doublesex and sexual dimorphism

Organizer: Caihong Han
Participants: Qionglin Peng, Xiangbing Su

12-28-2018
Doublesex is conserved in animal kingdom. Doublesex regulates experienced-dependent courtship behavior. We are interested in sexual dimorphic behaviors.
1. The evolutionary origination of *doublesex*---Han
2. *doublesex* determinates sexual differentiation---Peng
3. Sexual dimorphic behaviors are regulated by *doublesex*---Su
1. The evolutionary origination of *doublesex*---Han

2. *doublesex* determinates sexual differentiation---Peng

3. Sexual dimorphic behaviors are regulated by *doublesex*---Su
Origin and discovery

Structure and splicing

Evolutionary conservation

Discovery and Cloning

DM domain and OD Alternative splicing

C. Elegant Mouse other species
In 1965, *doublesex (dsx)* was first identified affecting sexual differentiation in *Drosophila*.

**DOUBLESEX, A RECESSIVE GENE THAT TRANSFORMS BOTH MALES AND FEMALES OF DROSOPHILA INTO INTERSEXES**

**PHILIP E. HILDERETH**

*Lawrence Radiation Laboratory and Department of Zoology, University of California, Berkeley*

Received December 23, 1964

Both sexes are transformed into intersexes, which designated “*doublesex* (dsx)”

DSX controls sex determination in *Drosophila*

**SEX AND THE SINGLE CELL. I. ON THE ACTION OF MAJOR LOCI AFFECTING SEX DETERMINATION IN DROSOPHILA MELANOGASTER**

BRUCE S. BAKER AND KIMBERLY A. RIDGE

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Manuscript received June 27, 1979
Revised copy received August 27, 1979

**TABLE 1**

<table>
<thead>
<tr>
<th>Loci (symbol)</th>
<th>Chromosome map position</th>
<th>Relevant properties</th>
<th>Major references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex determination mutants:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>transformer-2 (tra-2)</td>
<td>3-70</td>
<td>Transforms females into males; males sterile</td>
<td>Watanabe 1975, Fujitaka, Kawahara and Oishi 1978</td>
</tr>
<tr>
<td>(tra-2&lt;sup&gt;0&lt;/sup&gt;Y)</td>
<td></td>
<td>Incomplete transformation of females into males; males fertile</td>
<td></td>
</tr>
<tr>
<td>intersex (ix)</td>
<td>3-60.5</td>
<td>Transforms females into intersex; males normal</td>
<td>Kroger 1999</td>
</tr>
<tr>
<td>(ix&lt;sup&gt;a&lt;/sup&gt;)</td>
<td></td>
<td>Like ix</td>
<td></td>
</tr>
<tr>
<td>transformer (tra)</td>
<td>3-45</td>
<td>Transforms females into males; males normal</td>
<td>Sturtevant 1945, Seidel 1963, This report</td>
</tr>
<tr>
<td>(tra&lt;sup&gt;10&lt;/sup&gt;)</td>
<td></td>
<td>Like tra</td>
<td></td>
</tr>
<tr>
<td>doublesex (dxe)</td>
<td>3-48.1</td>
<td>Transforms both males and females into intersex</td>
<td>Hildebrand 1965, Fung and Gowen 1957, Duncan and Kaufman 1975</td>
</tr>
<tr>
<td>(dxe&lt;sup&gt;0&lt;/sup&gt;)</td>
<td></td>
<td>Dominant, dxe&lt;sup&gt;0&lt;/sup&gt;/+ transforms females into intersex; male unaffected</td>
<td></td>
</tr>
<tr>
<td>Masculinizer (Mas=dxe&lt;sup&gt;0&lt;/sup&gt;)</td>
<td></td>
<td>Like dxe&lt;sup&gt;0&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

The regulatory hierarchy controlling sex and dosage compensation in *D. melanogaster*.
Dosage compensation is the process by which organisms equalize the expression of genes between members of different biological sexes.

transcriptional upregulation of the male X chromosome and is regulated by the MSL complex in *Drosophila*.

MSL: Male Specific Lethal ribonucleoprotein
Molecularly cloning of $dsx$ locus by chromosomal walking

$Dsx$ locus is about 40kb length.
Both sex-specific transcripts appear at the end of the larval period.

Nucleotide sequences of male and female dsx cDNA and polypeptides


**COMMON**

```
ATGGTTTCGGGAAAGAACAGATAATGCGGATCGCGGCTGCAGTCTGGGCCGCGCCCTGGGCTGCTGTGCTGGGATCCCTAGTCCAGTCCAGGCGC
```

**MALE-SPECIFIC**

```
GCAAATTACAGTGTGGAATGACTTACCCGCTCAATCTTTAATGCTACGGGATGAGCTCGCCCGACACCGCGGAAGTATTTAGAAT
```

**FEMALE-SPECIFIC**

```
GCAAATTACAGTGTGGAATGACTTACCCGCTCAATCTTTAATGCTACGGGATGAGCTCGCCCGACACCGCGGAAGTATTTAGAAT
```

In 1988, *mab3* was first discovered in *C. elegans*, which prevents yolk protein production in males.

*mab* stands for “Male-ABnormal”
Mab3 promote male-specific development in C. elegans.

Evidence for evolutionary conservation of sex-determining genes

Christopher S. Raymond*, Caroline E. Shamu†‡, Michael M. Shen†‡, Kelly J. Seifert‡, Betsy Hirsch‡, Jonathan Hodgkin† & David Zarkower*

* Institute of Human Genetics and Department of Biochemistry and § Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, Minnesota 55455, USA
† MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Another DM domain gene, *mab-23*, required for the differentiation of male characteristics in *C. elegans*.

*mab-23* is required for male-specific muscle cell differentiation.

**DMRT gene family**

**Dmrt genes:** the family of *doublesex/mab-3* related transcription factors, characterized by the presence of the DNA-binding DM domain.

**DM domain:** DM = *dsx* and *mab-3*
In 1999, DM-genes were found to control sexual development in vertebrates.
The structure of *doublesex* in *Drosophila*

**doublesex pre-mRNA**

PRE: purine-rich element

Hertel K.J. et al. (1996)


The DM domain is a zinc finger DNA-binding motif that interacts with DNA in the minor groove.

a novel zinc module and disordered tail.

Mutations in either Zn2+-binding site or tail can lead to an intersex phenotype.

The splicing mechanism of *dsx*

**Six 13-nucleotide repeat sequences**

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCTCAAATCAACA</td>
<td>2742'</td>
</tr>
<tr>
<td>TCTCAAATCAACA</td>
<td>2799'</td>
</tr>
<tr>
<td>TCTCAAATCAACA</td>
<td>2856'</td>
</tr>
<tr>
<td>TCTCAAATCAACA</td>
<td>2877'</td>
</tr>
<tr>
<td>TCAACAAATCAACA</td>
<td>2908'</td>
</tr>
<tr>
<td>TCAACGAATCAACA</td>
<td>3013'</td>
</tr>
</tbody>
</table>

The sequences of each of the six tridecamer repeats found in the final exon of the female-specific dsx transcript are shown. The coordinates shown for the first and last nucleotides of each report correspond to those of the sequence in Figure 3.


**Sequence alignment of exon 4**

Six copies of a 13 nucleotides repetitive element is required for activation of the female-specific 3' splice site by TRA and TRA-2 in females.

Hertel KJ1, et al. (1996).
SR protein RBP1 recognizes dsx repeat region to regulate alternative splicing

The *Drosophila* SR protein RBP1 contributes to the regulation of *doublesex* alternative splicing by recognizing RBP1 RNA target sequences

Volker Heinrichs and Bruce S. Baker (1995)
**Sex-Specific Splicing and Protein Phosphorylation**

Protein Phosphorylation Plays an Essential Role in the Regulation of Alternative Splicing and Sex Determination in *Drosophila*

hs83-tra: tra female-specific mRNA

Conserved structure of *dmrt* gene

Zhang N. et al. (2014).
Phylogeny of the DM domain of vertebrate Dmrt proteins.

The function of Dmrt genes in vertebrate development: It is not just about sex

Table 2
Major phenotypes associated with loss or gain of function of Dmrt genes

<table>
<thead>
<tr>
<th>Genes</th>
<th>Species</th>
<th>LOF/GOF</th>
<th>Phenotypes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dmrt1</td>
<td>Human</td>
<td>M</td>
<td>Gonadal dysgenesis and XY sex reversal&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Veitia et al., 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gonadal dysgenesis and XY sex reversal&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Raymond et al., 1999b</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>KO</td>
<td>Variable XY sex reversal and mental retardation&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Muroya et al., 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KO</td>
<td>Ovotestes in genetic males and growth retardation&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Ounap et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Failure to differentiate (embryonic carcinoma cell line)</td>
<td>Koji et al., 2006</td>
</tr>
<tr>
<td>(Dmy)</td>
<td>Medaka</td>
<td>M</td>
<td>Female development in genetic males</td>
<td>Matsuda et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GOF</td>
<td>Male development in genetic females</td>
<td>Matsuda et al., 2007</td>
</tr>
<tr>
<td>Dmrt2</td>
<td>Human</td>
<td>M</td>
<td>Gonadal dysgenesis and XY sex reversal&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Raymond et al., 1999b</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>KO</td>
<td>Variable XY sex reversal and mental retardation&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Muroya et al., 2000</td>
</tr>
<tr>
<td>(Terra)</td>
<td>Zebrafish</td>
<td>GOF</td>
<td>Ovotestes in genetic males and growth retardation&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>Ounap et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KD</td>
<td>Embryonic somite patterning defects</td>
<td>Seo et al., 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KO</td>
<td>Increased apoptosis</td>
<td>Meng et al., 1999</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Randomization of left-sided-specific genes and desynchronization of the segmentation clock</td>
<td>Saude et al., 2005</td>
</tr>
<tr>
<td>Dmrt4</td>
<td>Mouse</td>
<td>KO</td>
<td>Females with polyovular follicles and males with copulatory behavior toward other males</td>
<td>Balciuniene et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Xenopus</td>
<td>KD</td>
<td>Impaired neurogenesis in the olfactory epithelium</td>
<td>Huang et al., 2005b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GOF</td>
<td>Promote neurogenesis in naïve ectoderm</td>
<td>Huang et al., 2005b</td>
</tr>
<tr>
<td>Dmrt7</td>
<td>Mouse</td>
<td>KO</td>
<td>Infertility with spermatogenic arrest at pachytene stage and abnormal sex chromatin modifications</td>
<td>Kawamata and Nishimori, 2006; Kim et al., 2007a</td>
</tr>
</tbody>
</table>

GOF, Gain of function; KD, Knockdown; KO, Knockout; LOF, loss of function; M, Mutation. (a), patients with deletion of a region of chromosome 9p containing DMRT1, DMRT2 and DMRT3 genes.

Dmrt genes integrate sex-specific, spatial, and temporal cues to induce sexually dimorphic differentiation.

Diverse roles of DMRT1 orthologues in vertebrate sex determination.

Summary

- Dsx has a DNA-binding domain and two oligomerization domains.

- Dsx regulates dimorphic trait development in *Drosophila*.

- Alternative splicing of *dsx* pre-mRNA requires two elements: six 13-nucleotide repeat sequences and SR protein.

- SR protein RBP1 promote alternative splicing by recognize target sequence in *dsx* pre-mRNA.

- Dmrt gene family is highly conserved in animals.
What was the regulatory activity of arthropod Dsx before the evolution of sex-specific splicing?

How does \textit{dsx} regulate other target gene instead of yolk protein?

What are the relationship between protein phosphorylation and sex-specific splicing?
1 The evolutionary origination of doublesex---Han

2 doublesex determinates sexual differentiation---Peng

3 Sexual dimorphic behaviors are regulated by doublesex---Su
Sex-determining pathway in model organisms

Zhang, N., et al. (2014)
dsx is at the interface of sex determination and sexual differentiation in insects

Morphological characteristics in the insect world

**Bumblebee**
- Male
- Worker
- Queen

**Stag Beetle**

**Butterfly**
- Male
- Non-mimetic female
- Mimetic female

**Fruit Fly**
External appearance of a gynandromorph (bumblebee)

Ugajin, A., et al. (2016)
dsx expression patterns of the gynandromorph (bumblebee)

Ugajin, A., et al. (2016)
Intraspecific sexual dimorphism and male variation in *Cyclommatus metallifer* (stag beetle)

Gotoh, H., et al. (2014)
dsx transcripts of *Cyclommatus metallifer* (stag beetle)

Gotoh, H., et al. (2014)
Intersex phenotypes of $dsx$ RNAi in females and males

Gotoh, H., et al. (2014)
dsx modulates the response to JH in a sex-specific manner.

Ace: acetone
JHA: juvenile hormone analog

Gotoh, H., et al. (2014)
Female-limited Batesian mimicry in *P. polytes*

doublesex is a mimicry supergene


Association mapping, based on full genome sequences of 30 P. polytes butterflies, revealed multiple perfect associations inside dsx but none outside the gene.

Kunte, K., et al. (2014)
Expression of female \textit{dsx} isoforms is female-biased

\textit{dsx} transcripts

\textit{dsx} isoform F1 and isoform F2 show wing-biased expression whereas isoform F3 expression is body-biased.

Kunte, K., et al. (2014)
Female *dsx* isoforms also show elevated expression in mimetic females

*dsx* isoform F1 and F2 show elevated expression in mimetic females relative to non-mimetic females.

Kunte, K., et al. (2014)
Chromosomal inversion of $dsx$

- $dsx(H)$  $dsx(h)$

Expression patterns of genes located on the \( H \) locus

$dsx(H)$ is involved in both mimetic and non-mimetic wing pattern formation.

dsx dictates sexual differentiation in *Drosophila*

**wild type control**

- XY WT ♂
- XX WT ♀

**dsx mutant female**

- XX dsx-null
- XX tra-null

**Overexpression of dsxF in male**

- XY UAS-dsxF; dsxGal4

**Overexpression of dsxM in female**

- XX UAS-dsxM/dsxGal4
- XX UAS-traIR; dsxGal4
- XX UAS-tra2IR/dsxGal4

Regulation of Body Pigmentation by the Abdominal-B Hox Protein and Its Gain and Loss in *Drosophila* Evolution

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² Microbial Genome Analysis and Annotation, The Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02141, USA
*Contact: sbcarrol@wisc.edu
DOI 10.1016/j.cell.2006.04.043
The Regulation and Evolution of a Genetic Switch Controlling Sexually Dimorphic Traits in *Drosophila*

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*Correspondence: sbcarrol@wisc.edu*

DOI 10.1016/j.cell.2008.06.052

**Diagram:**
- **Female (♀):** ABD-B, DSXF
- **Male (♂):** ABD-B, DSXM
- **bab1** is a dimorphic element.
- **bab on in A5 and A6:** Pigmentation repressed
- **bab off in A5 and A6:** Pigmentation expressed

Williams, T. M., et al. (2008)
Two cis-regulatory elements (CRE) direct \textit{bab1} expression in the abdomen

Two CRE

AE: Anterior element

DE: Dimorphic element

Williams, T. M., et al. (2008)
Abd-B behaves genetically as an activator of the DE and as a repressor of the AE

**AE: Anterior element**

**DE: Dimorphic element**

**Abd-B overexpression**

AE expression was repressed in both males and females

**Abd-B overexpression**

DE expression was expanded in females, but no effect in males

Abdominal-B (Abd-B): Hox gene, segment-specific regulator

Williams, T. M., et al. (2008)
Regulation of *bab1* expression by *dsx*

*dsx* acting as a repressor in males

*dsx* acting as an activator in females

**DE**: Dimorphic element

*dsx* : *dsx* mutant

*dsx* : *dsx* is spliced as *dsx*
Sex-specific DSX regulate female-specific activation and male-specific repression of *bab1*

Williams, T. M., et al. (2008)

DNase I footprinting assay
Identification of *Drosophila* DSX Target Genes - DSX occupancy and binding sites

Clough, E., et al. (2014)
DSX occupancy for the *gpp*, *Su(Tpl)*, and *lilli* loci

Clough, E., et al. (2014)
Function of DOT1 in Sex Differentiation

Model of DOT1

Clough, E., et al. (2014)
**Summary**

- *dsx* is involved in the gynandromorph of *bumblebee*, with male characters on the left side and female characters on the right side;

- *dsx* interacts with juvenile hormone (JH) signaling to determine the developmental fates and final sizes of *stag beetles*;

- A single gene, *dsx*, can switch the entire wing pattern among mimicry phenotypes in *butterflies*;

- **DSX**\(^F\) and **ABD-B** activate *bab1* expression in females, whereas **DSX**\(^M\) directly represses *bab1*, which allows for pigmentation in *fruit flies*.

- **DSX**\(^F\) and **DSX**\(^M\) bind thousands of the same targets, regulating sex-biased expression in multiple tissues in both sexes.
Questions

✓ Function of DSX target genes
✓ Find cis-regulatory elements (CRE)


1. The evolutionary origination of *doublesex*

2. *doublesex* determinates sexual differentiation

3. Sexual dimorphic behaviors are regulated by *doublesex---su*
**doublesex** control sex determination and sexual dimorphic behavior in *Drosophila*.

THE EFFECT OF TRANSFORMER, DOUBLESEX AND INTERSEX MUTATIONS ON THE SEXUAL BEHAVIOR OF DROSOPHILA MELANOGASTER

SCOTT P. McROBERT AND LAURIE TOMPKINS

Department of Biology, Temple University, Philadelphia, Pennsylvania 19122

Manuscript received September 5, 1984
Revised copy accepted May 14, 1985

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Courtship Anomalies Caused by doublesex Mutations in Drosophila melanogaster

Adriana Villella and Jeffrey C. Hall

Department of Biology, Brandeis University, Waltham, Massachusetts 02254
Manuscript received October 31, 1995
Accepted for publication January 29, 1996
Doublesex regulates multiple steps of sexual behavior:


Diagram showing the stages of sexual behavior:
1. Orientation
2. Tapping
3. Singing
4. Licking
5. Attempted copulation
6. Copulation
7. Postmatting
a model for the neural organization that controls courtship behavior

- vision
- olfaction
- gustation
- audition

- tapping
Expression of Gr68a is regulated by the sex-determination genes
Gr68a-expressing neurons are required during the tapping step

Doublesex regulates the courtship song


Arbeitman MN1, Newell NR. Dev Cell. 2016


7 Postmatting
Neurogenetics of *Drosophila* courtship song

**Hum: sine song**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No Fru^M^ neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>XY</td>
<td>105</td>
</tr>
<tr>
<td>XX tra/Df(tra)</td>
<td>101</td>
</tr>
<tr>
<td>(FRU^M^, DSX^M^)</td>
<td></td>
</tr>
<tr>
<td>XX fru^M^ /Df(fru)</td>
<td>75</td>
</tr>
<tr>
<td>XX fru^Δtra^ /Df(fru)</td>
<td>73</td>
</tr>
<tr>
<td>(FRU^M^, DSX^F^)</td>
<td></td>
</tr>
<tr>
<td>XY: dsx^{23}/Df(dsx)</td>
<td>83</td>
</tr>
<tr>
<td>(FRU^M^, DSX-null)</td>
<td></td>
</tr>
</tbody>
</table>

sine song are regulated by TN1A neurons and hg1 motorneuron

hg1 motoneuron regulates sine song

the TN1A Neurons Is Required for the Generation of Sine Song


dsx regulates the link between the TN1A neurons and hg1 motoneuron

The TN1A neurons and hg1 motoneuron are functionally coupled

\[ \text{Chrismon in TN1A} \]

\[ \Delta F/F \text{ in hg1 motoneuron} \]

\[ \text{time (seconds)} \]

\[ 0 \]

\[ 10 \]

\[ 20 \]

\[ -10 \]

\[ 30 \]

\[ 60 \]

\[ 90 \]

\[ d sx \text{ Regulates the Link between the TN1A Neurons and hg1 Motoneuron} \]

Shirangi TR et al. Dev Cell (2016)
Neuronal and genetic basis of courtship sine song
The involvement of pCd and pC1 neurons in female receptivity

Activation of Latent courtship circuitry in the brain of females induces male-like behaviors

dsx-pC1 neurons induce male-typical courtship behaviors in females

Copulation are regulated by *dsx*


Sexually dimorphic dsx/glutamatergic neurons control genital coupling during copulation.

Sexually dimorphic \textit{dsx}/GABAergic neurons control genital uncoupling during copulation.

model of circuit organization underlying copulation in males

dsx regulates the postmating behavior


mSP and SPR expression in dsx neurons influence postmating behaviors

Behavioral Effects of mSP Expression in dsx Neurons in Virgin Females

Knocking Down SPR Expression in dsx Neurons Reduces Postmating Behaviors in Mated Females
Dsx neurons are part of the circuitry involved in sensing SP
characterization of intersected *dsx*-Abg neurons and associated projections

neuronal circuitry involved in sex peptide function.
mab-23 males are defective in specific steps of male mating behavior.

Robyn Lints and Scott W. Emmons, Genes Dev. 2002
Hierarchy of sexual differentiation genes in *C. elegans* and *Drosophila*.

Robyn Lints and Scott W. Emmons. Genes Dev. 2002
1. Dsx neurons are dimorphic in flies.

2. Dsx regulates the expression of Pheromone receptor GR68a.

3. Sine song are regulated by $dsx$.

4. Dsx regulates the copulation by GABAergic and Glut neurons.

5. Dsx neurons are part of the circuitry involved in sensing SP.
Questions

1. What are the neural circuits that receive inputs from the pCd and pC1 neurons?

2. Whether the pC2 neurons also function in female sexual behaviors.

3. How does the courtship step are translated correctly in fruit fly?


