© Indian Academy of Sciences

REVIEW ARTICLE

The role of the *Drosophila* LAMMER protein kinase DOA in somatic sex determination

LEONARD RABINOW* and MARIE-LAURE SAMSON

Université Paris Sud 11, CNRS UMR 8195, Centre de Neurosciences de Paris Sud, Bâtiment 442 bis, 91405 Orsay Cedex, France

Abstract

DOA kinase, the *Drosophila* member of the LAMMER/Clk protein kinase family, phosphorylates SR and SR-like proteins, including TRA, TRA2 and RBP1, which are responsible for the alternative splicing of transcripts encoding the key regulator of sex-specific expression in somatic cells of the fly, DOUBLESEX. Specific *Doa* alleles induce somatic female-to-male sex transformations, which can be enhanced when combined with mutations in loci encoding SR and SR-like proteins. The *Doa* locus encodes six different kinases, of which a 69-kDa isoform is expressed solely in females. Expression of this isoform is itself under the regulation of the somatic sex determination regulatory network, thus forming a putative positive autoregulatory loop which would reinforce the choice of the female cell-fate. We speculate that this loop is part of the evolutionary ancestral sex-determination machinery, based upon evidence demonstrating the existence of an autoregulatory loop involving TRA and TRA2 in several other insect species.

[Rabinow L. and Samson M.-L. 2010 The role of the *Drosophila* LAMMER protein kinase DOA in somatic sex determination. *J. Genet.* **89**, 271–277]

Somatic sex determination in *Drosophila* is established by a cascade of alternative splicing

Somatic sexual identity in *Drosophila melanogaster* is under the control of a well-characterized genetic cascade which ultimately functions to regulate the alternative splicing of transcripts encoding the key transcription factor DOUBLESEX (DSX), as well as the less generalized but important neuronal transcription factor FRUITLESS (FRU) (Schutt and Nothiger 2000; Forch and Valcarcel 2003). This cascade is initiated by the early transcription of *Sex-lethal* (*Sxl*) in female embryos between stages 12 and 14, in response to an X to autosome (X : A) ratio of 1 : 1.

SXL protein is expressed only in females due to the inclusion of exon 3 in males, which introduces a premature stop codon. In females, SXL protein binds to its own primary transcript, inhibiting inclusion of exon 3, enabling the production of functional protein. SXL also binds directly to transcripts of *transformer* (*tra*), the next gene in the somatic sex regulatory cascade, inhibiting the use of an upstream 3′ splice acceptor site in exon 2, which would otherwise introduce an in-frame stop codon. Curiously, the inhibition of splicing at the *tra* upstream splice acceptor by SXL is only about 50% efficient (Nagoshi *et al*. 1988). Skipping the upstream splice acceptor and the premature stop codon in the following exonic sequences leads to use of a weak downstream acceptor splice site and production of full-length TRA protein, which is thus expressed only in females.

TRA promotes the formation of a functional splicing complex containing additional splicing factors, including TRA2 and RBP1, on the exonic splicing enhancer (ESE) in exon 4 of the *doublesex* (*dsx*) transcript (figure 1). Formation of the splicing complex on the *dsx* ESE activates the weak female-specific splice-acceptor site in exon 4, precluding use of the stronger male-specific splice acceptor in exon 5. Without the presence of the splicing complex, exon 4 is skipped in favour of the stronger 3′ splice-acceptor bordering exon 5, as occurs in males. The DSX transcription factor is thus expressed as either a male isoform, thought to induce male-specific transcripts and repress female-specific genes, or a

*For correspondence. E-mail: Leonard.Rabinow@u-psud.fr.

Keywords. phosphorylation; alternative splicing; SR proteins; sex determination.

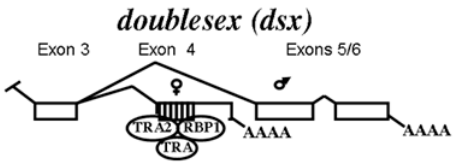

Figure 1. Sex-specific alternative splicing of *dsx* is under control of the SR and SR-like proteins TRA, TRA2 and RBP1. Female-specific splicing to the relatively weak splice acceptor in exon 4 of *dsx* pre-mRNA is stimulated by binding of the SR and SR-like proteins to the exonic splicing enhancer (ESE), a set of six repeats of a 13-nucleotide sequence, and a single iteration of a purine-rich sequence. TRA protein is expressed only in females, and in its absence default splicing occurs to the strong male-specific splice acceptor in exon 5.

female-specific isoform performing the opposite functions. However, although a consensus *in vitro* binding site for DSX protein has been determined (Erdman *et al*. 1996), very few direct DSX-bound promoters are known. The only characterized direct DSX targets are the promoters of yolk proteins 1 and 2 (YP1 and YP2) (Burtis *et al*. 1991; Coschigano and Wensink 1993), the transcrition factor *bric-a-brac* (*bab1*) (Williams *et al*. 2008), and the female-specific hydrocarbon-hormone-synthesizing enzyme *desaturase-F* (*desat-F*) (Shirangi *et al*. 2009). Other potential DSX targets have been identified through genomic analyses (e.g. Lebo *et al*. 2009), and several labora tories are currently using various immunoprecipition or chromatin-marking strategies to identify additional DSX target genes.

Phosphorylation of SR and SR-like proteins by DOA, the LAMMER protein kinase of *Drosophila*

The RBP1, TRA and TRA2 proteins which bind the *dsx* ESE possess domains rich in arginine–serine repeats (RS domains), and are members of the SR and SR-'like' protein families. Members of these families participate in multiple functions in RNA metabolism, from pre-mRNA splicing to translation and mRNA stability (Long and Caceres 2009; Shepard and Hertel 2009; Zhong *et al*. 2009). SR and SR-like proteins are regulators of sex determination in several Dipteran species, as well as in Hymenoptera, notably the products of *csd* and *fem* loci in *Apis* (Beye *et al*. 2003; Hasselmann *et al*. 2008) and apparently also the *tra* orthologue of *Nasonia* (Werren *et al*. 2010). These proteins are subjected to phosphorylation and dephosphorylation to regulate their activity, localization and half-lives. They are phosphorylated *in vitro* by a number of different kinases, including SRPK (Wang *et al*. 1998; Ngo *et al*. 2005), LAMMER/CLK (Colwill *et al*. 1996a; Savaldi-Goldstein *et al*. 2000; Nikolakaki *et al*. 2002), AKT (Blaustein *et al*. 2005), prp4 (Kojima *et al*. 2001), and others, including cdc2 (Okamoto *et al*. 1998). LAMMER, SRPK, prp4 and AKT kinases also activate SR-protein-induced alternative splicing in cultured cell models.

LAMMER kinases are found ubiquitously throughout eukaryotes, and indeed have been identified as one of the core group of eukaryotic 'signature' proteins (Hartman and Federov 2002). These kinases are best known for their phosphorylation of SR and SR-like proteins with subsequent effects on alternative splicing, but other substrates have also been identified, including PTP1B in mammals (Moeslein *et al*. 1999) and EF1γ in *Drosophila* (Fan *et al*. 2010). Plant LAMMER kinases are also known (Bender and Fink 1994). In tobacco they are induced by the hormone ethylene, and similarly to their animal counterparts also phosphorylate SR and SR-like proteins to affect alternative splicing (Sessa *et al*. 1996; Savaldi-Goldstein *et al*. 2000, 2003).

Four LAMMER kinase paralogues exist in vertebrate genomes, but only a single orthologue is found throughout the genus *Drosophila* and most of the other insect species for which genome sequence is available, including *Nasonia vitripenis, Apis mellifera, Tribolium castaneum, Bombyx mori, Aedes aegypti, Culex pipiens, Pediculus humanus corporis*, and *Acyrthosiphon pisum* (L. Rabinow and M. L. Samson, unpublished data). Preliminary analysis suggests that a gene duplication occurred in *A. gambiae*, and two different contigs carry LAMMER kinase catalytic domains of different organization and sequence. These observations are based upon preliminary BLAST analysis using the highly conserved C-terminal catalytic domain of the kinase, but the various genomes have not been scanned to determine whether multiple non-catalytic N-termini exist, such as those described for the *Drosophila* orthologue (see below) (Kpebe and Rabinow 2008a).

Of particular interest here, the *Drosophila* LAMMER protein kinase, DOA (Darkener-of-apricot), possesses essential functions in the cascade of alternative splicing regulating somatic sex determination via the phosphorylation of TRA, TRA2 and RBP1 (Du *et al*. 1998). DOA kinase phosphorylates these three SR and SR-like proteins *in vitro*, as well as all others tested (Nikolakaki *et al*. 2002). Protein–protein interactions between the kinase and the SR proteins are mediated through the latter's RS domains since truncated constructs lacking these repeated motifs fail to interact in yeast two-hybrid assays (figure 2).

Genetics of the *Doa* locus

Virtually all of the more than 30 available *Doa* alleles are recessive lethal. Despite extensive efforts to characterize point mutations in the locus, none have been identifed, although two different deletions of 14 bp introducing premature stop codons and truncated proteins were described in the *γ3A* and *γ3C* mutants (Kpebe and Rabinow 2008b). Other alleles, such as *105* and *γ3B* were induced by chromosomal rearrangements (Rabinow and Birchler 1989; Yun *et al*. 1994). Several transposon-induced alleles were also mapped, and two of these affect specific kinase isoforms (Kpebe and Rabinow 2008b). For example, the *HD* allele is caused by the insertion of a *copia* retrotransposon which specifically reduces levels of the transcripts encoding 55-kDa and 69-kDa kinase isoforms (figure 3).

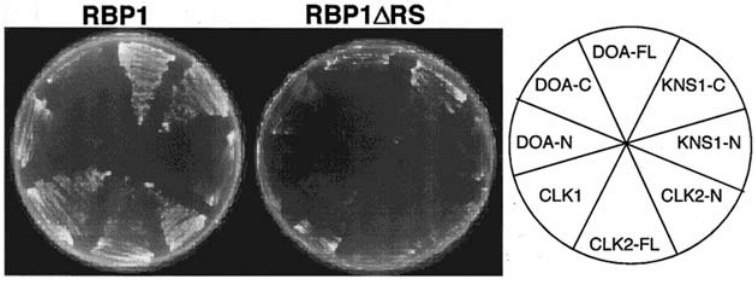

Figure 2. Interactions between LAMMER protein kinases, including DOA, and SR proteins, require the RS domain. Prototypical SR proteins contain 1 or 2 RNA-recognition motifs (RRMs), followed by a Ser–Arg-rich domain, which undergoes multiple phosphorylation events. Interaction between LAMMER kinases and SR proteins require the RS domain, as demonstrated here in a yeast two-hybrid test for protein interaction. The *Drosophila* SR protein RBP1 was used in this test, including (left), and deleted for (right) its RS domain. LAMMER kinases used were *Drosophila* DOA, mouse CLK1, human CLK2 and KNS1 from *S. cerevisiae*. FL, full-length protein; N, N-terminal non-catalytic domain of the kinase; C, C-terminal catalytic domain of the kinase. Growth of the yeast indicates protein–protein interaction between the SR protein and the kinase (Du *et al*. 1998).

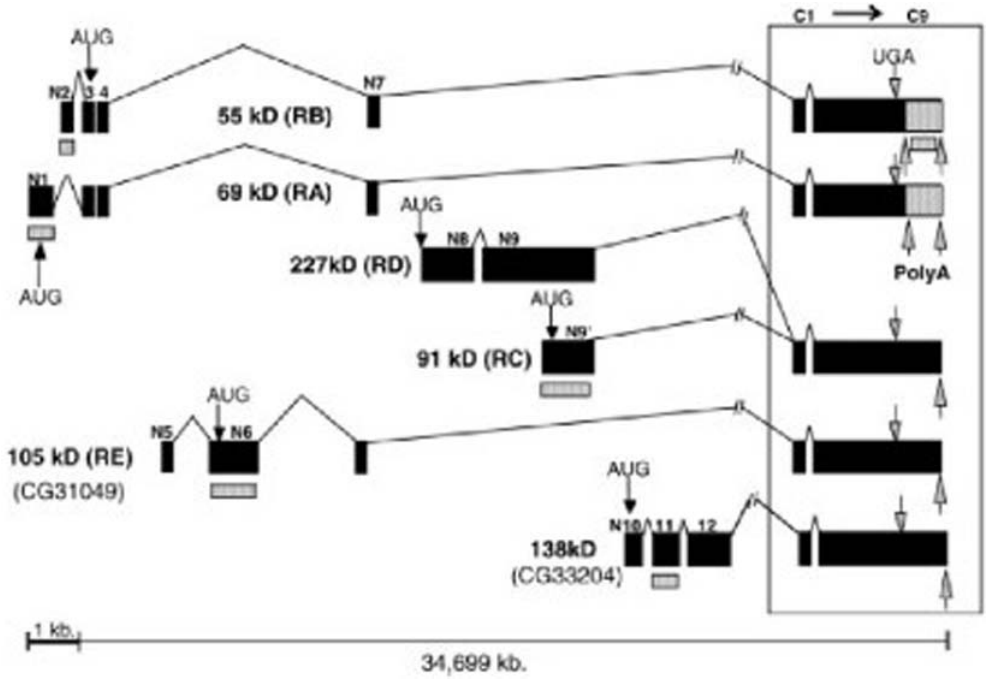

Figure 3. Structure of *Doa* transcript isoforms. Six isoforms of DOA kinase are produced via the use of alternative promoters (Kpebe and Rabinow 2008a). These proteins possess virtually identical catalytic domains but different non-catalytic N-termini. The 69-kDa kinase is expressed only in female bodies, but is not restricted to the germ line.

In addition to being recessive lethal for the whole animal, strong and null *Doa* alleles are also cell-lethal (Yun *et al*. 2000). Therefore, in order to study effects of strong reduction in *Doa* function in whole animals, specific combinations of alleles producing animals escaping lethality have been used to document phenotypes in adult flies (Rabinow and Birchler 1989; Rabinow *et al*. 1993). Among other phenotypes, heteroallelism at *Doa* induces sex-transformations to varying degrees (Du *et al*. 1998; Kpebe and Rabinow 2008b). For example, sex transformations are not visible in the *Doa* allelic pair *HD/105*, while in other cases they are essentially cryptic, such as in *HD/DEM* heteroallelic flies (figure 4C).

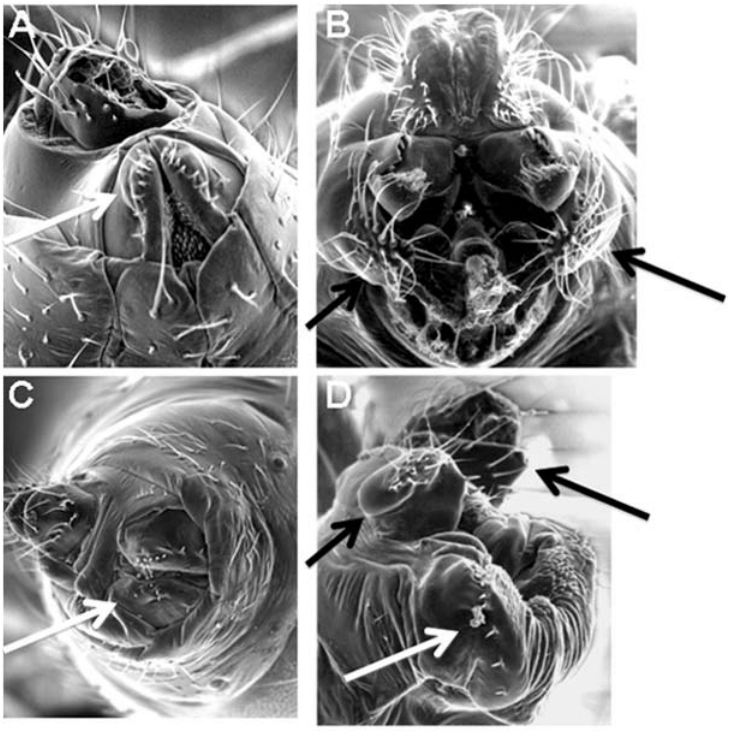

Figure 4. Female-to-male sex transformations induced by heteroallelism at *Doa*. A) wild-type female; B, wild-type male; C, Doa^{HD}/Doa^{DEM} female; D, Doa^{E786}/Doa^{DEM} female. Note the symmetric rows of vaginal bristles in (A), which are slightly perturbed in (C) white arrows. In (B) and (D), male claspers are easily visible (black arrows), but XX; *Doa/Doa* females also possess vaginal structures (white arrow).

These transformations are quite subtle, and were not recognized until specifically looked for. They consist of occasional asymmetry or missing bristles in the vaginal sex-comb. Much more dramatic sex transformations phenocopying the dsx^D phenotype can also be observed in *Doa* heteroallelic animals however, such as in combinations for the alleles *DEM/E786*, where the appearance of a male-specific terminal tergite is observed, along with the growth of male 'claspers' (figure 4D). In addition to inducing sexual phenotypes on their own, sex transformations in heteroallelic *Doa* combinations are strongly enhanced by heterozygosity for *tra* alleles, which normally show no sex transformations whatsoever. Specifically, XX; $tra^1 Doa^{HD}/+$ Doa^{DEM} females show male sex combs, enhancement of male pigmentation and development of extra tissue from the genital plates of undetermined origin (Du *et al*. 1998), in contrast to the cryptic sex transformations usually observed in the Doa^{HD}/Doa^{DEM} combination. This is presumably due to the 'sensitization' of the sex-determination machinery in the $tra/+$ genotype due to the reduction of TRA protein levels in these flies, since the tra^1 allele is due to a near complete removal of all coding sequences.

We have recently made use of the enhancement of *Doa*-induced sex transformations as a means of identifying interacting loci. For example, SRm160 is a SR-like protein whose mammalian orthologue interacts with human TRA2 in cultured cells (Blencowe *et al*. 1998). It is a likely LAMMER kinase substrate based upon the presence of an RS domain, several excellent matches for the DOA and human CLK2 kinase consensus phosphorylation sites (Nikolakaki *et al*. 2002), and because it is also one of the most highly phosphorylated proteins in both human (Beausoleil *et al*. 2004; Molina *et al*. 2007) and *Drosophila* nuclei (Bodenmiller *et al*. 2008; Zhai *et al*. 2008). We recently induced a mutation in the *Drosophila* orthologue of *SRm160*, and found that heterozygosity for it strongly enhances Doa^{HD}/Doa^{DEM}-induced sex transformations, phenocopying dsx^D (Y. Fan, A. Gittis, F. Juge and L. Rabinow, unpublished data).

Irrespective of the cuticular phenotype, females (XX) of all *Doa/Doa* genotypes examined via RT-PCR, including the *HD/105* allelic pair, accumulate the male-specific *dsx* product in addition to the female-specific isoform (figure 5A). Thus, effects on female sex-specific splicing occur at the molecular level well before their phenotypes are detectable on the cuticle of the adult fly.

It was further shown through epistasis experiments that *Doa* intervenes in the genetic hierarchy regulating somatic sex determination at the same level or in parallel to *tra*

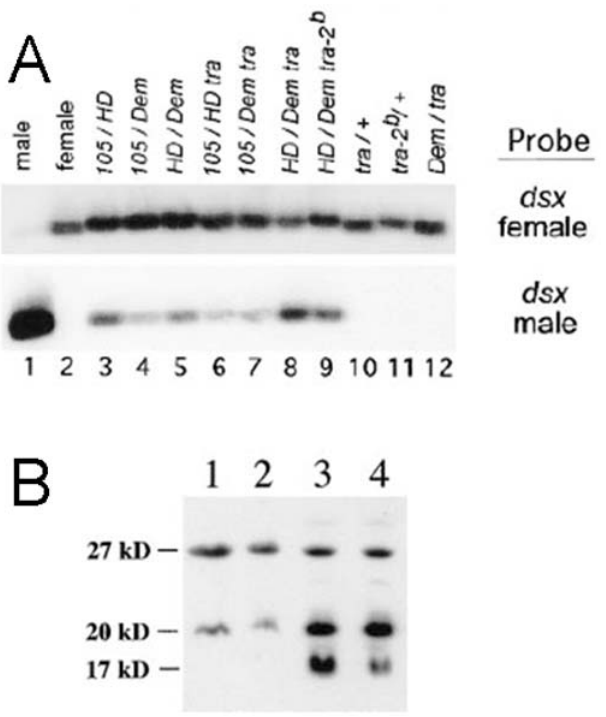

Figure 5. A) Accumulation of male-specific *doublesex* mRNA in *Doa/Doa* females. RT-PCR products were gel fractionated and probed with labelled oligonucleotides for female (top) and male (bottom) specific transcripts. Wild-type males accumulate only male-specific *dsx* product, while wild-type females accumulate only female-specific product. In contrast, females of all *Doa* heteroallelic genotypes accumulate both female and male *dsx* splice variants (Du *et al*. 1998). B) Hypophosphorylation of the SR protein RBP1 in protein extracts from *Doa* mutants. An immunoblot probed with an antibody to the SR protein RBP1, with proteins derived from: 1, w^a; +/+ males; 2, w^a; +/+ females; 3, w^a; Doa^{HD}/Doa^{105} males; 4, w^a; Doa^{HD}/Doa^{105} females. The RBP1 protein has a predicted molecular weigh of approximately 17 kDa. The higher molecular weight proteins detected in extracts obtained from flies wild-type for *Doa* (lanes 1&2) are due to RBP1 hyperphosphorylation. In proteins obtained from *Doa* mutants (lanes 3&4), both some hyperphosphorylated and hypophosphorylated proteins are observed. This is consistent with the fact that the *Doa/Doa* flies retain low levels of activity of the gene (Du *et al*. 1998).

(Du *et al*. 1998). Since TRA protein participates directly in the protein complex inducing female-specific splicing of the *dsx* pre-mRNA, it was deduced that TRA and or TRA2 must be DOA kinase substrates. Since it was also shown that the kinase phosphorylates both proteins *in vitro*, both may well be substrates *in vivo* as well.

The sex transformations observed in *Doa/Doa* flies are accompanied by the de-localization of TRA2 from chromatin into nuclear 'speckles' in spermatocytes, presumably due to its hypo-phosphorylation (Du *et al*. 1998). Nuclear speckles are sites for storage of de-phosphorylated SR and other splicing-related proteins (Sacco-Bubulya and Spector 2002), whereas phosphorylated SR proteins are often associated with actively transcribed chromatin, consistent with the coincidence of transcription and splicing. The idea that dephosphorylated TRA2 accumulates in speckles is supported by the observation that transfection of CLK1 kinase, one of the four mammalian LAMMER kinase paralogues, causes the uniform dispersion of SR proteins from nuclear speckles, presumably due to their hyperphosphorylation (Colwill *et al*. 1996b).

In addition to cuticular sex transformations, aberrant *dsx* splicing in females and delocalization of TRA2 to nuclear speckles, the hypophosphorylation of RBP1 was observed in protein extracts derived from *Doa* heteroallelic animals (figure 5B). This observation confirms that the *in vivo* function of the kinase in somatic sex-determination occurs at least partially through the direct phosphorylation of the SR and SR-like proteins.

Splicing of *fru* pre-mRNA is also regulated by TRA and TRA2. Interestingly however, no effects of *Doa* alleles were found on this RNA, suggesting that TRA and TRA2 must be insensitive to their phosphorylation state when regulating this splicing event (Du *et al*. 1998). This observation in turn argues that TRA and TRA2 must function differently when activating the 3′ splice acceptor site in *dsx* pre-mRNA, as opposed to their apparent repression of a 5′ splice-donor site in *fru* pre-mRNA.

Multiple isoforms of DOA kinase perform different

Recently we have found that DOA is expressed as six distinct protein isoforms via the use of alternative promoters (Kpebe and Rabinow 2008a) (figure 3). Essentially these six kinases share identical catalytic domains but possess variable N-terminal non-catalytic domains. Genetic complementation suggests that at least three different functions, including somatic sex determination, are performed by these six isoforms (Kpebe and Rabinow 2008b). As noted above, the 55-kDa isoform is particularly predominant, expressed at all developmental stages and in all tissues examined, and is nuclearly localized (Yun *et al*. 1994, 2000). In contrast, an isoform of 105 kDa is exclusively cytoplasmic (Yun *et al*. 2000). The 105-kDa kinase isoform is not thought to play an important role in somatic sex determination, since both RNA interference specific to this isoform, as well as heteroallelism for an allele specifically affecting it fail to reveal any cuticular sex transformations (Kpebe and Rabinow 2008b). However, RT-PCR analysis of *dsx* transcripts was not performed.

GAL4-directed overexpression and structural data suggest that the 55-kDa and 69-kDa proteins share functions, which is perhaps not surprising since they differ only in the addition of 69 amino-acid residues at the N-terminus of the larger protein. The 55-kDa protein is primarily if not exclusively nuclear (Yun *et al*. 2000). Although localization of the 69-kDa protein has not been determined, it is likely to be nuclear given the nearly complete overlap in its structure with the 55-kDa protein as well as computer predictions (Farkas *et al*. 2009).

We hypothesize that the predominant 55-kDa and 69-kDa isoforms are responsible for the observed phosphorylation of the SR and SR-like proteins, and are required for alternative splicing leading to somatic sex determination. In the case of the 55-kDa protein, this hypothesis is due to its ubiquitous expression and nuclear localization. Additionally, the *HD* allele specifically reduces expression of 55 kDa-coding and 69 kDa-coding transcripts, and in specific heteroallelic combinations produces the sex-transformation phenotypes described.

Expression of a female-specific DOA isoform is regulated by TRA, TRA2 and DSX

Interestingly, the 69-kDa DOA isoform is female-specific (Kpebe and Rabinow 2008a). While expressed ubiquitously in early embryos of both sexes, the transcript is expressed only in females during later developmental stages. Our recent data demonstrates that the 69-kDa isoform is itself under the control of the *Drosophila* sex-determination cascade, thus forming an apparent positive autoregulatory loop capable of reinforcing the choice of female fate through the phosphorylation of substrates necessary for the sex-specific splicing of the *dsx* primary transcript, such as TRA, TRA2, RBP1 and SRm160 (A. Kpebe, M. Li, M. L. Samson and L. Rabinow, unpublished data).

This potential autoregulatory loop in *Drosophila* leads to the speculation that the female-specific 69-kDa DOA isoform is included in the evolutionarily-conserved *tra-tra2-dsx* autonomous sex-determination loop which has been described in several other Dipteran species (Ruiz *et al*. 2007), including *C. capitata* (Pane *et al*. 2002; Salvemini *et al*. 2009), *L. cuprina* (Concha and Scott 2009), and most recently *M. domesticus* (Hediger *et al*. 2010). The persistence of this loop in *D. melanogaster* could be explained by the apparently recent evolutionary installation of *Sxl* at the top of the somatic sex-determination hierarchy, consistent with the 'bottom-up' theory of the evolution of sexual regulatory mechanisms (Sheaman 2002). The existence of a *Drosophila* gene paralogous to *Sxl* with no apparent role in sex determination (Traut *et al*. 2006) lends support to the idea that the *tra-tra2-dsx* autoregulatory loop described in other dipteran species is the evolutionary ancestor of the alternative splicing cascade described in *D. melanogaster*, and that the female-specific *Doa* isoform is a participant in the more ancient sex-determination mechanism found in other Diptera. The characterization of the *Doa* orthologues and their sex-specific expression from these and other species promises to be exciting.

Acknowledgments

Work in the laboratory of LR and MLS is supported by the Université Paris Sud 11 and the Centre National de la Recherche Scientifique (CNRS).

References

Beausoleil S. A., Jedrychowski M., Schwartz D., Elias J. E., Ville J., Li J., Cohn M. A. *et al*. 2004 Large-scale characterization of HeLa cell nuclear phosphoproteins. *Proc. Natl. Acad. Sci. USA* **101**, 12130–12135.

Bender J. and Fink G. R. 1994 *AFC1*, a LAMMER kinase from *Arabidopsis thaliana*, activates STE12-dependent processes in yeast. *Proc. Natl. Acad. Sci. USA* **91**, 12105–12109.

Beye M., Hasselmann M., Fondrk M. K., Page R. E. and Omholt S. W. 2003 The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. *Cell* **114**, 419–429.

Blaustein M., Pelisch F., Tanos T., Munoz M. J., Wengier D., Quadrana L. *et al*. 2005 Concerted regulation of nuclear and cytoplasmic activities of SR proteins by AKT. *Nature Struct. Mol. Biol*. **12**, 1037–1044.

Blencowe B. J., Issner R., Nickerson J. A. and Sharp P. A. 1998 A coactivator of pre-mRNA splicing. *Genes Dev*. **12**, 996–1009.

Bodenmiller B., Campbell D., Gerrits B., Lam H., Jovanovic M., Picotti P., *et al*. 2008 PhosphoPep–a database of protein phosphorylation sites in model organisms. *Nature Biotechnol*. **26**, 1339–1340.

Burtis K. C., Coschigano K. T., Baker B. S. and Wensink P. C. 1991 The doublesex proteins of *Drosophila melanogaster* bind directly to a sex-specific yolk protein gene enhancer. *EMBO J*. **10**, 2577–2582.

Colwill K., Feng L. L., Yeakley J. M., Gish G. D., Caceres J. F., Pawson T. and Fu X. D. 1996a SRPK1 and Clk/STY protein kinases show distinct substrate specificities for Serine/Arginine-rich splicing factors. *J. Biol. Chem*. **271**, 24569–24575.

Colwill K., Pawson T., Andrews B., Prasad J., Manley J. L., Bell J. C. and Duncan P. I. 1996b The Clk/STY protein kinase phosphorylates SR splicing factors and regulates their intranuclear distribution. *EMBO J*. **15**, 265–275.

Concha C. and Scott M. J. 2009 Sexual development in *Lucilia cuprina* (Diptera, Calliphoridae) is controlled by the transformer gene. *Genetics* **182**, 785–798.

Coschigano K. T. and Wensink P. C. 1993 Sex-specific transcriptional regulation by the male and female doublesex proteins of *Drosophila. Genes Dev*. **7**, 42–54.

Du C., McGuffin M. E., Dauwalder B., Rabinow L. and Mattox W. 1998 Protein phosphorylation plays an essential role in the regulation of alternative splicing and sex determination in *Drosophila. Mol. Cell* **2**, 741–750.

Erdman S. E., Chen H. J. and Burtis K. C. 1996 Functional and genetic characterization of the oligomerization and DNA binding properties of the *Drosophila* doublesex proteins. *Genetics* **144**, 1639–1652.

Fan Y., Schlierf M., Cuervo Gaspar A., Dreux C., Kpebe A., Chaney L. *et al*. 2010 *Drosophila* translational elongation factor-1g is modified in response to doa kinase activity and is essential for cellular viability. *Genetics* **184**, 141–154.

Farkas R., Kovacikova M., Liszekova D., Beno M., Danis P., Rabinow L. *et al*. 2009 Exploring some of the physico-chemical properties of the LAMMER protein kinase DOA of *Drosophila. Fly (Austin)* **3**, 130–142.

Forch P. and Valcarcel J. 2003 Splicing regulation in Drosophila sex determination. *Prog. Mol. Subcell Biol*. **31**, 127–151.

Hartman H. and Federov A. 2002 The origin of the eukaryotic cell: A genomic investigation. *Proc. Natl. Acad. Sci. USA* **99**, 1420–1425.

Hasselmann M., Gempe T., Schiott M., Nunes-Silva C. G., Otte M. and Beye M. 2008 Evidence for the evolutionary nascence of a novel sex determination pathway in honeybees. *Nature* **454**, 519–522.

Hediger M., Henggeler C., Meier N., Perez R., Saccone G. and Bopp D. 2010 Molecular characterization of the key switch F provides a basis for understanding the rapid divergence of the sex-determining pathway in the housefly. *Genetics* **184**, 155–170.

Kojima T., Zama T., Wada K., Onogi H. and Hagiwara M. 2001 Cloning of human PRP4 reveals interaction with Clk1. *J. Biol. Chem.* **276**, 32247–32256.

Kpebe A. and Rabinow L. 2008a Alternative promoter usage generates multiple evolutionarily conserved isoforms of *Drosophila* DOA kinase. *Genesis* **46**, 132–143.

Kpebe A. and Rabinow L. 2008b Dissection of DOA kinase isoform functions in *Drosophila. Genetics* **179**, 1973–1987.

Lebo M. S., Sanders L. E., Sun F. and Arbeitman M. N. 2009 Somatic, germline and sex hierarchy regulated gene expression during *Drosophila* metamorphosis. *BMC Genomics* **10**, 80.

Long J. C. and Caceres J. F. 2009 The SR protein family of splicing factors: master regulators of gene expression. *Biochem. J.* **417**, 15–27.

Moeslein F. M., Myers M. P. and Landreth G. E. 1999 The CLK family kinases CLK1 and CLK2 phosphoryate and activate the tyrosine phosphatase PTP-1B. *J. Biol. Chem.* **274**, 26697–26704.

Molina H., Horn D. M., Tang N., Mathivanan S. and Pandey S. 2007 Global proteomic profiling of phosphopeptides using electron transfer dissociation tandem mass spectrometry. *Proc. Natl. Acad. Sci. USA* **104**, 2199–2204.

Nagoshi R. N., McKeown M., Burtis K. C., Belote J. M. and Baker B. S. 1988 The control of alternative splicing at genes regulating sexual differentiation in *D. melanogaster. Cell* **53**, 229–236.

Ngo J. C., Chakrabarti S., Ding J. H., Velazquez-Dones A., Nolen B., Aubol B. E. *et al*. 2005 Interplay between SRPK and Clk/Sty kinases in phosphorylation of the splicing factor ASF/SF2 is regulated by a docking motif in ASF/SF2. *Mol. Cell* **20**, 77–89.

Nikolakaki E., Du C., Lai J., Cantley L., Giannakouros T. and Rabinow L. 2002 Phosphorylation by LAMMER protein kinases: determination of a consensus site, identification of *in vitro* substrates and implications for substrate preferences. *Biochemistry* **41**, 2055–2066.

Okamoto Y., Onogi H., Honda R., Yasuda H., Wakabayashi T., Nimura Y. and Hagiwara M. 1998 cdc2 kinase-mediated phosphorylation of splicing factor SF2/ASF. *Biochem. Biophys. Res. Commun*. **249**, 872–878.

Pane A., Salvemini M., Delli Bovi P., Polito C. and Saccone G. 2002 The transformer gene in Ceratitis capitata provides a genetic basis for selecting and remembering the sexual fate. *Development* **129**, 3715–3725.

Rabinow L. and Birchler J. A. 1989 A dosage-sensitive modifier of retrotransposon induced alleles of the *Drosophila white* locus. *EMBO J.* **8**, 879–889.

Rabinow L., Chiang S. L. and Birchler J. A. 1993 Mutations at the *Darkener of apricot* locus modulate transcript levels of *copia* and *copia*-induced mutations in *Drosophila melanogaster. Genetics* **134**, 1175–1185.

Ruiz M. F., Milano A., Salvemini M., Eirin-Lopez J. M., Perondini A. L., Selivon D. *et al*. 2007 The gene transformer of anastrepha fruit flies (Diptera, tephritidae) and its evolution in insects. *PLoS ONE* **2**, e1239.

Sacco-Bubulya P. and Spector D. L. 2002 Disassembly of interchromatin granule clusters alters the coordination of transcription and pre-mRNA splicing. *J. Cell Biol.* **156**, 425–436.

Salvemini M., Robertson M., Aronson B., Atkinson P., Polito L. C. and Saccone G. 2009 Ceratitis capitata transformer-2 gene is required to establish and maintain the autoregulation of Cctra, the master gene for female sex determination. *Int. J. Dev. Biol.* **53**, 109–120.

Savaldi-Goldstein S., Sessa G. and Fluhr R. 2000 The ethylene-inducible PK12 kinase mediates the phosphorylation of SR splicing factors. *Plant J.* **21**, 91–96.

Savaldi-Goldstein S., Aviv D., Davydov O. and Fluhr R. 2003 Alternative splicing modulation by a LAMMER kinase impinges on developmental and transcriptome expression. *Plant Cell* **15**, 926–938.

Schutt C. and Nothiger R. 2000 Structure, function and evolution of sex-determining systems in Dipteran insects. *Development* **127**, 667–677.

Sessa G., Raz V., Savaldi S. and Fluhr R. 1996 PK12, a plant dual-specificity protein kinase of the LAMMER family, is regulated by the hormone ethylene. *Plant Cell* **8**, 2223–2234.

Sheaman D. C. 2002 The evolution of sex determination systems in dipteran insects other than *Drosophila. Genetica* **116**, 25–43.

Shepard P. J. and Hertel K. J. 2009 The SR protein family. *Genome Biol.* **10**, 242.

Shirangi T. R., Dufour H. D., Williams T. M. and Carroll S. B. 2009 Rapid evolution of sex pheromone-producing enzyme expression in *Drosophila*. *PLoS Biol.* **7**, e1000168.

Traut W., Niimi T., Ikeo K. and Sahara K. 2006 Phylogeny of the sex-determining gene Sex-lethal in insects. *Genome* **49**, 254–262.

Wang H. Y., Lin W., Dyck J. A., Yeakley J. M., Songyang Z., Cantley L. C. and Fu X. D. 1998 SRPK2: a differentially expressed SR protein-specific kinase involved in mediating the interaction and localization of pre-mRNA splicing factors in mammalian cells. *J. Cell Biol.* **140**, 737–750.

Werren J. H., Richards S., Desjardins C. A., Niehuis O., Gadau J., Colbourne J. K. *et al*. 2010 Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science* **327**, 343–348.

Williams T. M., Selegue J. E., Werner T., Gompel N., Kopp A. and Carroll S. B. 2008 The regulation and evolution of a genetic switch controlling sexually dimorphic traits in *Drosophila. Cell* **134**, 610–623.

Yun B., Farkas R., Lee K. and Rabinow L. 1994 The *Doa* locus encodes a member of a new protein kinase family, and is essential for eye and embryonic development in *Drosophila melanogaster. Genes Dev.* **8**, 1160–1173.

Yun B., Lee K., Farkas R., Hitte C. and Rabinow L. 2000 The LAMMER protein kinase encoded by the *Doa* locus of *Drosophila* is required in both somatic and germ-line cells, and is expressed as both nuclear and cytoplasmic isoforms throughout development. *Genetics* **156**, 749–761.

Zhai B., Villen J., Beausoleil S. A., Mintseris J. and Gygi S. P. 2008 Phosphoproteome analysis of *Drosophila melanogaster* embryos. *J. Proteome Res.* **7**, 1675–1682.

Zhong X. Y., Wang P., Han J., Rosenfeld M. G. and Fu X. D. 2009 SR proteins in vertical integration of gene expression from transcription to RNA processing to translation. *Mol. Cell* **35**, 1–10.

Received 18 March 2010, in revised form 21 April 2010; accepted 27 April 2010

Published on the Web: 6 September 2010