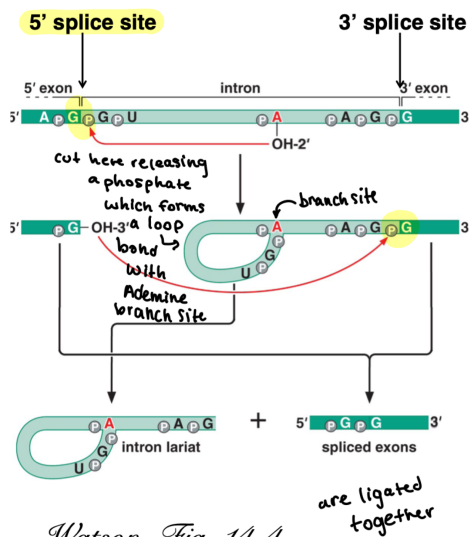


- In euk cell needs to process primary transcript to produce functional translatable mRNAs

↳ For many processing regulated at RNA processing especially by alternative splicing

- Typically introns removed & exons flanking each intron are spliced together by phosphodiester bonds

Overview of the splicing reaction



Watson, Fig. 14-4

Splicing occurs in two steps:

- The **5' splice site** is cut, with the 5' end of the intron forming a covalent bond to a **branch site** in the intron.

- The **3' splice site** is then cut, and the lariat-shaped intron is removed. A new phosphodiester bond is formed joining the ends of the two exons.

Both reactions are catalyzed by a molecular machine called the **spliceosome**.

- This is normal but **alternative splicing** is very common which allows for single pre-mRNA to form different **isoforms**

- Alternative splicing allows a single gene to synthesize 2 similar but **structurally & functionally distinct protein isoform**

Factors that Influence Splice Sites

① Cell can turn splice sites on & off by expressing proteins that will bind to pre-mRNA near splice site

- * Splice Repressor → Spliceosome away
- * Splice activator → Spliceosome to use

② Some splice sites prefer to pair due to specific features of their **RNA sequence**

- Both 5' & 3' splice sites are marked w/ somewhat variable base sequences

↳ Spliceosome will use splice site if sequence >60% consensus

- Spliceosome will preferentially bind 5' & 3' splice sites because have 'match' in base sequence

③ Multiple promoters effect Alternative Splicing

- If gene transcribed by multiple promoters it'll have multiple isoforms with different 5' exons

Drosophila

- Have XY, XX like humans but XY chromosomes are not homologous to XY of humans they have different sets of genes

* In humans presence of Y determines if male or female (Y = anatomically male)

- In Drosophila the ratio of X chromosome to autosomes (non sex chromosomes) determine gender

X:A → 1 ratio Female
X:A → .5 Male

- Have no X-inactivation in flies so XXY would develop as female unlike humans

Alternative Splicing effect on Sex determination

7 Key genes

- Sisterless a (Sis-a,b)
- Sisterless b
- Deadpan (dpp)
- Sex-lethal (Sxl)
- transformer (tra)
- transformer-2 (tra-2)
- doublesex (dsx)

- All except Sisterless are an autosome meaning they're in equal amount in both genders

- Transcription factors Sisterless (Sis-a,b) from X chromosome

* When you have more sisterless you activate early promoter (pe) of autosomal gene SxI

↳ pe will create active SxI without a splice repressor

- later on pe is silenced and PM is activated

↳ Pm can only make active SxI in females so males **Never** have an active SxI

* early SxI required for later SxI formation (early only in female)

- SxI allows expression of Tra proteins

↳ Tra allows female specific Dsx isoform to form

- Because males have no SxI they have no tra-2 so they form a different male specific Dsx

↳ male Dsx activates male development and female Dsx causes female development

* PE is only used in early stages of embryonic development as embryo develops switch to Pm (maintenance promoter) which has separate +1 site and produces different Exon 1

- Doublesex gene (dsx) has zinc finger transcription factor so has distinct isoform in either gender. It is expression of Dsx isoform that causes formation of gender organs

Male Isoform
1+3

Female Isoform
1+2

*Exon 2 includes poly A signal sequence at 3' end so inclusion in female terminates transcription before it reaches exon 3. In males exon 2 is skipped so 3 transcribed

What determines gender specific splicing of dsx gene

↳ 3 splice regulatory proteins

Sex lethal (Sxl) which encodes splice repressor protein

Transformer (tra) encode splice activator protein

Transformer-2 (tra-2) encode splice activator protein

Female Development

- The Sxl^F protein binds to both its own Sxl pre-mRNA and the tra, tra-2 pre-mRNAs

↳ Sxl **splice repressor** prevents the inclusion of male specific exon in final mRNA

- Tra^F & Tra-2^F proteins bind to dsx pre-mRNA which act as **splice activator** which attract spliceosome to exon 2 so it's included in female isoform of Dsx protein

*Tra is allosteric

Male development

- Have no expression of female "Sxl" splice receptor repressor

↳ The spliceosome includes the male specific exon in the Sxl mRNA

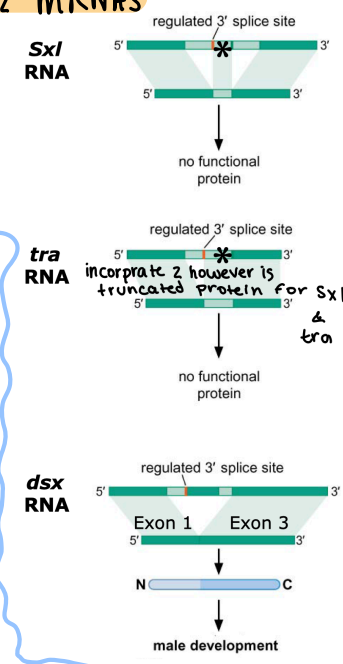
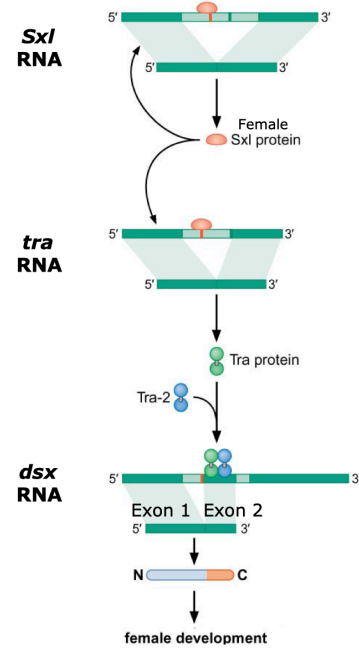
- The male-specific exon contains an in-frame stop codon so even though male mRNA is longer than the female mRNA it produces a short non-functional isoform of Sxl protein

- Due to no Sxl repressor protein have inclusion of male-specific exons in tra and tra-2 mRNAs

- These exons have premature stop codons so they produce functionless Tra splice enhancer protein

↳ Since no Tra & Tra-2 protein exon 1 of dsx mRNA is spliced to exon 3 producing male isoform

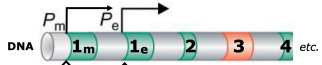
*Exon 2 is skipped b/c no splice enhancer protein present in male



Sxl Gene

- Sxl gene has 2 core promoters (Pe) used in early development and (Pm) later on

↳ These 2 promoters have different exons which effect splicing downstream



The Pe or "early promoter" is only used during the earliest stages of embryonic development.

As the embryo matures, transcription switches to the Pm or "maintenance promoter". It has a different +1 site and produces a different Exon 1.

- Transcription of Pe depends on concentration of **activator TF's Sisterless-a & Sisterless-b** and the **repressor TF Deadpan**

*DPN is autosomal so same in both sexes

*Sis-a, b genes are on X chromosome so females have **2x activator** than repressor so can transcribe pe promoter unlike males

* Use of 2 Sxl promoters introduce another form of alternative splicing for Sxl pre-mRNA

- When transcribe at pe promoter don't need Sxl protein to skip male specific exon

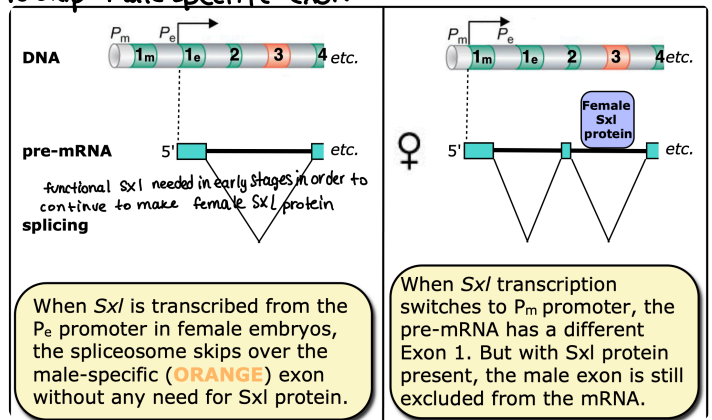
↳ still being transcribed but not spliced

↳ The Sxl feedback and bind own RNA to prevent male specific exon from being spliced in so only make female

* Transcription of pm contain a different exon 1 so require functional Sxl (**splice repressor**) to skip over male specific exon

* males have less sisterless so don't activate pe and only use pm

↳ They don't have Sxl protein and male exon is included in mature mRNA



When Sxl is transcribed from the Pe promoter in female embryos, the spliceosome skips over the male-specific (ORANGE) exon without any need for Sxl protein.

When Sxl transcription switches to Pm promoter, the pre-mRNA has a different Exon 1. But with Sxl protein present, the male exon is still excluded from the mRNA.

Summary of X Chromosome ratio to Sex determining

- X:A Ratio determines Sex
 - ↳ due to expression of TF's *Sisterless A & B* from chromosome X
- ↑ *sisterless* = *Pe promoter of Sxl = Female*
- *Pe* creates *Sxl* protein without exon 3 & makes protein without need of a splice receptor
- *Pe* is silenced by *Pm*
 - ↳ *Pm* will only skip exon 3 if *Sxl* splice repressor protein is available which only females have
- * Exon 3 has stop codon so males **Never** have active *Sxl*
- *Sxl* also represses inclusion of stop codon in *tra* genes
 - ↳ *Tra* gene has splice enhancers that include female specific Exon 2 into *Dsx*
 - ↳ Exon 2 of *Dsx* has polyA+ signal that terminates transcription of female gene
- Males have no *Sxl* so can't have *Tra2* so males can't include exon 2 of *Dsx* so they include exon 3

What you should know

- mRNAs can be selectively spliced so that each gene may have multiple isoforms
- Splicing is carried out by the spliceosome, which recognizes specific sequences at splice junctions and removes the intron as a lariat structure
- Splicing is regulated by splice activators and splice repressors.
- Genes can also have alternative start sites with alternative first exons.
- Understand how sex determination occurs in *Drosophila*
 - X:A Ratio determines Sex
 - ↳ due to expression of TF's *Sisterless A & B* from chromosome X
 - ↑ *sisterless* = *Pe promoter of Sxl = Female*
 - *Pe* creates *Sxl* protein without exon 3 & makes protein without need of a splice receptor
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Sis-a Sis-b mutation

- have more *deadpan* (repressor) therefore will have **male** regardless of XY or XX since *Pm* is activated

Deadpan mutation

- Have no repressor thus have more *Sis-a* and *Sis-b* so *Pe* promoter is chosen thus regardless of XY or XX will be **female**

Sex-lethal mutation

- Have no *Tra* protein therefore will have no female specific *Dsx* isoform thus will have male since **males** never express *Sxl*

tra tra-2 mutation

- Act as splice activator protein when bind to *dsx*-pre-mRNA which attract spliceosomes to exon 2 which causes termination before 3 thus if not functional would include exon 3 and since no splice enhancer will be **male**

Protein Sufficient to determine sex of fly

- DSX (Drosophila Sex)

- Including exon 3 will produce nonfunctional Sxl so produce male
- not including exon 3 will produce a functional Sxl and will produce a female

Why Do male and female fly produce variant of protein

Alternative Splicing produce isoforms of DSX

<u>male</u>	<u>Female</u>
1+3	1+2

- Both male and female splice Tra but only functional in female b/c of Sxl (Tra protein made)
- Tra targets DSX & splices in manner which will repress expression of male specific gene