Lecture 18: Gene regulation and Drosophila sex determination

Post transcriptional regulation

microRNA

splicing

Sex-determination in Drosophila- a case study

Fig. 18.6-18.18; 18.19a; 18.20-24
Read: p664-676
Mechanisms of post transcriptional gene regulation

- Some genes are regulated after transcription.
  - RNA splicing can regulate expression.
  - RNA stability controls amount of gene product.
  - mRNA editing can affect biological properties of protein.
  - Noncoding sequences in mRNA can modulate translation.
  - Protein modification after translation can control gene function.
Micro-RNAs mediate RNA interference.

- 2000-2005 – micro-RNAs identified and characterized
  - RNA interference – trans-acting single stranded micro-RNAs that regulate eukaryotic gene expression
  - from introns of protein coding transcripts
  - from products of primary transcripts devoid of ORFs
  - Number of miRNAs may exceed the number of protein coding genes.
Fig. 18.17

(a) Primary transcripts from different types of miRNA genes

STEM-LOOP STRUCTURE

1. 5' cap

2. 5' cap

3. 5' cap

4. 5' cap

(b) Examples of predicted pri-miRNA stem loops

PROTEIN-CODING REGION
Micro-RNA processing and modes of action

Immediately after transcription, pri-miRNAs are recognized by Drosha which crops out pre-miRNA stem loops from larger RNA. Pre-miRNAs undergo active transport from nucleus to cytoplasm where they are recognized by Dicer. Dicer reduces the pre-miRNA into a short-lived miRNA:miRNA duplex which is released and picked up by RISC.
Two modes of RNA interference

1. If miRNA and its target mRNA contain perfectly complementary sequences, miRISC cleaves the mRNA. RNase rapidly degrades cleavage product.
2. If miRNA and its target mRNA have only partial complementarity, cleavage does not occur. miRISC remains bound to its target and represses its movement across ribosomes.
You Tube movie
Protein modifications after translation provide a final level of control over gene function.

- **Ubiquitination targets proteins for degradation.**
  - Ubiquitin – small, highly conserved protein
    - Covalently attaches to other proteins
    - Ubiquitinized proteins are marked for degradation by proteosomes

![Diagram of protein modifications and ubiquitination process](Fig. 18.19 a)

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RNA splicing helps regulate gene expression.
Sex determination in Drosophila
A comprehensive example of gene regulation

Sex specific traits in Drosophila

- Antenna
  - Sensillae
- Foreleg
  - Chemosensory axons
  - Sex comb in male
- Thoracic ganglion
  - Courtship behaviors
- Abdomen
  - Pigmentation
  - Male-specific muscle
- Genitalia
  - In male:
    - Testes/spermatogenesis
    - Accessory gland peptides
    - Ejaculatory duct proteins
  - blue = specific to males
  - red = specific to females
  - green = found in different forms in the two sexes
- Brain
  - Regions determining courtship behaviors
  - More Kenyon fibers in female mushroom body
- Fat body
  - Yolk proteins in female
- Gonads and reproductive tract
  - In female:
    - Ovaries/oogenesis
    - Yolk, chorion, and vitelline membrane proteins

Fig. 18.20
<table>
<thead>
<tr>
<th>Sex Chromosomes</th>
<th>X:A</th>
<th>Sex Phenotype</th>
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<tbody>
<tr>
<td><strong>Autosomal Diploids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XO</td>
<td>0.5</td>
<td>Male (sterile)</td>
</tr>
<tr>
<td>XY</td>
<td>0.5</td>
<td>Male</td>
</tr>
<tr>
<td>XX</td>
<td>1.0</td>
<td>Female</td>
</tr>
<tr>
<td>XXY</td>
<td>1.0</td>
<td>Female</td>
</tr>
<tr>
<td><strong>Autosomal Triploids</strong></td>
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<td></td>
</tr>
<tr>
<td>XXX</td>
<td>1.0</td>
<td>Female</td>
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<tr>
<td>XYY</td>
<td>0.33</td>
<td>Male</td>
</tr>
<tr>
<td>XXY</td>
<td>0.66</td>
<td>Intersex</td>
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</tbody>
</table>
The X:A ratio regulates expression of the Sex lethal (sxl) gene.

- **Key factors of sex determination:**
  - Helix-loop-helix proteins encoded by genes on the autosomes
    - Denominator elements
  - Helix-loop-helix proteins encoded by genes on the X chromosome
    - Numerator elements – monitor the X:A ratio through formation of homodimers or heterodimers
    - sisterless-A and sisterless-B
Fig. 18.21

Gene expression leads to gene products, which undergo dimerization. Female (XX: AA = 1) and male (X: AA = 1/2) differ in the number of Sxl proteins produced. Transcription of Sxl is activated in females, leading to Sxl protein production and activation of female sex determination pathways. In males, no Sxl protein is produced, and male sex determination pathways are activated. Extra monomers in the male pathway are indicated.
Fig. 18.16 b

Female DNA

Sxl protein from early development binds to later RNA at exon 3

RNA

Full-length open reading (indicated by purple boxes)

Productive splice

More Sxl protein

Male DNA

Truncated open reading frame

Stop codon

Unproductive splice

No Sxl protein

<table>
<thead>
<tr>
<th>DNA</th>
</tr>
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<tbody>
<tr>
<td>P_L</td>
</tr>
<tr>
<td>P_e</td>
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</table>

<table>
<thead>
<tr>
<th>RNA</th>
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<tr>
<td>Orange boxes</td>
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<table>
<thead>
<tr>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple boxes</td>
</tr>
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Sxl triggers a cascade of splicing.

- Sxl influences splicing of RNAs in other genes.
  - e.g., *transformer (tra)*
    - Presence of Sxl produces functional protein.
    - Absence of Sxl results in nonfunctional protein.

![Diagram of Sxl splicing](a) **tra splicing**

Results of *tra* splicing when Sxl protein is present (♀)

- Sxl protein blocks splice site
- Full length open reading frame
- Functional Tra protein

Results of *tra* splicing in absence of Sxl protein (♂)

- Stop codon
- Truncated open reading frame
- No functional Tra protein

Fig. 18.22 a
Cascade of splicing continues

- e.g., *doublesex (dsx)*
  - Tra protein synthesized in females along with Tra2 protein (produced in males and females) influences splicing of dsx.
    - Females - Produces female specific Dsx-F protein
    - Males – No Tra protein and splicing of *Dsx* produces Dsx-M protein

(b) *dsx* splicing

Results of splicing when *tra* is present (♀)

1 —— 2 —— 3 —— 4 → Dsx–F

Results of splicing when *tra* is absent (♂)

1 —— 2 —— 3 —— 4 —— 5 —— 6 → Dsx–M

Fig. 18.22 b
Dsx-F and Dsx-M are transcription factors that determine somatic sexual characteristics.

- Alternative forms of Dsx bind to YP1 enhancer, but have opposite effects of expression on YP1 gene.
  - Dsx-F is a transcriptional activator.
  - Dsx-M is a transcriptional repressor.
Tra and Tra-2 proteins also help regulate the expression of *Fruitless*.

- Primary *fru* mRNA transcript made in both sexes
  - Presence of *tra* protein in females causes alternative splicing encoding *fru-F*.
  - Absence of *tra* protein in males produces *fru-M*.

Fig. 18.24
## TABLE 18.3  
*Drosophila* Mutations That Affect the Two Sexes Differently

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Phenotype of XY</th>
<th>Phenotype of XX</th>
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</thead>
<tbody>
<tr>
<td>$Sx^{fl}*$</td>
<td>Male</td>
<td>Dead</td>
</tr>
<tr>
<td>$Sx^{ML}**$</td>
<td>Dead</td>
<td>Female</td>
</tr>
<tr>
<td><em>transformer (tra)</em></td>
<td>Male</td>
<td>Male (sterile)</td>
</tr>
<tr>
<td><em>doublesex (dsx)</em></td>
<td>Intersex</td>
<td>Intersex</td>
</tr>
<tr>
<td><em>fruitless</em></td>
<td>Male with aberrant courtship behavior</td>
<td>Female</td>
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