Dispatches

Sexual Behavior: How Sex Peptide Flips the Postmating Switch of Female Flies

Drosophila **male Sex Peptide elicits an amazing variety of postmating responses in mated females, some of which are transmitted via a receptor on specific neurons of the female genital tract. New work shows that neurons expressing the sex-determination gene *doublesex* (*dsx*) play a pivotal role in the female postmating switch.**

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Males and females differ, anatomically and behaviorally. In the fruitfly *Drosophila melanogaster*, most of the neuronal circuitry responsible for accurate sexual behavior develops under the control of well-studied sex-specific transcription factors encoded by the genes *fruitless*, *doublesex*, and others [1,2]. A paper by Rezaval *et al.* in this issue of *Current Biology* [3] reports that neurons expressing the gene *doublesex* play a crucial role in the females' postmating responses.

Virgin *Drosophila* females lay only a few eggs per day and readily mate when confronted with a courting male. Mating, however, drastically changes the physiology and the behavior of the female [4]: egg-laying is dramatically increased (up to 80 eggs per day per female!) and courting males are vigorously rejected by kicking, fending, decamping or, most effectively, by extruding the ovipositor. A 36 amino-acid-long male peptide, dubbed Sex Peptide (SP), is the major player in eliciting these postmating responses [5,6]. Together with other peptides, SP is synthesized in the accessory glands of the male and transferred during copulation to the female [4]. The actions of SP have been well characterized. Besides inducing increased oviposition and reducing the receptivity of the female, SP has many other effects (Figure 1) [7–10]. How does such a small peptide achieve all these various features?

Biochemical 'dissection' of SP has shown that it contains at least three functional regions, each responsible for different actions in the female [11]. While the amino-terminal part is responsible for juvenile hormone induction and binding to sperm, the hydroxyproline-motif of the middle region is required for inducing the immune system, and the carboxy-terminal end for the changes in oviposition and receptivity, and, very likely, food intake, food choice, siesta sleep and longevity. Hence, SP may be a ligand for several different 'receptor' proteins. Recent NMR studies of the solution conformation of SP revealed a rather flexible peptide that lacks a compact folded structure in solution [12]. This latter finding fits well with the multitasking job of the 'Swiss army knife Peptide' in the mated female (Figure 1).

The carboxy-terminal part of SP is the part that interacts with the Sex Peptide receptor (SPR), a G-protein coupled protein isolated by Yapici *et al.* [13]. Congruous localization of SPR by immunohistology and binding of ^{125}I-SP to cryostat sections of whole mount females was found in many afferent nerves, the ventral nerve cord (VNC) and specific brain parts [7,13]. These findings suggested that all labeled sites may be involved somehow in transmitting the SP signal. However, two groups have recently shown that SP primarily acts via a subset of sensory neurons in the female reproductive system [14,15]. These neurons co-express the sex-determination gene *fruitless* (*fru*) and the proprioceptive neuronal marker *pickpocket* (*ppk*). Thus, only

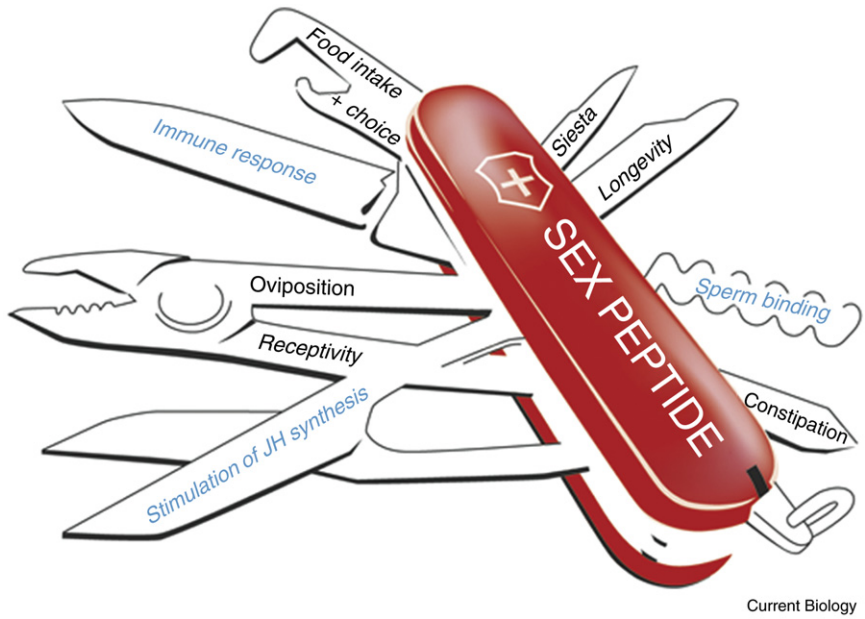

Figure 1. The many functions of Sex Peptide in the mated female.

Sex Peptide induces ovulation, juvenile hormone synthesis, the immune response, constipation, enhanced food intake and reduces receptivity. It also influences food choice, inhibits siesta sleep, and decreases the life span of the mated female. Furthermore, SP binds to sperm and, thus, prolongs the postmating responses for about a week [7–10]. Neuronally transmitted functions are indicated in black. Functions indicated in blue are very likely transmitted via hemolymph, or mediated via other proteins (sperm binding [4]).

a subset of SPR-expressing neurons seems to be involved in relaying the SP stimulus. Besides the neurons described by Häsemeyer *et al.* [14] and Yang *et al.* [15], SP-regulated female sexual behavior requires also a subset of ascending VNC neurons [16]. But little is known about the circuitry that finally leads to the female-specific behavioral outputs in response to SP. Since neurons expressing the sex-determination gene *dsx* play a decisive role in pre- and post-copulatory female sexual behavior [2], Rezaval *et al.* [3] set out to determine its involvement in the SP circuitry.

Expression of a membrane-bound SP (mSP) fusion protein in specific cells of virgin females is sufficient to elicit the postmating responses [17]. Rezaval *et al.* [3] used this approach by targeted expression of mSP under the control of dsx^{Gal4} in virgin females. Such virgins show elevation of egg-laying and reduction of receptivity comparable to mated control females and, as observed in mated wild-type controls, they also show increased extrusion of the ovipositor and reduced locomotion while being courted. The latter leads to a reduced male courting index — males court significantly less as a consequence of the reduced locomotion of their mating partners. Inhibition of the *Gal4*-driven mSP expression in *dsx*-neurons by either *elav-Gal80* or *syb-Gal80* co-expression (both inhibiting mSP expression) led to reversal of the postmating phenotype of the virgin females. Hence, expression of mSP in *dsx*-neurons is sufficient to induce complex postmating behaviors in females, and elicits adequate responses in potential mates.

These results were nicely confirmed by a complementary experiment in which SPR function was knocked-down specifically in *dsx*-expressing cells. As mentioned above, SPR is broadly expressed in the fly central nervous system (CNS) and is needed for mediating the SP responses [13]. Knocking down SPR in *dsx*-neurons by expressing *dsx-Gal4* driven SPR-RNA interference (RNAi) resulted in neither elevated oviposition nor reduced receptivity after mating with wild-type males. On the other hand, expression of SPR in *dsx*-neurons in a SPR-null genetic background restored all postmating responses induced by mating. Thus, SPR function in *dsx*-neurons is necessary and sufficient to respond to the SP-induced signal leading to the postmating responses.

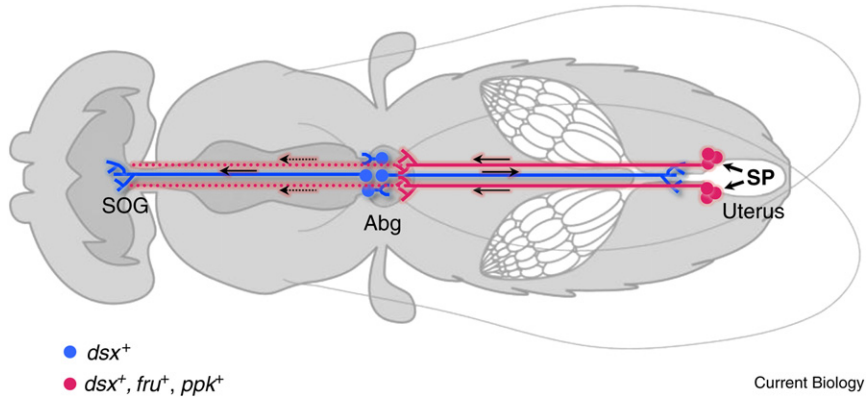

Figure 2. Neuronal circuitry involved in Sex Peptide function.

Primary targets of SP are the $dsx^+/fru^+/ppk^+$ neurons in the genital tract (red dots). Their axons project to two target regions in the central nervous system (CNS), the abdominal ganglion (Abg; red, drawn out) and, possibly, the subsoesophagal ganglion (SOG, red stippled) [14,15]. In addition, Rezaval *et al.* [3] have identified ascending neurons that target the brain, local interneurons and descending neurons innervating the genital tract (blue = *dsx* expression). The primary sites of action of SP are the sensory nerve endings in the lumen of the genital tract (red dots). In addition, SP may act on presynaptic terminals of the SP-sensing neurons in the CNS [14,15] and via modulating input in SP-binding afferent neurons (see text for details) [16]. In addition, enteric neurons (not shown) mediate SP-induced changes in intestinal physiology [9]. (Adapted from [20].)

Do these *dsx*-neurons overlap with the SPR-expressing fru^+/ppk^+ neurons that innervate the lumen of the uterus and the lower oviduct [14,15]? By using two specific molecular markers Rezaval *et al.* [3] demonstrated co-localization of *dsx* in most *ppk* sensory neurons found in the female genitalia. In a similar experiment it was shown that co-expression of *dsx* and *fru* was restricted to three *fru*-neurons on each side of the uterus. Thus, the primary target neurons in the female genital tract were narrowed down to two bilateral clusters of three $dsx^+/fru^+/ppk^+$ neurons (Figure 2).

About 50 neurons distributed in eight clusters in the brain and about 300 neurons of the abdominal ganglion (Abg) express *dsx* [2]. Are they involved in mediating the SP signal downstream of the SP-sensory cluster in the genital tract? Using whole mount preparations of CNSs and entire reproductive systems of dsx^{Gal4}*/UAS-mCD8::GFP* females, Rezaval *et al.* [3] were able to show that a sub-population of *dsx*-Abg neurons sends two bilateral fascicles along the abdominal nerve trunk to ramify on the uterus and vaginal plates (Figure 2). By implementing a sophisticated FLP/FRT strategy, functionally defined subsets of *dsx* neurons were identified that affect only parts of the postmating response. These neurons map in the Abg and show extensive presynaptic arborisations within the posterior brain (Figure 2). Furthermore, two descending fibers of the *dsx*-Abg neurons were found to directly innervate specific areas of the reproductive system, e.g. the uterus (Figure 2). They might direct motor outputs to achieve specific postmating responses, such as ovulation and oviposition.

Is this the whole story? Is SP useless after hitting its receptor localized in the few $dsx^+/fru^+/ppk^+$ neurons in the female genital tract? There are some findings that suggest otherwise. Expression of mSP (see above) in the head of virgin females elicits the postmating responses [17]. Injection of native or synthetic SP into the hemolymph of a virgin female elicits strong oviposition and reduction of receptivity [7,8]. These postmating responses can also be induced by ectopic expression of SP in the fat body of transgenic virgins [18]. Ectopically expressed SP secreted into the hemolymph seems not to enter the calyx and the oviduct [11] — SP secreted into the hemolymph seems not to have access to the terminals of the SP-sensor neurons innervating the lumen of the uterus and the lower oviduct (Figure 2). Indeed, SP has been

found in the hemolymph of mated wild-type females where it is degraded into smaller non-functional peptides [19]. Taken together with the almost ubiquitous distribution of the SPR (see above) to many peripheral neurons and specific parts of the VNC and the brain [13], these findings suggest that SP may have additional effects. The *dsx*$^{+}$*/fru*$^{+}$*/ppk*$^{+}$ neurons are very likely the primary targets. But SP entered into the hemolymph may in addition act by modulating the activity of the SP circuitry by modifying sensory input via binding to afferent nerve axons and/or directly on presynaptic terminals [7,14–16]. Finally, stimulation of juvenile hormone synthesis and inducing the immune response in various organs very likely occurs via SP entered into the hemolymph [7].

In sum, Rezaval *et al.* [3] have identified ascending neurons that target the brain, local interneurons and descending neurons that innervate the reproductive system. All express *dsx* and are involved in the SP response, hence confirming that *fru* and *dsx* are involved in establishing sexual dimorphic features of neural circuitry in fruit flies [1]. This is a major step towards understanding the complex function of a fascinating small male peptide. It will be interesting to learn how *fru* and *dsx* shape the development of the relevant neuronal circuitries.

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Cancer and Inflammation: An Aspirin a Day Keeps the Cancer at Bay

Live imaging of the interactions between oncogene-transformed cells and leukocytes in zebrafish reveals that PGE_2 promotes the survival and proliferation of cancer cells. Non-steroid anti-inflammatory drugs, like aspirin, are the effective inhibitors of PGE_2 production and could be used with other anti-tumor agents in the treatment of cancer.

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The relationship between cancer and inflammation has been a hot subject in research and medicine for centuries. The original observations by Galenius (2000 b.C), Virchow [1] and Dvorak [2] all suggested a close link between inflammation and cancer. As leukocytes are the first line of defense in immune responses, their presence in tumors has been hypothesized to have anti-cancer activity. In recent years, it has become clear, however, that the majority of tumor-associated leukocytes are there to promote tumor growth, tumor angiogenesis, invasion and metastasis. Two years ago, in a paper published in *PLoS Biology*, Feng *et al.* [3] reported that oncogene-transformed cells are able to attract leukocytes immediately after transformation, demonstrating an early trophic support provided by leukocytes to growing tumors. In a new study reported in this issue of *Current Biology*, Feng *et al.* [4], using the same cancer model, now identify the signal that leukocytes provide to cancer cells to promote their survival.

To address these questions, Feng *et al.* [4] used genetic tools and chemical inhibitors to image cancer and inflammation in transparent zebrafish larvae. These authors employed a novel model of oncogene-induced transformation that targets a population of very superficial cells (the mucous-producing cells of the skin). These single cells, closely related to sebaceous gland mucous