

$fs(1)A1304^{1}$ is a 5' UTR deletion of the essential gene small ovary in Drosophila

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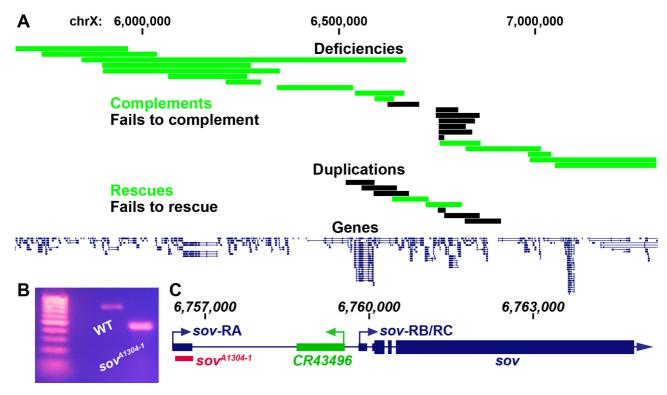


Figure 1: A) Complementation mapping of *fs(1)A1304*¹. Boxes represent either the portion of the chromosome deleted or duplicated. For deficiencies, green indicates complementing deletions and black indicates non-complementing deletions. For duplications, green indicates rescuing fragments while black indicates non-rescuing fragments. Numbers indicate genomic coordinates in bases along the X chromosome. B) Genomic PCR of wildtype (WT) and *sov*^{A1304-1} flies. Primers were designed to amplify genomic DNA encoding the 5' UTR region of the *sov*-RA transcript. C) Cartoon of the *sov* locus. Dark blue represents the *sov* gene region with the left arrow representing the *sov*-RA transcriptional start site and right arrow representing the *sov*-RB/RC transcriptional start site. Green represents the *CR43496* gene region with the arrow representing the transcriptional start site. Red box represents the deleted segment in *sov*^{A1304-1} flies. Small rectangles represent untranslated regions while large boxes represent translated regions. Numbers indicate genomic coordinates in bases along the X chromosome.

Description

X-linked female sterile screens in *Drosophila* have led to a tremendous increase in our understanding of the genetic control of oogenesis (Gans *et al.* 1975; Mohler 1977; Komitopoulou *et al.* 1983). However, many of the loci in these screens have not been mapped to a single gene and therefore remain a rich resource for further elucidating the genetic control of female fertility. $fs(1)A1304^1$ is one such allele that is germline dependent and results in a degenerative ovary phenotype (Gans *et al.* 1975; Khipple and King 1976; Mulligan 1981; Wieschaus *et al.* 1981; Mulligan and Rasch 1985; Lamnissou and Gelti-Douka 1985). We were interested in determining the mutation that leads to sterility in $fs(1)A1304^1$ females. Previous recombination mapping had placed $fs(1)A1304^1$ at 19 ± 2 cM on the X chromosome (Gans, Audit, and Masson 1975; Khipple and King 1976). We confirmed the previous mapping interval by meiotically mapping $fs(1)A1304^1$ to the right of *crossveinless* (12 cM) and to the left of *singed* (22 cM). We began complementation tests for female sterility with known deficiencies tiling the *crossveinless* and *singed* region and placed the lesion within a roughly 235 kb region (Figure 1A, non-complementing Df(1)BSC276, BSC285, BSC286, BSC297, BSC351, BSC535, and sov) (Parks *et al.* 2004;

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Cook *et al.* 2012). Two duplications within this narrow region rescued $fs(1)A1304^1$ sterility and thus further narrowed down the possible location of the causal mutation (Figure 1A, Dp(1;3)DC486 and Dp(1;3)DC026) (Venken *et al.* 2010). The mapping results were somewhat ambiguous within this narrow region (discussed below). However, the smallest noncomplementing deficiency, Df(1)sov, contains only the protein coding gene *small ovary* (sov) and non-coding RNA gene CR43496. We therefore decided to complementation test $fs(1)A1304^1$ with known alleles of sov. Flies homozygous for hypomorphic alleles of sov show a similar female sterility phenotype to flies bearing $fs(1)A1304^1$ while amorphic sov alleles are embryonic lethal (Wayne *et al.* 1995; Jankovics *et al.* 2018; Benner *et al.* 2019). We found that amorphic alleles sov^{EA42} and sov^{ML150} failed to complement $fs(1)A1304^1$ female sterility while the hypomorphic sov^2 complemented $fs(1)A1304^1$ sterility. Collectively this indicates that $fs(1)A1304^1$ is a sov allele ($sov^{A1304-1}$).

To determine the molecular lesion, we performed paired-end DNA sequencing on $sov^{A1304-1}$ females. The sov locus contains three annotated transcripts; sov-RA has an annotated upstream transcriptional start site while sov-RB/RC are annotated to use a downstream transcriptional start site (Thurmond et~al.~2019). Our sequencing data suggested that $sov^{A1304-1}$ flies contained a deletion within the sov gene region that would delete a majority of the sov-RA 5' UTR. Genomic PCR of this potential deletion confirmed the presence of a deletion in $sov^{A1304-1}$ flies (Figure 1B). Sanger sequencing of the $sov^{A1304-1}$ genomic PCR product showed that there was a 324 nucleotide deletion (chrX:6,756,385-6,756,709) and a 10 nucleotide insertion (TCAACCTTCG) in the sov-RA 5' UTR and would therefore remove most of the annotated 5' UTR and donor splice site (Figure 1C).

We are unsure why a duplication (Dp(1;3)DC026) and a deficiency (Df(1)BSC535) to the left of the sov region rescued and failed to complement sov^{A1304-1}, respectively. We also found that the small duplication of just sov and CR43496 (Dp(1:3)sov^{tCH322-191E24}) failed to rescue. We were not able to find any deleterious mutations or structural variants in our sequencing data to the left of sov that might indicate the presence of a second-site suppressor or long-range genomic interactions with the sov locus that are necessary for its proper expression. It is interesting that sov^{A1304-1} had not been previously mapped to sov since the Mohler and Gans X-linked female sterile collections had been previously complementation tested inter se (Perrimon et al. 1986). We found that one of the original Mohler alleles, sov², complemented $sov^{A1304-1}$ sterility and is thus possible that the other two Mohler alleles, sov^1 and sov^3 , behaved similarly, providing an explanation as to why sov^{A1304-1} was not previously recognized as belonging to the sov locus. It would be interesting to determine if the 5' UTR deletion of the sov-RA transcript found in sov^{A1304-1} flies affects sov activity in other tissues of the body other than the ovary. There is no indication that sov-RA, or sov-RB/RC, is differentially expressed in the ovary or other adult tissues (Benner *et al.* 2019). Pole cell transplantation studies of sov^{A1304-1} indicated that defects are germline dependent (Wieschaus et al. 1981; Lamnissou and Gelti-Douka 1985), however, sov is an essential gene that has been shown to dominantly suppress position-effect variegation in tissues such as the eye (Jankovics et al. 2018; Benner et al. 2019). It is possible that the deletion solely affects sov-RA and that the Drosophila ovary is more sensitive to loss of sov-RA, or sov transcripts in general, in comparison to other tissues since sov^{A1304-1} females are viable but sterile. However, we have not directly measured the deletions effects on sov-RB/RC transcript levels, which might also be perturbed. The nature of the $sov^{A1304-1}$ deletion therefore provides a unique mechanism to further elucidate the function of Sov at potentially both the transcript and regulatory level in *Drosophila*.

Methods

Flies were cultured on 'Fly Food A' (LabExpress, Ann Arbor, MI) under standard laboratory conditions at 25°C. Genomic DNA was extracted from 30 homozygous $fs(1)A1304^1$ flies with a Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany) according to the manufacturers insect protocol. DNA-sequencing libraries were made with Illumina Nextera DNA Library Prep Kit (San Diego, CA). 50 nucleotide paired-end sequencing was performed (Illumina HiSeq 2500, CASAVA base calling). Sequencing reads were mapped with Hisat2 to the FlyBase r6.25 genome and are available at the SRA (SRP238927) (Kim *et al.* 2015; Thurmond *et al.* 2019). Variant calling was completed with mpileup and bcftools from SAMtools within the X chromosome region 6625450-6860753 (Li *et al.* 2009; Li 2011) followed with variant annotation software snpEFF (Cingolani *et al.* 2012). For structural variant calling, we used BreakDancer software (Chen *et al.* 2009). Sanger sequencing was completed by Genewiz (Plainfield, NJ).

Reagents

Deficiencies and duplications in order as they appear in Figure 1 (top to bottom).

Deficiencies:

Df(1)ED6802 = BDSC 8949 (or FBst0008949)

Df(1)*BSC*654 = BDSC 26506 (or FBst0026506)

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Df(1)dx81 = BDSC 5281  (or FBst0005281)
Df(1)ED418 = BDSC 8032  (or FBst0008032)
Df(1)ED6829 = BDSC 8947  (or FBst0008947)
Df(1)Exel6238 = BDSC 7712 (or FBst0007712)
Df(1)BSC640 = BDSC 25730  (or FBst0025730)
Df(1)Exel6239 = BDSC 7713 (or FBst0007713)
Df(1)Exel6240 = BDSC 7714 (or FBst0007714)
Df(1)e02477-d06059 = BDSC 39617 (or FBst0039617)
Df(1)BSC535 = BDSC 25063  (or FBst0025063)
Df(1)BSC285 = BDSC 23670  (or FBst0023670)
Df(1)BSC351 = BDSC 24375  (or FBst0024375)
Df(1)BSC297 = BDSC 23681 (or FBst0023681)
Df(1)BSC286 = BDSC 23671 (or FBst0023671)
Df(1)BSC276 = BDSC 23661 (or FBst0023661)
Df(1)sov = Benner et al., 2019
Df(1)ED6878 = BDSC 9625 (or FBst0009625)
Df(1)BSC882 = BDSC 30587 (or FBst0030587)
Df(1)BSC867 = BDSC 29990  (or FBst0029990)
Df(1)Sxl-bt = BDSC 3196  (or FBst0003196)
Df(1)Sxl^{fP7B0} = BDSC 58489  (or FBst0058489)
Duplications:
Dp(1;3)DC158 = BDSC 30296  (or FBst0030296)
Dp(1;3)DC159 = BDSC 32268  (or FBst0032268)
Dp(1;3)DC160 = BDSC 30297 (or FBst0030297
Dp(1;3)DC026 = BDSC 30226  (or FBst0030226)
Dp(1;3)DC486 = BDSC 32306  (or FBst0032306)
Dp(1;3)sov^{tCH322-191E24} = Venken et al., 2010 (or FBal0243261)
Dp(1;3)DC163 = BDSC 32269  (or FBst0032269)
Dp(1;3)DC164 = BDSC 32270  (or FBst0032270)
Alleles:
fs(1)A1304^{1} (sov<sup>A1304-1</sup>) = BDSC 4314 (or FBst0004314)
sov^2 = BDSC 4611  (or FBst0004611)
sov^{EA42} (synonymous with l(1)6Dc^3) = FBal0007068
sov^{ML150} = BDSC 4591 (or FBst0004591)
Primer fs(1)A1304<sup>1</sup> Forward = TGACCATGTTGCATCTAAGCCA
Primer f_s(1)A1304^1 Reverse = AGTAGAGCTCGCAATACGCC
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