponsible for producing all progeny of cells that make up either the right half or left half of the body during embryogenesis. So, in the case of bilateral gynander, the original XO cell gives rise to that half of the body showing maleness, while all cells on the other half derived from the original XX cell, show female characters. Depending on the orientation of the spindle during the first mitotic division, the 'line' demarcating male versus female development occurs at almost any place along or across the fly's body.

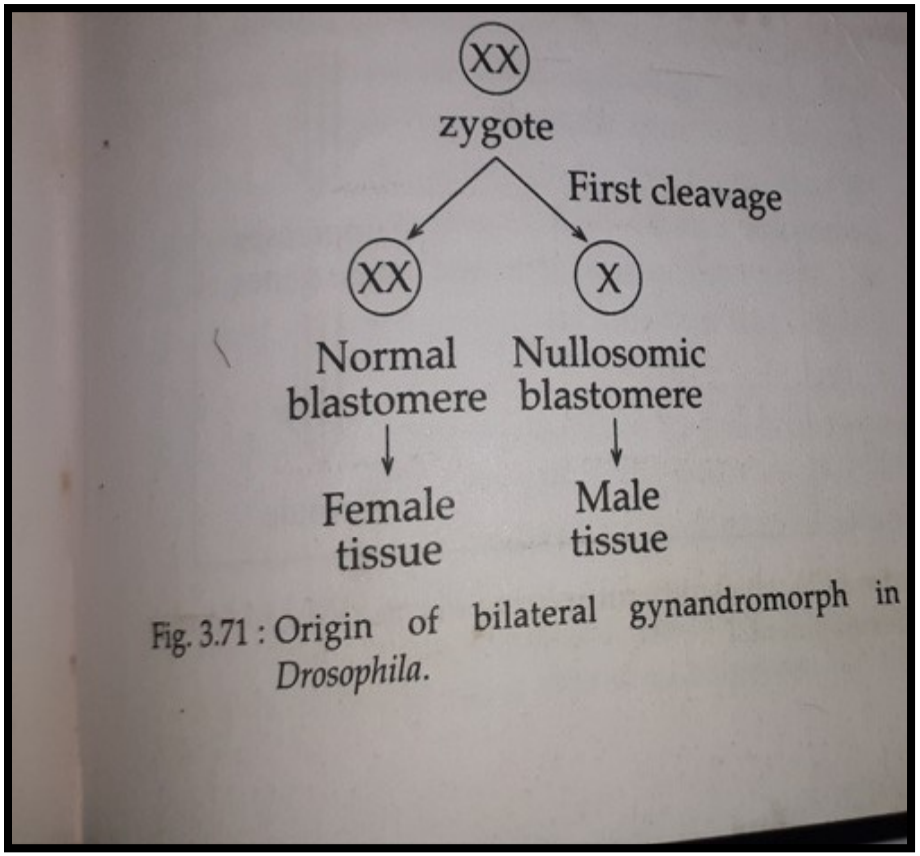


Fig. 3.71 : Origin of bilateral gynandromorph in *Drosophila*.

Factors influencing sex determination

1. **Role of sex-linked lethal genes** : Due to presence of a sex-linked recessive lethal gene (*l*) sometimes sex ratio becomes 2 : 1 for female : male. In such cases, the only possible cross is between heterozygous female and normal male. This cross is as follows :

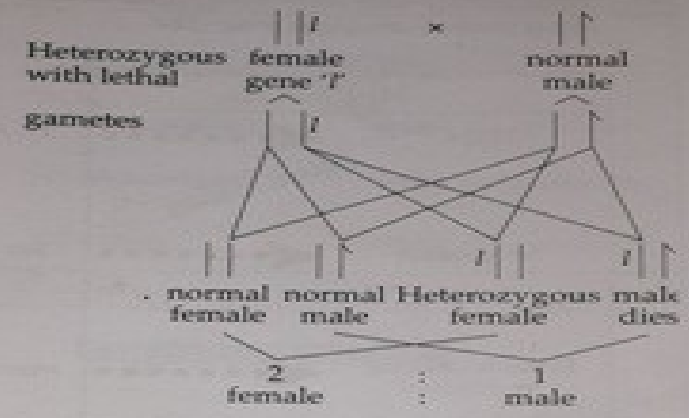


Fig. 3.72 : Cross between heterozygous female for lethal gene 'l' and normal male, produces 2 : 1 for female : male offspring.

2. **Meiotic drive** : Meiosis is normal in male, but rarely due to some X-borne factor, Y-bearing sperms are incapable of normal functioning. So males are reduced in number amongst offspring. The factor has no effect in female, but may be transmitted by them to males.

3. **Segregation disorder** : Sometimes a 'segregation disorder' or SD factor remains present on second chromosome. SD has no effect in female, but has effect only in heterozygous or $SD^+//SD$ males. In $SD^+//$ SD males, meiosis is normal, but sperms having SD^+ gene are non-functional. This may alter expected 1 : 1 sex ratio.

4. **Spirochaete-induced lethality** : Sometimes selective lethality of male embryos, larvae and pupae may alter the expected sex ratio. The death of male results from transmission of a spirochaete, probably of the genus *Treponema* from the haemolymph and ooplasm of the mother, into the developing male embryos (via the egg).

5. **Thermal effect** : It has been found that intersexes raised at high temperature are more female-like and those raised at low temperature are more male-like.

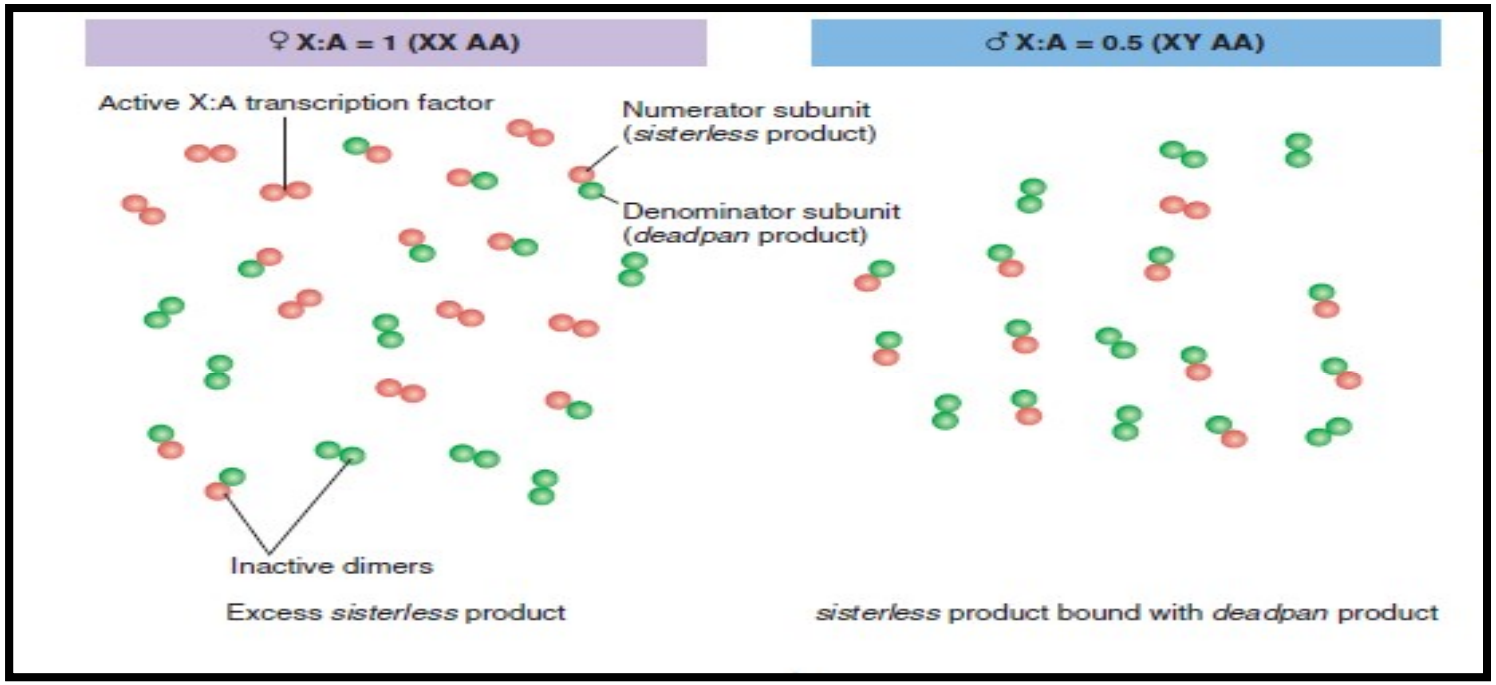
6.Role of genes in sex determination (polygenic determination) : The pathway of sex determination in *Drosophila* involves interaction between a series of genes in which alternative splicing events distinguish male and female. (Mention the genes involved in the cascade discussed in later slides)

Factors influencing Sex determination

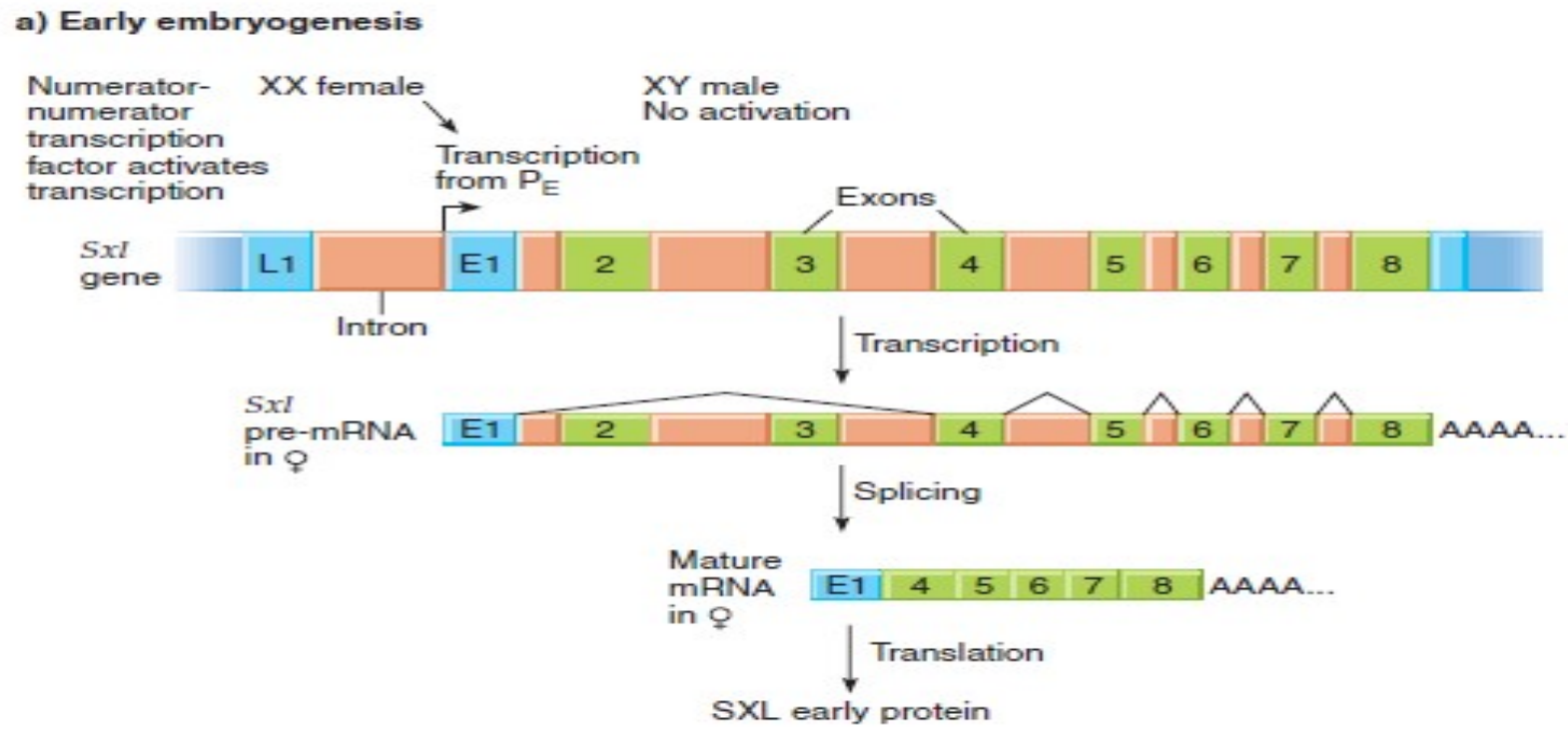
Mechanism:

The number of X chromosomes : sets of autosomes (X:A) ratio determines sex in Drosophila. First, the X:A ratio is read during development. For wild-type Drosophila, the ratio that sets the initial switch for development into females (XX) is 2X : 2 sets of , and the ratio that sets the initial switch for development into males
(XY) is 1X : 2 sets of autosomes=0.5.
This information is transmitted to the sex determination genes, which make the choice between the alternative female and male developmental pathways, starting with the master regulatory gene Sex-lethal (Sxl). Loss-of-function mutants of Sxl are lethal for female embryo development (meaning that Sxl needs to be active in females), but they have no effect on male embryo development (meaning that Sxl expression is not necessary for male development).
However, gain-of-function mutants are lethal for male embryo development, which means that Sxl needs to be inactive in males. Alternative splicing of the Sxl pre-mRNA in embryos destined to become females or males sets in motion the two different pathways.
Steps in each pathway are regulated by alternative splicing of pre-mRNAs.

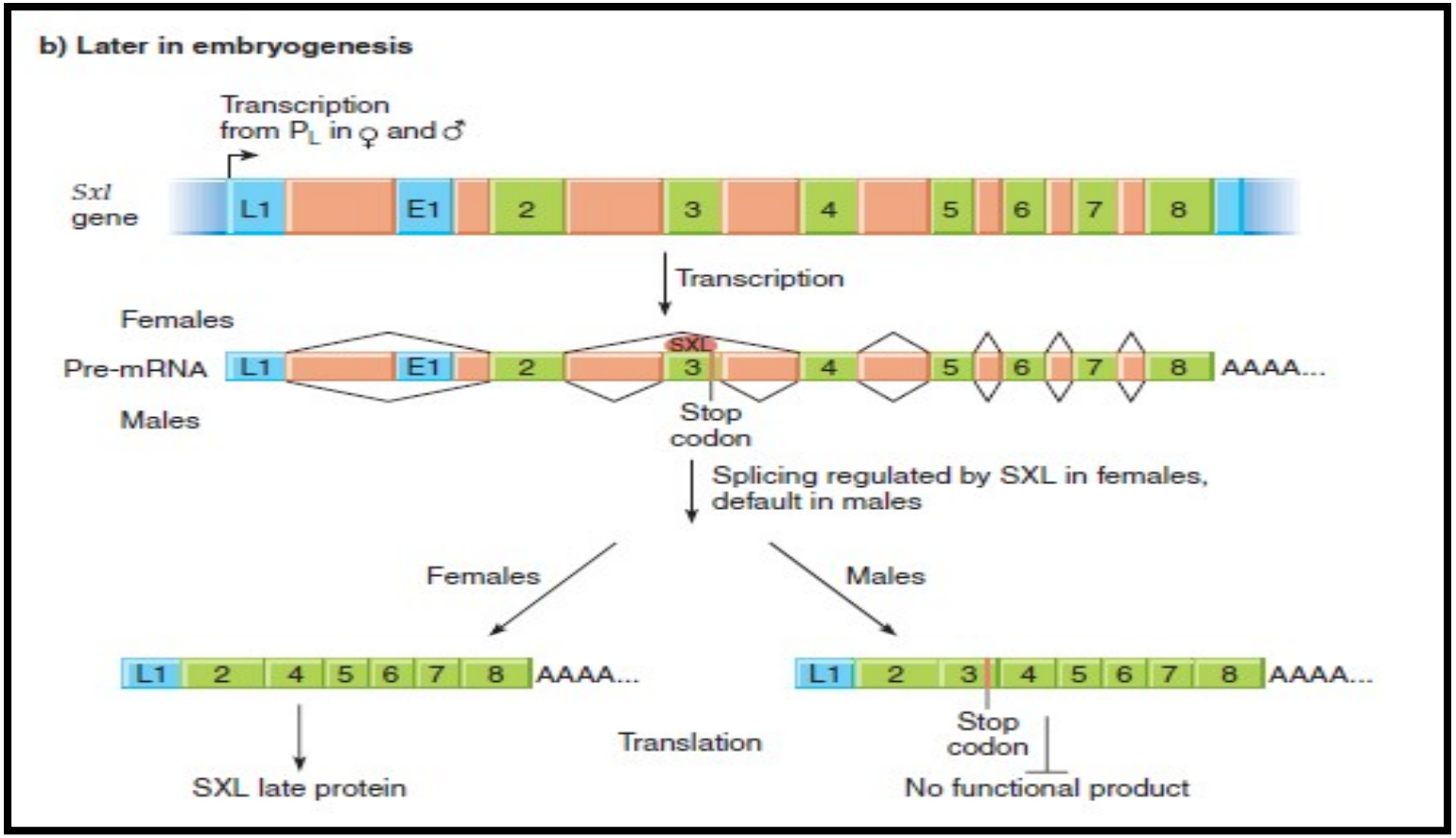
How is the X:A ratio detected? On the X chromosome are the *sisterless numerator genes sis-a, sis-b, and sis-c,runt and* on an autosome is the *deadpan (dpn) denominator gene.* The numerator genes are expressed to produce protein subunits that can form either homodimers or heterodimers with the subunit encoded by the denominator gene.



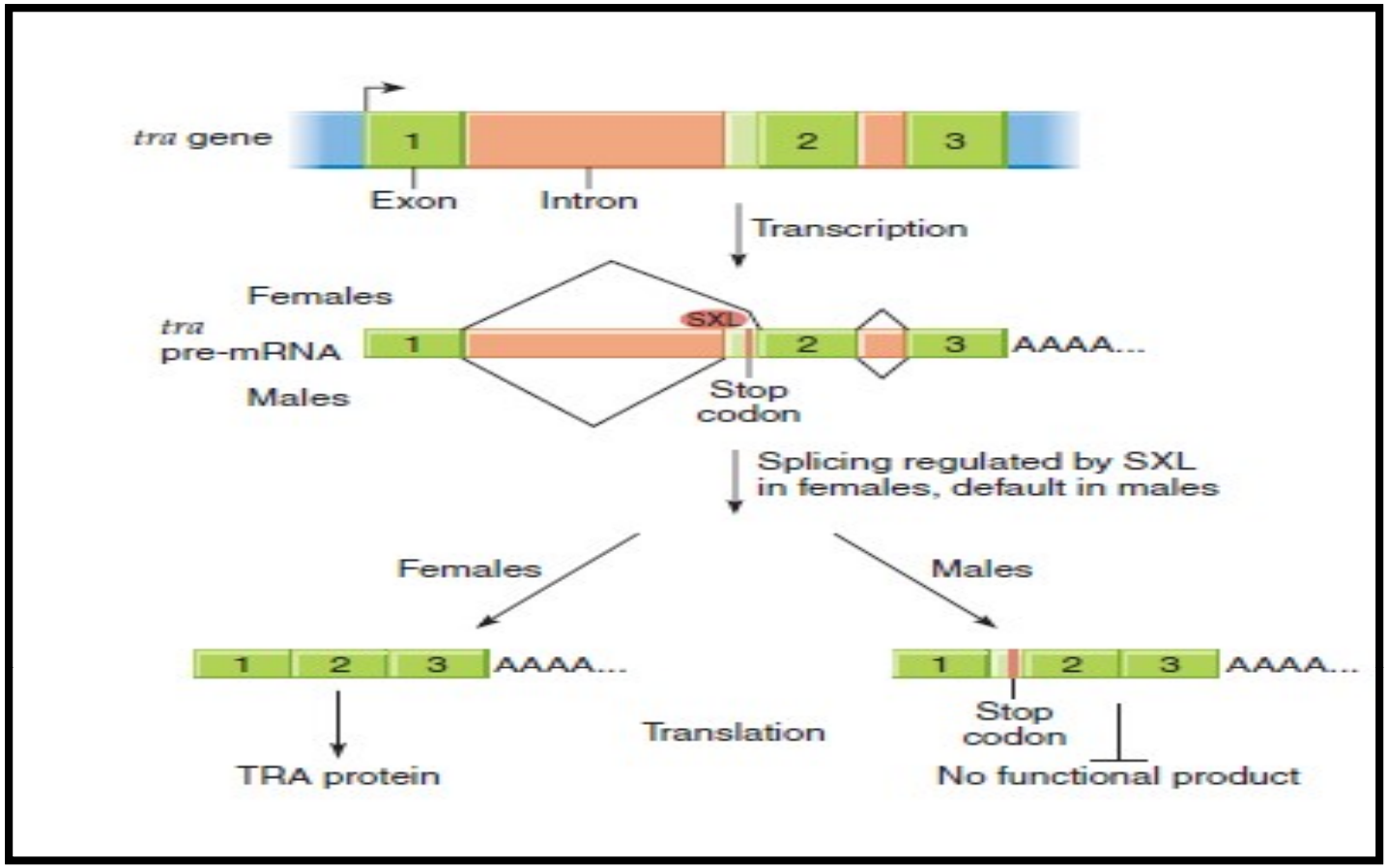
In females, the excess of numerator proteins produces numerator numerator dimers that function as transcription factors to activate *Sxl.* In males, *Sxl expression* from PE does not occur because sufficient numeratornumerator transcription factors are absent: No SXL proteinis produced in males.



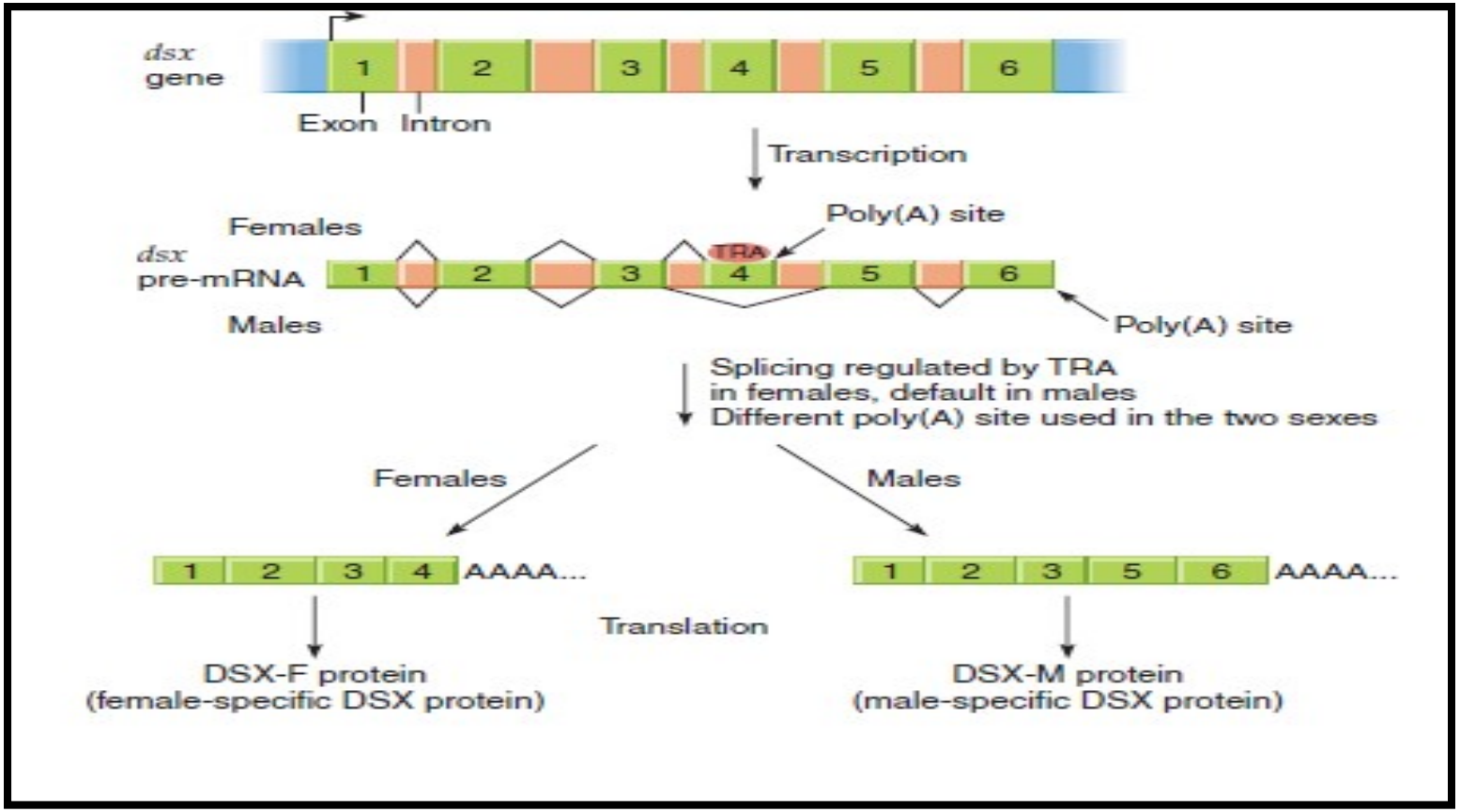
Expression of Sex-lethal (Sxl) early in embryogenesis: In early embryogenesis in females, the numerator-numerator dimer transcription factors activate transcription of *Sxl from PE. Splicing of* the pre-mRNA skips exons 2 and 3.The resulting mRNA is translated to produce SXL early protein.In males, *Sxl expression* from PE does not occur because sufficient numerator numerator transcription factors are absent: No SXL protein is produced in males.



Expression of Sex-lethal (Sxl) later in embryogenesis*:*Later in embryogenesis, the *Sxl* gene is transcribed constitutively from PL in both female and male embryos. The pre-mRNA is spliced in a regulated fashion in female embryos owing to the presence of SXL early protein, and in a default fashion in male embryos owing to the absence of SXL early protein. As a result, SXL late protein is produced in female embryos, but not in male embryos.

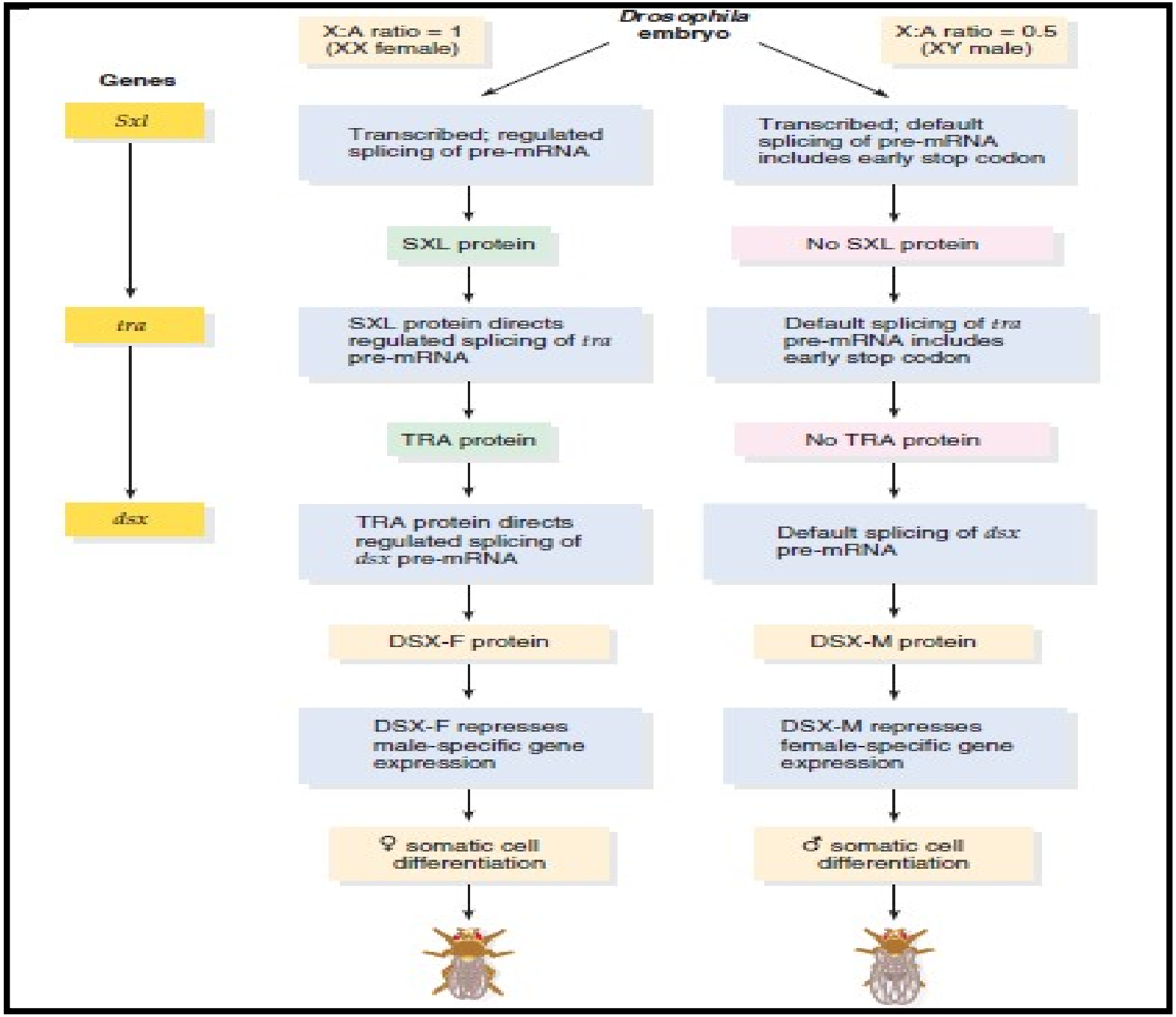


Expression of *transformer (tra) during embryogenesis* :SXL late protein in female embryos regulates splicing of *tra premRNA;* the resulting mRNA is translated to generate the TRA protein that regulates splicing of the *doublesex transcript*. In male embryos, *tra pre-mRNA is spliced in a* default fashion because SXL late protein is absent. The resulting mRNA has a stop codon prior to exon 2 and so no TRA protein is made.



Expression of *doublesex (dsx) during* embryogenesis: TRA protein in female embryos regulates splicing, so exon 4 is included and cleavage and polyadenylation occurs at the poly(A) site following exon 4. In male embryos, default splicing occurs in the absence of TRA protein, leading to the exclusion of exon 4, but to the inclusion of exons 5 and 6 because of cleavage and polyadenylation at the poly(A) site following exon 6. Translation of the two different mRNAs produces the female-specific DSX protein, DSX-F, in females, and the malespecific DSX protein, DSX-M, in males

Fig:Regulatory cascade for sex determination in *Drosophila*.



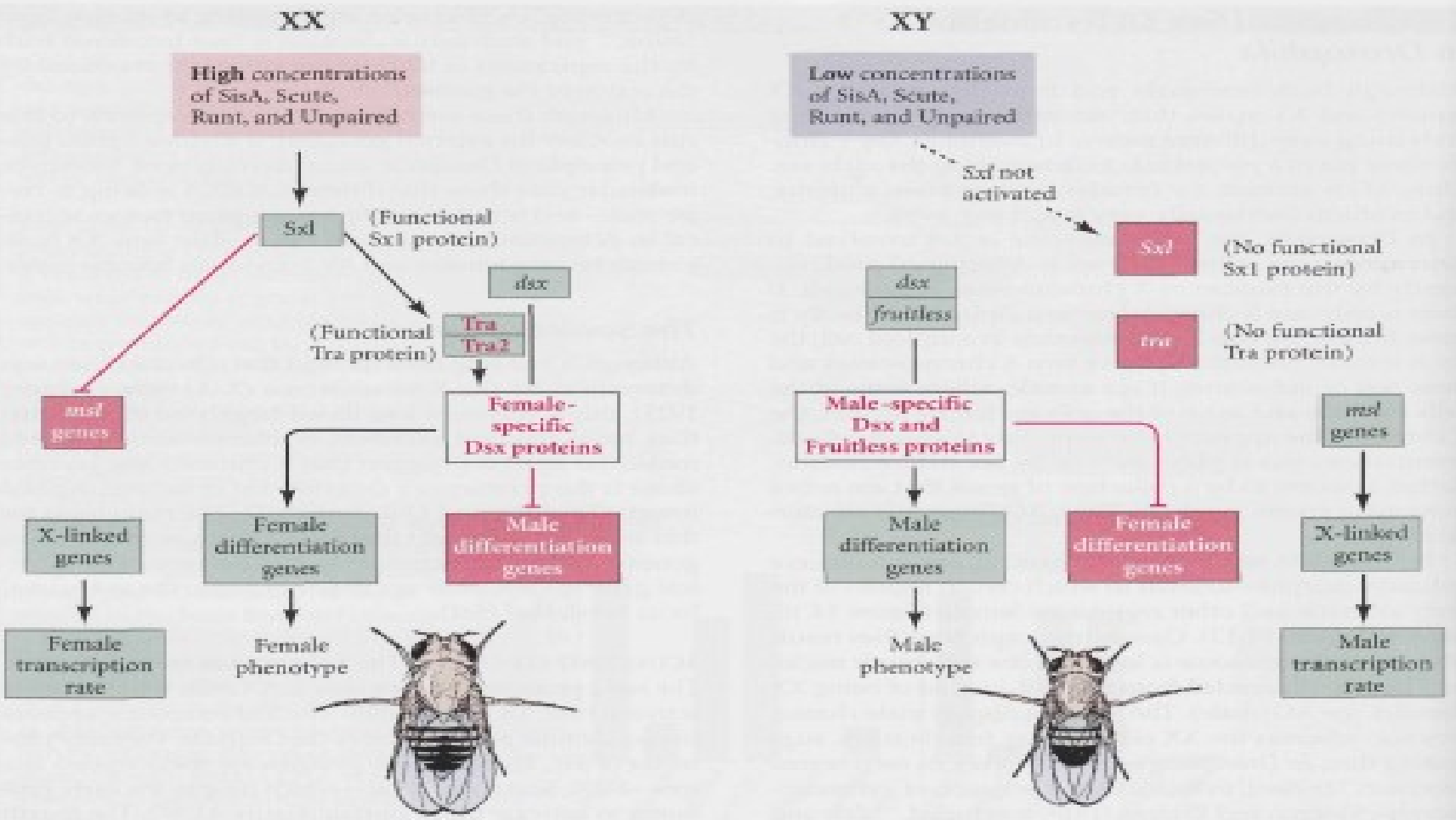


FIGURE 14.17 Proposed regulatory cascade for *Drosophila* somatic sex determination. Transcription factors from the X chromosomes and autosomes compete to activate or repress the *Sxl* gene, which becomes active in females (XX) and inactive in males (XY). The Sex-lethal protein performs three main functions. First, it activates its own transcription, ensuring further Sxl production. Second, it represses the translation of *msl2* mRNA, a factor that facilitates transcription from the X chromosome. This equalizes the amount of transcription from the two X chromosomes in females with that of the single X chromosome in males. Third, Sxl activates the *transformer* (*tra*) genes. The Tra proteins process *doublesex* pre-mRNA in a female-specific manner that provides most of the female body with its sexual fate. They also process the *fruitless* pre-mRNA in a female-specific manner, giving the fly female-specific behavior. In the absence of Sxl (and thus the Tra proteins), *dsx* and *fruitless* pre-mRNAs are processed in the male-specific manner. (After Baker et al. 1987.)

Description Of the Regulatory Cascade:

Early in embryogenesis in the female, the numerator numerator dimer transcription factor activates transcription of the Sxl gene from PE (promoter early), one of two promoters for this gene, the other being a more upstream promoter, PL . The premRNA transcribed from PE has eight exons; exons 2 and 3 are skipped to produce the mature mRNA consisting of exons E1, 4, 5, 6, 7, and 8. Translation of this mRNA produces the SXL early protein. In males, Sxl expression from PE does not occur because sufficient numerator numerator transcription factors are absent: No SXL protein is produced in males.

Later in embryogenesis (after gastrulation), Sxl is transcribed constitutively from the late promoter, PL, in all cells, regardless of the X:A ratio . This transcription does not depend on the numerator transcription factors. The pre-mRNA produced is longer than the transcript from PE and is subject to alternative splicing depending on the presence or absence of SXL early protein.

In females, the SXL early protein binds to the Sxl pre-mRNA and causes regulated splicing: exons E1 and 3 are skipped, resulting in a mature mRNA with exons L1, 2, 4, 5, 6, 7, and 8. Translation of this mRNA produces the SXL late protein. In males, the absence of SXL early protein results in default splicing of the pre-mRNA and a mature mRNA is produced that includes exon 3. Exon 3 has a stop codon in frame with the start codon at the beginning of exon 2, so no functional SXL late protein is produced in males.

These events set the switch to either female or male differentiation. A cascade of alternative splicing events follows. In the female embryo, SXL late protein regulates splicing of transformer (tra) pre-mRNA .In this case, a stop codon-containing exon segment upstream of and contiguous with exon 2 is removed, resulting in an mRNA with exons 1, 2, and 3. Translation of this mRNA produces the active TRA protein. In males, default splicing occurs as a result of the absence of SXL late protein. This means that the stop codon-containing segment is not removed. Translation of the resulting mRNA halts at the stop codon in that segment; no functional TRA protein is produced.

TRA protein is also an RNA splicing regulator. The target is the pre-mRNA of the doublesex (dsx) gene .In females, TRA-regulated splicing gives rise to female dsx mRNA. This mRNA encodes the DSX-F (F for female) protein, a transcription factor that represses male-specific gene expression in all cells. As a result , female-specific somatic cell differentiation occurs. In males, the absence of functional TRA protein results in default splicing of the dsx pre-mRNA to produce male dsx mRNA. This mRNA encodes the DSX-M (M for male) protein, a transcription factor that represses female specific gene expression in all cells. As a result, male specific somatic cell differentiation occurs. Knockout mutants of dsx have a mixture of male and female characteristics, which occurs because of the lack of repression of male- and female-specific genes.

Dosage Compensation:

Any mechanism in organisms with genotypic sex determination for equalizing expression of genes on the sex chromosomes in males and females.

Organisms with sex chromosomes have an inequality in gene dosage (the number of gene copies) between the sexes; that is, there are two copies of X-linked genes in females and one copy in males. In many such organisms, if gene expression on the X chromosome is not equalized, the condition is lethal early in development.

Normally, each gene is present in two copies. Departures from this condition, either up or down, can cause abnormal phenotypes, and sometimes even death.

How is the numerical difference of X-linked genes accommodated?

Three mechanisms may compensate for this difference:

(1) each X-linked gene could work twice as hard in males as it does in females

(2) one copy of each X-linked gene could be inactivated in females

(3) each X-linked gene could work half as hard in females as it does in males.

Extensive research has shown that all three mechanisms are utilized, the first in *Drosophila,* the second in mammals, and the third in the nematode *Caenorhabditis elegans.*

- In mammals, dosage compensation occurs by decreasing transcriptional activity of X chromosome genes in females to match that in males. In *Drosophila, the opposite occurs:* Transcriptional activity is increased twofold in males to match that in females, who have twice the number of X chromosomes. In both cases, chromatin remodeling is involved in regulating transcriptional activity.
- Key malespecific lethal genes are *mle (maleness), msl-1 (malespecific lethal-1), msl-2, msl-3, and mof (males absent on the first). Males with mutations in these genes die at* the late larval stage, while females with the same mutations develop normally. The products of these genes are collectively called the male-specific lethal (MSL) proteins.
- The SXL late protein plays a key role in dosage compensation.In females,the SXL late protein binds to the transcript of *msl-2, blocking its* translation; no MSL2 protein is produced.
- In males, the *msl-2 transcript can be translated because SXL late protein* is absent. MSL2 forms a complex with the other MSL proteins, MLE, MSL1, MSL3, and MOF. This MSL complex binds to about 35 chromatin entry sites (CES) on the *Drosophila male X chromosome and then MSL* complexes spread from those sites in both directions into the flanking chromatin.
- The MOF protein of the MSL complex is a histone acetyltransferase (HAT), and its chromatin remodeling activity spreads along the X chromosome and is responsible for the twofold higher level of transcription of X chromosome genes in males than in females.
- In females, the MSL proteins other than MSL2 are produced. However, because MSL2 is essential for the binding of the MSL complex to the X chromosome, no chromatin remodeling can occur in XX females.