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Review

Sex-peptides: seminal peptides of the *Drosophila* male*

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Abstract. Mating affects the reproductive behaviour of insect females: the egg-laying rate increases and courting males are rejected. These post-mating responses are induced mainly by seminal fluid. In *Drosophila melanogaster,* males transfer two peptides (sex-peptides, = Sps) that reduce receptivity and elicit increased egg laying in their mating partners. Similarities in the open reading frames of the genes suggest that they have arisen by gene duplication. In females, Sps bind to specific sites in the central and peripheral nervous system, and to the genital tract. The binding proteins of the nervous system and genital tract are membrane proteins, but they differ molecularly. The former protein is proposed to be a receptor located at the top of a signalling cascade leading to the two post-mating responses, whereas the latter is a carrier protein moving Sps from the genital tract into the haemolymph. Sps bind to sperm. Together with sperm they are responsible for the persistence of the two post-mating responses. But Sps are the molecular basis of the sperm effect; sperm is merely the carrier.

Key words. Behaviour; *Drosophila*; mating; oogenesis; oviposition; receptivity; sex-peptides; sperm.

Introduction

Reproduction is a fundamental characteristic of life. Hence, it is no surprise that evolution has produced a bewildering variety of mechanisms to secure the production of offspring. Many organisms are also astoundingly ingenious in their control of reproductive behaviours. Insects, the species-richest animal class, reflect this situation beautifully [1, 2]. Most female insects carefully adjust their investments in mating and egg production depending upon their reproductive state [3–5]. Whereas virgin females may lay a few eggs, after mating the egg-laying rate is considerably increased. Furthermore, virgin females readily accept males, but mated females reject courting males, i.e. their receptivity is reduced. These so-called post-mating responses (see table 1 for a compilation of terms used in this article) are often induced by substances of the seminal fluid. They are transferred to the female during copulation together with sperm [6]. Both post-mating responses make 'economic sense'. Increased egg laying is appropriate only once sperm are available for fertilization. On the other hand, the activity of a fertilized female, as long as sperm are available, is frequently better invested in the search for food to produce an optimal amount of eggs rather than wasted with additional copulations. Indeed, in *Drosophila melanogaster*, the two post-mating responses are coupled to the presence of sperm; once sperm stores are depleted, the reproductive behaviour of a female becomes 'virgin' again [7, 8].

Seminal proteins and peptides, most of them synthesized in the male accessory glands, additionally affect sperm storage, sperm competition and mating-plug formation, protect the reproductive tract or gametes from microbial attack and have an influence on female life span [for re-

* This article is dedicated to the 85th birthday of the discoverer of the sex-peptide, Prof. Dr. Pei Shen Chen, Zoological Institute, University of Zürich, Switzerland. P. S. Chen has served on the Editorial Board of Experientia (now CMLS) from 1974 to 1988.

Table 1. Some definitions and bioassays [reproduced and modified from ref. 18 with the permission of the publisher.]

Oviposition	Deposition of eggs by the female. Measured as number of eggs laid by the female within a given time period. Standard procedure: 30 females, 24-h time period.
Ovulation	Transfer of an egg from the ovary to the uterus. Measured as percentage of females containing an egg in the uterus. Standard procedure: 30 females. Ovulation is induced by chilling the females on ice, or, if an egg is not spontaneously extruded, by gently squeezing the tip of the abdomen with forceps.
Post-mating responses	The two responses elicited by the sex-peptides: increase in oviposition (or ovulation*) and reduction of receptivity.
Receptivity	Readiness of a female to mate. It is high in mature virgin females, low in immature virgins, and suppressed after mating or injection of sex-peptides. Hence, lack of receptivity can be either due to lack of sexual maturity, or to the effect of sex-peptides. It is measured as the number of individuals copulating within a given time period. Standard procedure: 30 females. Three females each are confronted with seven males for 1 h in a tube containing standard food.
Rejection behaviour	Active refusal of the courting male by the female, observed during male-female encounters. Extrusion of the ovipositor (fig. 1F) is one component of the changes induced in the female by mating which leads to reduced receptivity. Standard procedure: 10 females. Each female is confronted with one male during 15 min and observed. Ovipositor extrusions are counted.
Sex-peptide response cascade	Biochemical processes elicited by sex-peptides from the entrance of the sex-peptide into the female body down to the two postmating reactions: increase in egg-laying rate and reduction of receptivity.

* In wild-type females, oviposition and ovulation are positively correlated. Hence, both are interchangeable as measures of the action of sex-peptides in the female.

cent reviews see refs 6, 9–11]. The 'love potion' transferred by the male affects many fitness traits of both sexes in a complex manner. Thus, investigations of components of the seminal fluid and their effects in females should not only deal with proximate causations ('how questions'), but also with historical and evolutionary factors (ultimate causations, 'why questions'). Indeed, interactions between males and females cannot be fully understood without taking into account both types of question.

Post-mating responses have been observed in females of most species of higher insects. Some of the relevant male substances eliciting the changes in female reproductive behaviour have been identified as have the sources [5, 6, 9, 12]. Thus, potentially, these substances open a door for insect control. In recent years, most progress has been made by studying the synthesis and function of accessory gland proteins and peptides (ACPs) of *D. melanogaster*. The reason is mainly the amenability of this species to genetics and gene manipulation. In a systematic approach, Wolfner and collaborators have identified nearly 100 ACPs [11]. Among them are prohormone precursors, glycoproteins, proteases, protease inhibitors, lipases and many novel proteins. The physiological functions of some of them have been established [10, 11, 13]. Two peptides (sex-peptides, Sps) eliciting the two post-mating responses have been studied in detail by Chen and associates [14] and in the author's laboratory [15]. Since several recent reviews summarize our knowledge about the other seminal peptides and proteins [9–11, 13], this article concentrates on the two Sps: sex-peptide (SP) and ductus ejaculatorius peptide 99B (DUP99B). SP was discovered about 10 years before DUP99B [14, 15], and therefore most experiments have been performed with SP. Thus, the emphasis here will be on this peptide. Results obtained with DUP99B will be mentioned when relevant. In this review SP (ACP70A) refers to the peptide isolated and characterized by Chen et al. in 1988 [14], DUP99B to the peptide recently described by Saudan et al. [15], and Sps to both peptides.

Sexual behaviour of *Drosophila* and the Sps

When injected into the abdomen of virgin *D. melanogaster* females in physiological concentrations, SP and DUP99B induce an increase in egg laying and reduction of receptivity. This is to say they elicit a physiological and a behavioural response as also observed after a normal mating. Injection into the abdomen delivers the peptides into the haemolymph. Hence, the molecular targets of the Sps are very likely accessible via the haemolymph [16–18]. A range of questions arise. How many receptors exist for the two responses? How many target organs? How many signal transduction cascades? Which parts of the peptides are functionally important? Are different domains of the peptides responsible for each of the two post-mating responses? Are SP and DUP99B redundant, and if so, to what degree?

I will first describe the mating behaviour of *D. melanogaster* and the assays developed to track the activity of the two peptides. Then, I summarize what is known about the genes encoding the Sps and their expression. Finally, I describe our current knowledge about homologous peptides in other *Drosophila* species.

The mating ritual

The mating ritual of *D. melanogaster* consists of five major phases: orientation, love song, licking, attempted copulation and copulation (fig. 1 A–E) [19]. In sexually mature wild-type flies, the ritual usually lasts a few minutes and mating about 15–20 min [20–22]. Superficially, the male alone appears to play the active role. However, the female is by no means passive [23, 24]. Detailed studies have shown that female courtship behaviour can be categorized into at least eight elements, and male courtship behaviour into at least nine [25, 26]. Shortly after mating, the females become less receptive, and about 2–3 h afterwards, they start to lay eggs at high rates (up to 70–80 eggs/day for a *D. melanogaster* Oregon R wild-type female). Both post-mating responses last about 1 week until most of the sperm has either been lost or used up for fertilization [7, 8]. Three assays have been developed to asses the post-mating responses [14, 16]. The terms ovulation, oviposition and receptivity are described in table 1 along with the standard procedures for quantitative measurements.

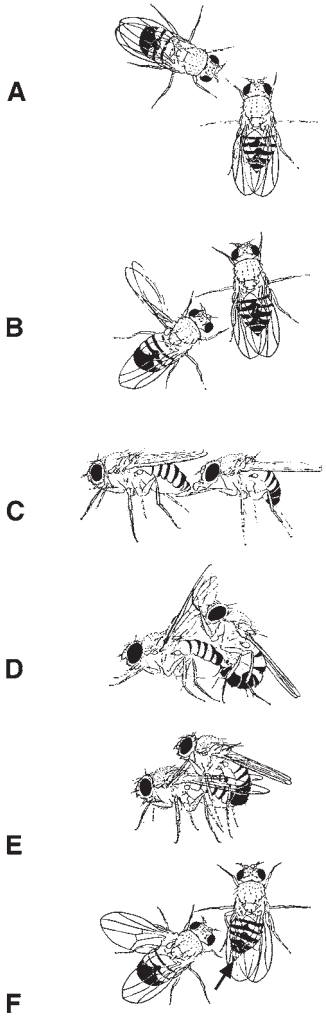

Figure 1. Courtship behaviour in *D. melanogaster*. (*A*) Orientation of the male towards the female (male, left: smaller, and tip of the abdomen black). (*B*) Love song (wing vibration) of the male. (*C*) The male licks the genitalia of the female. (*D*) Attempted copulation. (*E*) Copulation. (*F*) A rejection response by the female. The female turns her abdomen towards the male and extends her ovipositor (arrow). This is one of the typical rejection responses of a mated female and also of a female injected with Sps. [Reproduced from ref. 18 with the permission of the publisher.]

The Sp gene family

Three male peptides affecting egg deposition and/or receptivity have been characterized in *D. melanogaster*. All three are synthesized in the male genital tract. The ACP Ovulin (ACP26Aa) stimulates the release of oocytes from the ovary [27]. It clears the ovary of stage 14 oocytes and thus releases oogenesis from feedback inhibition. Stimulation of oviposition by Ovulin is restricted to the first day after mating [28]. Thus, Ovulin affects ovulation and oviposition. SP and DUP99B, the two peptides investigated in the author's laboratory, elicit both post-mating responses when injected into the haemolymph of virgin females [14, 15]. Ectopic expression of SP in virgin females also induces the post-mating responses either transiently or constitutively, depending on the properties of the promoters driving *SP* gene transcription in the transgenic females [16, 29]. Whereas SP is synthesized in the accessory glands, DUP99B is made in the ejaculatory duct of the male. In addition DUP99B is also synthesized in the cardia of both sexes [14, 15, 30, 31]. Since all three male peptides are transferred as part of the seminal fluid into the female during copulation [31–33], they qualify as sex pheromones (as defined by Karlson and Lüscher [34]).

The genes encoding SP and DUP99B have been cloned and sequenced [15, 30]. Each gene is a single copy and contains an intron at exactly the same site (fig. 2). Both peptides are synthesized via precursors. Upon secretion from the main cells of the accessory glands, SP is cleaved in its N-terminal part and releases a signal peptide of 19 amino acids from the 55-amino-acid precursor. The precursor of the ductus ejaculatorius peptide contains 54 amino acids and is also cleaved in the N-terminal part. Furthermore, the last two C-terminal amino acids (RK) of the DUP99B precursor are also cleaved off (fig. 2).

Mature SP and DUP99B are 36 and 31 amino acids long, respectively. Both have a cyclic C-terminal part (disulphide bridges between amino acids in position 24 and 36 and 19 and 31, respectively) and a linear N-terminal part. In the cyclic parts, 10 out of 12 amino acids are identical. Both peptides were prepared synthetically [15, 17].

High sequence similarity is found in the C-terminal parts of the two mature peptides. The corresponding amino acids are encoded by the second exon of each gene. The signal peptides and the N-terminal parts of the mature peptides are encoded by the first exon. Since the signal

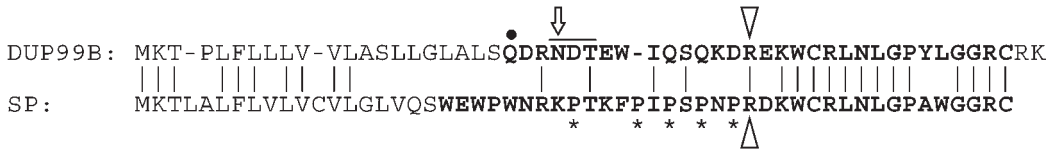

Figure 2. Amino acid sequences of the precursors and the mature peptides (bold). The mature DUP99B contains 31 amino acids, the mature SP, 36 amino acids. The DUP99B precursor contains an N-terminal 21-amino-acid signal peptide and two amino acid residues at the C terminus that are cleaved off during the peptide maturation process. The SP precursor contains a 19-amino-acid signal peptide at its N terminus. The two peptides show high sequence similarities in the N-terminal regions of the signal peptides, and in the C-terminal parts of the mature peptides. Identical amino acids are indicated by vertical bars. Open triangles, sites of insertion of the introns in the genomic sequences; open arrow, glycosylation site in the mature DUP99B peptide; filled circle, pyroglutamine; overlined amino acids, glycosylation consensus sequence; stars, hydroxyprolines. [Reproduced from ref. 15 with the permission of the publisher.]

peptides also share sequence similarity, the two genes are believed to have arisen by gene duplication. Based on this argument and on the partial functional homology of the two peptides (see below), we consider the two peptides as the founding members of the SP pheromone family.

SP and DUP99B are modified peptides, i.e. additional functional groups are added after translation. Mature SP contains five hydroxyprolines and, probably, one hydroxyleucine (fig. 2). DUP99B contains a glycosyl group in the N-terminal region of the peptide (fig. 2). Neither of the modifications is essential to elicit the post-mating responses [15, 17]. For SP, the putative biological function of the amino acid modifications has not been determined. DUP99B glycosylation decreases the amount of peptide needed to elicit ovulation in an injection assay [15]. The presence of the glycosyl group could increase the stability of the native peptide and/or increase the affinity of the peptide for the putative receptor.

The sequences of both Sp genes, their cDNAs and the peptides have been determined for all members of the *D. melanogaster* species subgroup and some other *Drosophila* species [14, 35–38; T. Schmidt and E. Kubli, unpublished data]. The closely related species *D. sechellia* contains a SP with only three amino acid changes [35]. *D. suzukii* also contains a SP homologous to the *D. melanogaster* SP [36]. The *D. suzukii* SP elicits the post-mating responses in *D. melanogaster* and vice versa. But *D. suzukii* males produce an additional ovulation-stimulating substance (OSS) with a sequence partially different from *D. melanogaster* SP [Y. Fuyama, personal communication]. OSS, however, does not reduce receptivity. *D. biarmipes* males synthesize two OSSs [38]. One is synthesized in the accessory gland, the other in the ejaculatory duct. They may correspond to *D. melanogaster* SP and DUP99B, respectively. Cirera and Aguadé [37] have characterized the genes homologous to *SP* in *D. subobscura*. Interestingly, the *SP* gene of this species has recently been duplicated. However, whether both genes are functional is not known.

The sequences of the SPs of all species belonging to the *D. melanogaster* subgroup show two regions of high similarity. The first five amino acids of the N-terminal ends (WEWPW) are identical in all SPs, and the C-terminal parts encoded by the second exon are very similar. All SPs (and DUP99B) contain two cysteines at the same locations, one of which is localized at the very C-terminal end of the peptides. Both conserved regions of SP are functional, as expected. Their functions are described in the following sections.

Structure-function relationships of the C-terminal part of SP

The C-terminal parts of all SPs belonging to the *D. melanogaster* subgroup are highly similar (see above). By injecting SP and its fragments into the abdomen of virgin females, this part has been shown to be is essential for eliciting the two post-mating responses [17]. Removal of the first ten N-terminal amino acids does not affect these responses (see below). However, all fragments lacking the C-terminal end, or with a destroyed disulphide bridge, are inactive. As shown below, the C-terminal region is the part needed for binding of SP to specific sites of the nervous system and the genital tract of females. This also holds for DUP99B, since the two peptides are nearly identical in this region (fig. 2). SP fragments elicit either both or none of the post-mating responses. Dose-response curves show that a critical concentration of about 0.6 pmol/female is needed to elicit ovulation, oviposition or reduction of receptivity [17, 18]. Taken together, these findings suggest that one type of molecular receptor resides on the top of the SP response cascade leading, finally, to the post-mating responses. However, these data do not provide information about the target tissue(s) of SP.

The physiological response

After mating, a *D. melanogaster* female lays about 300 eggs in 1 week, depending on the stock and the rearing conditions [8, 18, 28]. However, ovaries of mature females contain only about 80–100 oocytes of the terminal stage 14. Thus, after mating, oogenesis must be stimulated to sustain oviposition for one week. Since SP is involved, this process has been dubbed the ‘physiological response’ of SP.

Table 2. Dissociation constants K_d and minimal concentrations for binding of SP and DUP99B to the antennal nerve and the uterus.

	Fusion protein	Genital tract	Nervous system
K_d (nmol/l)	AP-SP_{1-36}	0.64	1.07
	AP-DUP99B	14.65	(6.4)
Minimal concentration for binding (nmol/l)	AP-SP_{1-36}	0.5–1	1–2
	AP-DUP99B	5–10	30–40

The affinity of the same peptide differs for the two tissues, and the affinities of the two peptides differ for the same tissue. First two rows: dissociation constants (K_d) for binding of AP-SP to antennal nerve and genital tract, of AP-DUP99B to the genital tract, and, in parentheses, of ^{125}I-labelled DUP99B to the antennal nerve [63]. Third and forth rows: minimal concentrations needed for binding of the fusion proteins AP-SP_{1-36} or AP-DUP99B to the genital tract and the nervous system. Minimal concentrations were determined by calculation of the signal intensities in the genital tract and the nervous system. The minimal concentrations refer to the average signal intensity below 50 (arbitrary units) as calculated with the NIH image program. [Reproduced from ref. 64 with the permission of the publisher.]

Stimulation of juvenile hormone synthesis

Juvenile hormone (JH) is synthesized in the corpus allatum and plays an important role in the oogenesis of many insects. In *D. melanogaster,* JH is important during sexual maturation and for egg production in the sexually mature female [39, 40]. Incubation of isolated *D. melanogaster* corpora allata -corpora cardiaca complexes in vitro with SP stimulates JH synthesis [41]. Stimulation is maximal in complexes isolated from virgin, sexually mature females. Experiments with SP fragments have shown that the N-terminal part of SP is essential [42]. This result is in accord with the finding that DUP99B is not active in this assay. The amino acid sequence of DUP99B differs from SP in the N-terminal part of the peptide (fig. 2). These results allocate a function to the highly conserved N terminus of SP and show, at least with regard to this function, that SP and DUP99B are not redundant. The same N-terminal fragments of SP (SP_{1-11} and SP_{1-22}) also stimulate JH synthesis in isolated corpora allata complexes of the moth *Helicoverpa armigera* [42]. Thus, very likely a similar peptide stimulates JH synthesis in a species belonging to the order of Lepidoptera.

Oocyte progression, yolk protein synthesis and uptake

D. melanogaster oocyte development has been divided into 14 oocyte stages [43]. Early vitellogenesis starts at stages 8 and 9. But considerable growth, combined with a renewed massive incorporation of yolk proteins (YPs), starts at stage 10. Wilson [44] has proposed a control point for vitellogenic oocyte development. He found that JH deficiency causes degeneration of stage 8 and 9 oocytes, while stage 10 oocytes continue to develop to stage 14 without further JH requirement. Three YPs belong to the major protein components of the *Drosophila* egg [45]. The YPs are synthesized in the female fat body and in the follicle cells of the ovary. The YPs of the fat body are secreted into the haemolymph, and those synthesized in the follicle cells are secreted unidirectionally towards the oocyte membrane. Proteins from both sources are taken up by the oocyte membrane by receptor-mediated endocytosis. Since JH and 20-hydroxyecdysone (20E) are believed to play a role in the regulation of the three *yp* genes [45, 46] Soller et al. [47, 48] investigated the role of SP, JH and 20E in oocyte maturation and in YP synthesis and uptake.

Mating, SP injection and methoprene application (methoprene is a JH analogue) all stimulate oocyte progression through the putative control point located between oocyte stages 9 and 10 (fig. 3) [48]). In sexually mature virgin females, oocyte stages 8 and 9 are present in the same numbers as in mated, SP-injected females, or in females treated with methoprene. However, the number of stage 10 oocytes is considerably increased after mating, SP in-

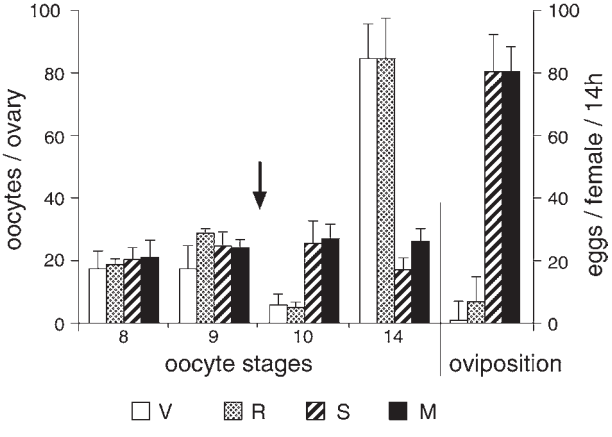

Figure 3. Oocyte stage distribution and oviposition 14 h after mating or SP injection. From the 14 oocyte stages, only stage 8, 9, 10 and 14 are presented. Oviposition is negatively correlated with number of stage 14 oocytes. Control point of oocyte progression between stage 9 and 10 (arrow). Oocyte stages of six pairs of ovaries were determined and are shown as means with the standard deviation for virgin (V), mated (M), Ringer's (R)- or SP (S)-injected females. The oviposition rate is shown as the mean number of laid eggs, with the standard deviation, within 14 h (n = 30). [Reproduced from ref. 47 with modification, with the permission of the publisher.]

jection or methoprene treatment (fig. 3) [48]. In virgin and methoprene-treated females, stage 14 oocytes are abundant, and egg laying is minimal. After mating or SP injection, the number of stage 14 cocytes drops considerably and egg laying increases. Whereas all three treatments induce oocyte progression, only mating or SP injection elicit egg laying [47, 48]. Hence, methoprene induces oocyte progression but not egg laying.

Transcription of the *yp* genes in the fat body is stimulated only moderately above the background level by mating, SP injection or methoprene application [47, 48]. However, uptake of YPs into the ovary and transcription of the *yp* genes in the ovary are stimulated by either treatment (fig. 4 A–E) [47, 48]. Thus, the JH analogue mimics the SP-mediated stimulation of oocyte progression and YP synthesis and uptake. Based on these results, Soller et al. [48] concluded that JH is a downstream component of the SP response cascade. However, as mentioned above, treatment of virgin females with the JH analogue does not induce egg laying nor does it reduce receptivity [48]. Thus, SP must have an additional separate mechanism to evoke the two post-mating responses.

A model for SP action

Injection of 20-hydroxy-ecdysene into sexually mature, virgin females induces apoptosis in stage 9 oocytes [48]. Hence, this hormone acts as an antagonist of JH. Simultaneous application of 20E and methoprene relieves this effect. The balance of the two hormones seems to determine whether stage 9 oocytes progress through the control point or undergo apoptosis. This view is supported by results of Harshman et al. [49], who found that *D. melanogaster* virgins contain more 20E in their haemolymph than mated females. Based on these data, Soller et al. [48] proposed a model for the control of vitellogenic oocyte maturation by SP in virgin and mated females (fig. 5). In sexually mature, virgin females, stage 14 oocytes are abundant. Stage 9 oocytes are resorbed, mediated by the high 20E level in the haemolymph when the JH titre is low. As a consequence, production of new eggs is prevented. YP synthesis in the fat body is continuous and YPs accumulate in the haemolymph of the sexually mature virgins. At mating, SP is transferred and stimulates JH synthesis in the corpora allata. JH stimulates the progression of oocytes through the control point at stage 9, and relieves the effect of 20E (low titre in mated females). The production of mature stage 14 oocytes resumes, and egg laying and reduction of receptivity are induced. Therefore, the corpus allatum seems to be a target for SP. However, since JH neither stimulates egg laying nor reduces receptivity, SP must also have additional targets. Reduction of receptivity involves a complex behavioural change, hence it seems appropriate to consider the nervous system, i. e. that SP causes a 'neuronal response'.

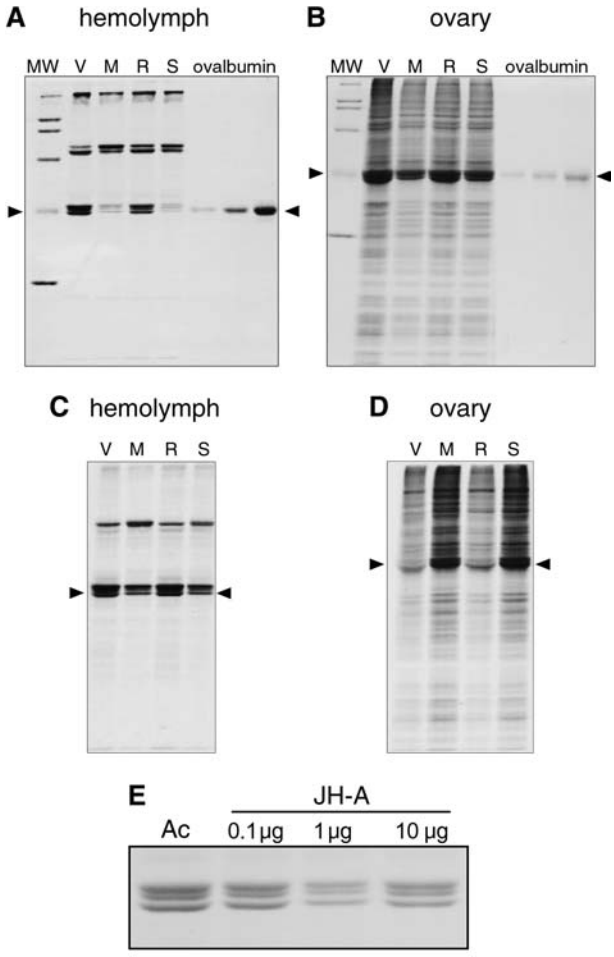

Figure 4. Concentration (*A*, *B*) and synthesis (*C*, *D*) of haemolymph (*A*, *C*) and ovarian proteins (*B*, *D*) after mating or SP injection. Proteins were labelled for 2 h with ^{35}S-methionine: Coomassie staining of proteins (*A*, *B*); autoradiography of the same gels (*C*, *D*). Mated (M) and SP-injected (S) females are compared to virgin (V) or Ringer's (R)-injected females. The three YPs are marked with arrowheads. Molecular weight markers were loaded on the left (MW) and ovalbumin (2.5, 5 and 10 µg) was loaded on the right (*A*, *B*). (*E*) Dose response of haemolymph YPs in methoprene-stimulated animals. The concentration of haemolymph YPs is correlated with the number of stage 10 oocytes. Application of 1 µg of the JH analogue (JH-A) methoprene results in a maximum of stage 10 oocytes [48] with a maximum uptake of YPs from the haemolymph into the ovary. The consequence is a minimal concentration of YPs in the haemolymph. Ac = acetone control. [See refs. 47 and 48 for experimental details. Reproduced from refs. 47 and 48 with the permission of the publishers.]

The neuronal response

Involvement of cyclic AMP and the mushroom bodies in the Sp response cascade

Females of the *D. melanogaster* mutant *dunce* have been reported to mate at least twice as often as females of wild-type stocks [49]. The gene *dunce* encodes a cAMP-specific phosphodiesterase II, leading to an elevated cAMP

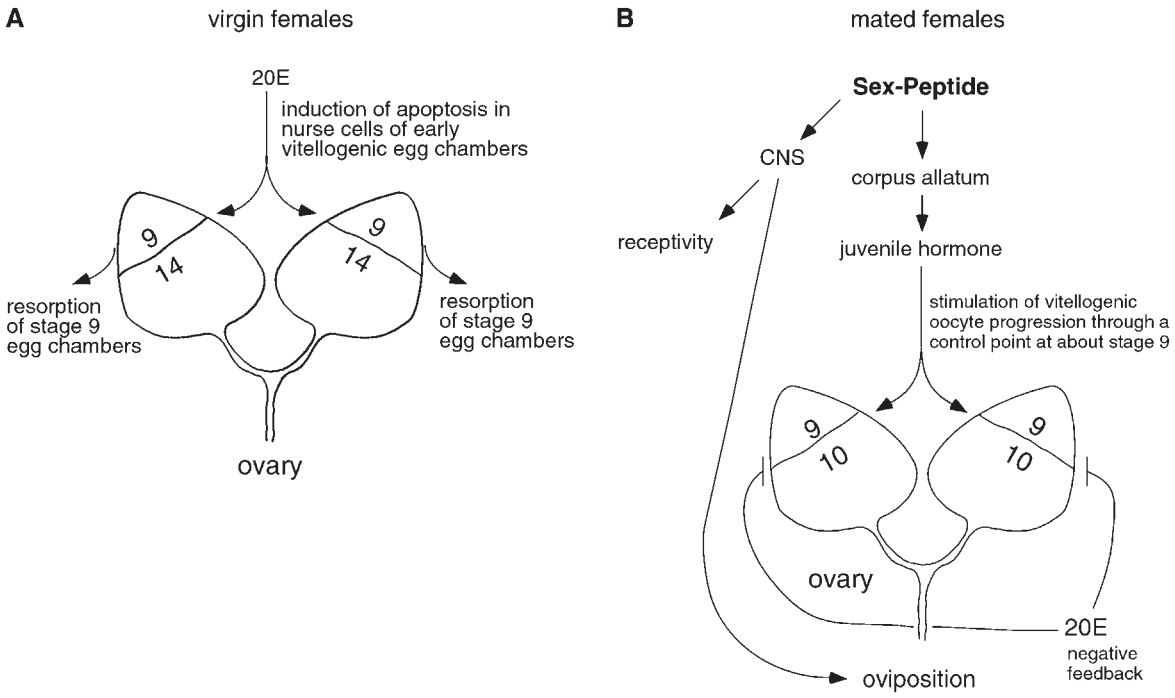

Figure 5. A model for the control of vitellogenic oocyte maturation in sexually mature virgin females (*A*) and after SP is provided by mating (*B*). (*A*) Once the ovary is filled with stage 14 oocytes during sexual maturation, supernumerary oocytes are removed in virgin females by 20E-mediated resorption at early vitellogenesis before oocytes enter the rapid growth phase. In virgin females, 20E levels are high in the haemolymph and low in the ovary [49]. (*B*) After mating, SP stimulates JH synthesis in the corpus allatum [41] and 20E levels are lowered in the haemolymph [49]. Progression of vitellogenic oocytes is then stimulated by JH, including an increased uptake of yolk proteins from the haemolymph and an increased synthesis of YPs in the ovary. JH also protects early vitellogenic oocytes from 20E-mediated resorption. Since in the presence of JH, elevation of 20E levels in the haemolymph slows down stage 10 development, under unfavourable conditions, e.g. starvation, 20E may function as a negative feedback signal. In the ovary of mated females, 20E levels are high [49] and thus 20E may also be necessary for further oocyte development as it is for pre-vitellogenesis. Since the JH analogue methoprene neither induces oviposition nor reduces receptivity, SP must have additional targets. Simultaneous application of SP and 20E to virgin females does not reduce oviposition, hence 20E does not act as a negative feedback signal on oviposition. [Reproduced from ref. 48 with the permission of the publisher.]

level in *dunce* mutants [50, 51]. Other abnormalities exhibited by *dunce* mutants include defects in associative and non-associative learning [51, 52], memory deficiency [53] and female sterility [51]. Bellen and Kiger [54] suggested that the *dunce* memory defect may be responsible for the increased mating rates, possibly as a result of a failure to recall the previous mating. However, *dunce* females remate 30 min after SP injection [55]. If the loss of the receptivity response was due to the *dunce* memory defect, then one might have expected a shortened, but initially still present response. Furthermore, *dunce* females showed no receptivity inhibition 90 min after SP injection [55]. Thus, the elevated mating rate of *dunce* females can be attributed at least in part to their inability to respond to SP.

The receptivity response of *dunce* females can be restored by ectopically expressing a *dunce* wild-type gene in transgenic females shortly before SP injection [56]. Injection of SP into these females reduces their remating rate. The partial restoration of the receptivity response in transgenic flies by expressing the cAMP phosphodiesterase II wild-type gene immediately before SP injection suggests that the enzyme function is needed at a physiological level, and not during development. Taken together, these results suggest that cAMP may be involved in the SP response cascade. If SP acts as a peptide hormone, it may be unable to trigger the 'second messenger' system of the cells in *dunce* females because of the already elevated levels of intracellular cAMP. The elevated cAMP level may render the cells unable to process the SP signal either because of detection problems at the receptor end, or because of habituation problems at the effector end [55, 56].

The *dunce* wild-type gene is complex and large [57]. It is expressed in many tissues, predominantly in the paired mushroom bodies (MBs) of the brain [58]. The MBs are involved in olfactory learning and memory [59], in visual learning [60] and in courtship behaviour [61]. Since the SP-induced receptivity response is affected in *dunce* mutants, Fleischmann et al. [56] investigated the role of the MBs in the SP response cascade. Each MB is made up of about 2400–3000 Kenyon cells [59]. Due to a devel-

opmentally strictly regulated dividing pattern of neuroblasts, the Kenyon cells can be almost completely ablated by feeding hydroxyurea (HU) to first-instar larvae (fig. 6A, C, D) [59, 62]. MB-ablated virgin females were injected with SP and subsequently assayed for ovulation, oviposition and reduction of receptivity [56]. Virgin females with chemically ablated MBs respond normally to SP injection: oviposition is increased, and receptivity is reduced as observed in the untreated controls. Hence, the MBs are not needed for a normal SP response. However, the background egg-laying rate in MB-ablated virgins was elevated above the level of egg laying observed in untreated virgins (fig. 6B, upper part). Ovulation, however, was not affected. In MB-ablated females, injection of SP further increases the egg-laying rate. Thus, although the MBs are not involved in the SP response cascade, the MBs suppress egg laying in virgin females. The model based on these data assumes that SP controls oviposition at two levels (fig. 6B, lower part) [56]. In untreated females (= 'normal' situation), SP relieves the MB-dependent suppression of egg laying and additionally stimulates oviposition in an MB-independent way via the SP response cascade. In HU-treated females, the MB-dependent suppression of egg laying in virgins is absent. Hence, the elevated virgin egg-laying rate. But application of SP can still stimulate oviposition via the MB-independent SP response cascade. These findings suggest that the SP response cascade does not contain a 'memory component'.

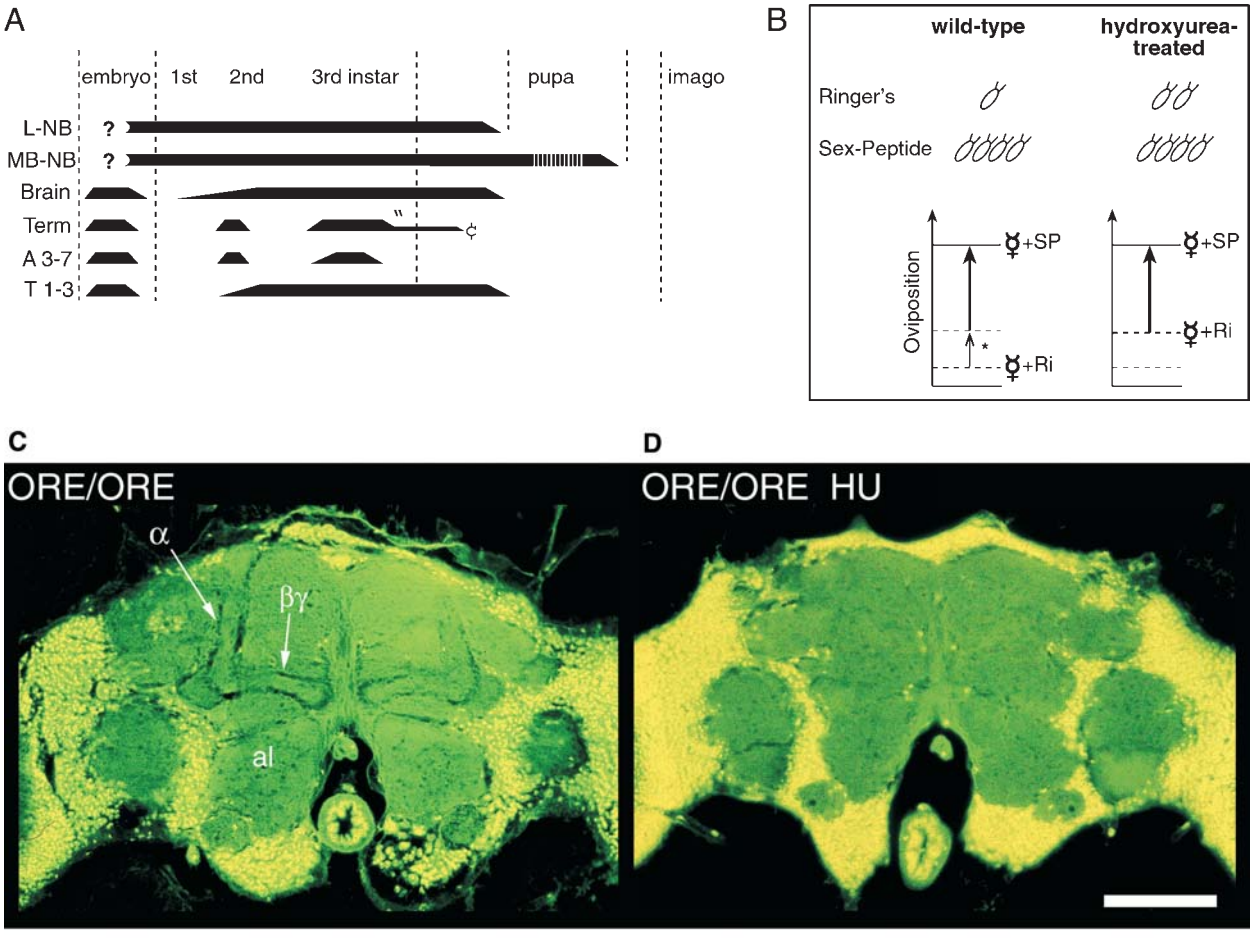

Figure 6. Ablation of the MB does not influence the post-mating responses, but enhances the virgin egg-laying rate. (*A*) Scheme of the ablation experiment. Neuroblasts divide only at specific times during development. During the first larval instar only lateral (L-NB) and MB neuroblasts (MB-NB) divide. Feeding HU during this stage kills dividing cells, resulting in adult flies lacking the MBs. (*C*, *D*) Frontal sections through brains of females raised on normal (*C*) and HU-containing (*D*) food. Arrows point to the α and $\beta\gamma$ lobes of the MBs; al, antennal lobe. The MBs are lacking in the flies raised on HU-containing food. Bar, 50 µm. (*B*) Above, schematic representation of the control of oviposition in wild-type and HU-treated flies. Number of eggs symbols are proportional to oviposition rate. Below, interpretation based on our results [56] and the results of Boulétreau-Merle [82]. Egg laying is under dual control by repression and activation. In untreated wild-type flies, SP derepresses the virgin egg-laying activity (↑*) and additionally activates oviposition (↑). In HU-treated wild-type flies, the background egg laying is elevated. Injection of SP further stimulates egg laying up to the level of the untreated SP-injected flies. Horizontal, bold, broken line: oviposition observed after Ringer's injection. Horizontal, bold, drawn-out line: oviposition observed after SP injection. [Reproduced and modified from refs. 56 and 83 with the permission of the publishers.]

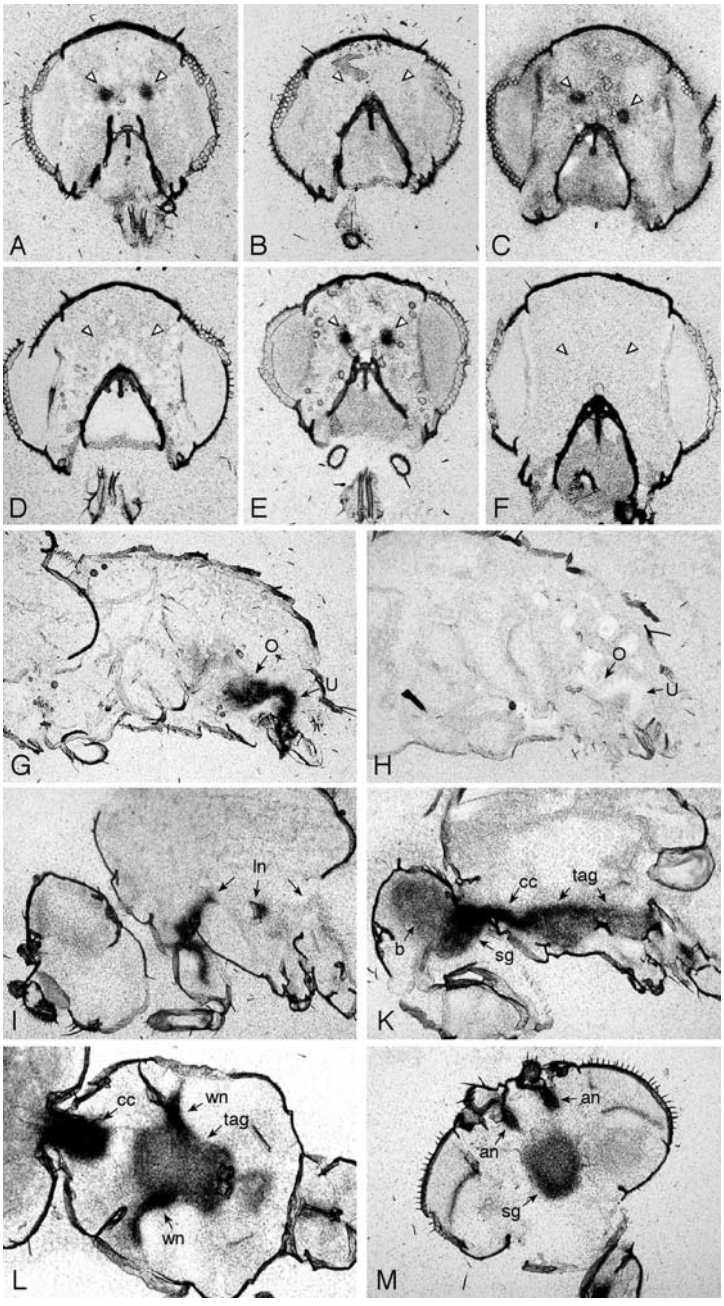

Figure 7. Localization of proteins binding Sps. Cryostat sections of adult females were incubated with ^{125}I-labelled peptides and subsequently autoradiographed. Specific parts of the central and the peripheral nervous system and the genital tract are labelled. SP and DUP99B bind to the same sites. For specific section planes see figure 8. (*A*–*F*) Frontal sections, plane a–a in figure 8. (*A*) Incubation with ^{125}I-DUP99B. The antennal nerve is labelled (open triangles). (*B*) Competition of ^{125}I-DUP99B with a 200-fold excess of cold DUP99B peptide. The label is absent. (*C*) Binding of ^{125}I-Y-SP to the antennal nerve. Note the high background specific for ^{125}I-Y-SP incubations. (*D*) Competition of ^{125}I-DUP99B with a 200-fold excess of cold SP. No label is observed, i. e. the two peptides compete for the same site. (*E*) DUP99B binds with its C-terminal part. A 200-fold excess of an N-terminal fragment of DUP99B ($DUP99B_{5-17}$) does not prohibit binding of full-size ^{125}I-DUP99B to the antennal nerve. (*F*) ^{125}I-DUP99B does not bind to the antennal nerve of *D. funebris*. This is consistent with the finding that SP does not elicit the post-mating responses in females of this species. (*G*, *H*) Longitudinal sections through the abdomen of a female. (*G*) Incubation with ^{125}I-DUP99B labels the uterus (U) and parts of the oviduct (O). (*H*) Competition of ^{125}I-DUP99B with a 200-fold excess of cold DUP99B peptide. The label is absent. (*I*, *K*) Longitudinal sections through heads and thoraces. The section planes are in the plane of the drawing in figure 8. (*I*) Longitudinal section off the midline. The leg nerves (ln) are labelled. (*K*) Longitudinal section through midline. Cervical connective (cc), suboesophageal ganglion (sg), brain (b), and thoracal-abdominal ganglion (tag) are labelled. (*L*) Horizontal section of thoraxin plane b–b in figure 8. (*M*) oblique section of the head in plane c–c in figure 8. In the thorax section, the cervical connective (cc), the wing nerve (wn), and the thoracal-abodominal ganglion (tag) are labelled. In the head section, the antennal nerve (an) and the subesophageal ganglion (sg) are labelled. [Reproduced and modified from ref. 63 with the permission of the publisher.]

Targets of the Sps in the nervous system

From the experiments described so far we can conclude that (1) the MBs are not part of the SP response cascade, but they control the level of egg laying in virgin females; (2) cAMP is very likely a component of the SP response cascade since its regulation is needed at a physiological level, and not during development; (3) putative target tissues are the corpora allata and the nervous system; (4) although SP targets more than one tissue, there exists probably only one type of molecular receptor for SP at the top of the signalling cascade eliciting the two post-mating responses. I will now describe approaches that have led to the identification of additional putative target tissues for SP and DUP99B.

The localization of proteins binding Sps at the tissue level was investigated by incubating cryostat tissue sections of females with radio-labelled ligands (fig. 7) [63]. Synthetic DUP99B and a synthetic analogue of SP with an additional tyrosine at the N terminus (Y-SP) were used for iodination giving ^{125}I-DUP99B and ^{125}I-Y-SP, respectively (native SP does not contain tyrosine). The iodinated Sps were shown to be biologically active. After incubation of the cryostat sections with the radio-labelled peptides, the slides were washed and autoradiographed. Although incubations with iodinated Y-SP and DUP99B yielded the same results (compare fig. 7A with 7C), incubations with ^{125}I-Y-SP resulted in a higher background (fig. 7C). Therefore, most experiments were done with labelled DUP99B. Labelling of specific parts of the central and peripheral nervous system and of the genital tract was observed (fig. 7A–E, G–M).

In the central nervous system, strong labelling was observed in the suboesophageal ganglion (fig. 7K, M), the cervical connective (fig. 7K, L) and in discrete parts of the thoracal-abdominal ganglion (fig. 7K, L). In the peripheral nervous system, labelling was detected in the proximal parts of leg nerves (fig. 7I), the wing (fig. 7L) and haltere nerves, and the antennal (fig. 7A, M), labial and accessory pharyngeal nerves.

The two peptides compete for the same binding sites on the cryostat sections (fig. 7D). This finding suggests that they bind with their very similar C-terminal parts. This view is supported by the fact that addition of a 250-fold-excess of an unlabelled N-terminal fragment of DUP99B does not compete with full-size iodinated DUP99B (fig. 7E). Analysis of the binding of ^{125}I-DUP99B to antennal nerves with Scatchard plots yielded a dissociation constant K_d of 6.4 nM/l, i.e. a value in the range expected for peptide hormone-receptor interactions. Since bioassays have shown that the C-terminal ends of the two peptides are essential for eliciting the two post-mating responses, we have very likely identified the targets of the Sps in the nervous system. A similar binding pattern was also found in male sections [63], but no binding was found to sections of *D. funebris*, a *Drosophila* species not responding to injection of *D. melanogaster* SP (fig. 7F). In sum, both peptides bind to specific parts of the central and the peripheral nervous system, very likely to the receptors located at the top of the SP response cascade leading to the post-mating responses.

The corpus allatum is very likely a target of SP, but labelling of this organ was not observed with iodinated SP. This could be due either to the introduction of an additional tyrosine at the N terminus of the iodinated peptide (the N terminus of SP is responsible for eliciting JH synthesis and, thus, expected to be the part of SP binding to the corpus allatum), and/or to the high background observed in all incubations with labelled SP (fig. 7C). The high background labelling could mask a putative binding to the tiny corpus allatum. Iodinated DUP99B does not label this tissue. This is in accord with the finding that DUP99B does not stimulate JH synthesis in vitro [42].

The maturation of the SP response system occurs in specific steps [41]. Although mature eggs are present in the ovary and are eventually laid after about one day, SP injection about 38 h after eclosion from the pupa can only partially induce ovulation. SP injection can reduce receptivity completely only 72 h after eclosion. These data correlate well with the finding that the binding sites in the nervous system appear at specific developmental stages [63]. Measurements of the labelling intensity at the antennal nerve at specific time points revealed that no labelling above background is present immediately after eclosion. Only 36 h after eclosion, the labelling intensity attains the same level as in 5-day-old sexually mature females. Since the complete number of axons in the antennal nerve is very likely present before eclosion, the increasing labelling intensity may reflect the accumulation of binding proteins during sexual maturation. In other parts of the fly, some neuronal binding sites appear immediately after eclosion. But the complete pattern is also established only 36 h afterwards. Thus, the development of the binding pattern of the nervous system shows a strong correlation with the maturation of the SP response cascade as determined by SP injection.

In the genital tract of females, the iodinated peptides label the uterus and the oviduct (figs 7G, 8). Hence, Sps probably encounter binding proteins immediately after entering the female genital tract. In contrast to the binding sites in the nervous system, these binding sites appear in the pupal stage P15. This finding suggests that the binding proteins may not be the same in the nervous system and the genital tract. This idea is corroborated by the results of experiments described below.

Sps bind to two molecularly different targets

Using fusion proteins containing alkaline phosphatase (AP) as a tag and SP or DUP99B as ligands, Ding et al. [64] extended these studies. AP was fused N-terminally to

the Sps localized at the C-terminal end of the fusion protein or vice versa. These chimeric proteins were used as probes for the incubation of cryostat sections. The sites of binding were determined by the very sensitive cytochemical detection of the enzymatic activity of the AP. Incubations with AP-SP and AP-DUP99B probes basically confirmed the results obtained with the iodination method. Thus, the two methods yield very similar results (fig. 8).

To further biochemically characterize the binding proteins in the nervous system (antennal nerve as the reference tissue) and the genital tract (uterus as the reference tissue), we determined the dissociation constants K_d and the minimal concentrations needed to obtain a signal on cryostat sections for both peptides (table 2) [64]. Taken together we found that the affinity of the same peptide differs for the two tissues, and the affinities of each of the two peptides differ for the same tissue. SP binds with higher affinity to the nervous system and to the genital tract. This finding suggests that SP may be more important than DUP99B in eliciting the post-mating responses.

Earlier studies of structure-function relationships with synthetic SP and its fragments had revealed that the C-terminal part is essential for eliciting the post-mating responses [17]. Ding et al. [64] constructed AP-SP fusion proteins containing different fragments of SP at the C terminus and also a protein containing SP at the N terminus of the fusion protein (SP-AP; table 3). These probes were used to determine the binding patterns and signal intensities on cryostat sections of females as described above. Two major conclusions can be drawn from the results. First, labelling of the nervous system is only possible with AP-SP probes containing SP fragments that also show activity of their own in the above-mentioned structure-function experiments (table 3). Second, binding in the genital tract was also observed with fusion proteins containing either a modified SP (terminal cysteine replaced by alanine) or fragments that did not elicit the post-mating responses in the structure-function assay. Although labelling was less prominent with these probes, the results indicate that binding in terms of amino acid specificity is less demanding in the genital tract than in the nervous system. This is a further indication that the properties of the binding proteins in the nervous system and the genital tract may not be identical.

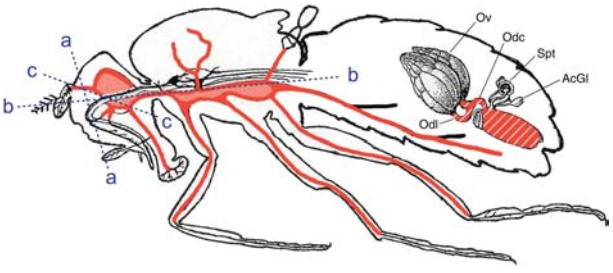

Figure 8. Schematic overview of binding sites of SP and DUP99B in the nervous system and the genital tract. The three section planes of figure 7 are indicated. Dark red, strong labelling; light red, weak labelling; black, no label. Based on biochemical and binding data (see text for details), the binding to the nervous system very likely represents binding to a receptor, whereas binding to the genital tract (hatched) reveals the presence of a carrier protein for SP and DUP99B. AcGl, female accessory gland; Odc, common oviduct; Odl, left oviduct; Ov, ovary; Spt, spermathecae. [Reproduced and modified from refs. 63 and 64 with the permission of the publishers.]

This view was further corroborated by a biochemical approach using affinity blots [64]. Cytosolic and membrane extracts were prepared from the abdomen and the head plus thorax fraction of sexually mature virgin females. The proteins were separated on a non-denaturing polyacrylamide gel and blotted onto a nitrocellulose filter. The filters were subsequently incubated with the AP-SP probe. Two proteins were detected in the membrane fractions. The proteins binding the AP-SP probe differ in their molecular properties, but since the gel was not denaturing, whether this is due to differing molecular weights and/or modification is not known. Since both proteins appear in the membrane fraction, they are very likely transmembrane proteins. The protein of the head plus thorax fraction probably corresponds to the binding protein of the nervous system, and the protein of the abdomen to the binding protein present in the genital tract (fig. 8). Their appearance at different stages of development, and the various binding studies with AP fused to SP and different SP fragments, respectively, suggest that the two proteins may also have different functions.

Interpretation of the binding studies

Based on these results, we conclude that two membrane proteins with differing affinities for the two Sps are located in the nervous system and the genital tract. The radio-labelled probes and the AP fusion proteins bind specifically to precise sites in the nervous system (fig. 8). Thus, the expression sites of putative receptors have very likely been localized [for a full discussion and a critical evaluation of the applied methods, see refs 63 and 64]. The labelling patterns along the peripheral nerves might indicate a common function of the labelled nerves, e.g. modification of sensory input (see below).

How do these results match with earlier findings of other laboratories? Experiments with gynandromorphs have shown that the focus for egg laying is localized in the thoracic ganglion [65], whereas the focus for receptivity is localized in the dorsal-anterior part of the brain [66]. Thus, the post-mating responses elicited by the two Sps may be controlled by two different centres. But these regions could also participate indirectly in the SP response cascade. The focus for receptivity contains cell bodies with axons projecting into the MBs [59, 67, 68]. However, chemical deletion of the MBs does not affect the SP responses. This finding is consistent with the lack of la-

Table 3. Binding of AP-SP, SP-AP and AP-Acp26Aa fusion proteins to the genital tract and the nervous system.

Fusion protein	Binding on genital tract	Binding on nervous system	Biological activity of synthetic peptide fragments
AP-SP_{1-36}	++++	++++	yes
AP-SP_{8-36}	++++	++++	yes
AP-SP_{11-36}	++++	++++	yes
AP-SP_{1-36A}	++++	–	no
AP-SP_{21-36}	++++	–	no
AP-SP_{8-23}	++	–	no
AP-SP_{25-36}	+	–	no
SP_{1-36}-AP	++	–	no
AP-Acp26Aa	–	–	nd

The colum on the right indicates the biological activity of synthetic SP and its fragments [data taken from ref. 17 and unpublished results]. Binding to the nervous system is only possible with biologically active peptides.
++++, strongest signal, signal intensity is over 200 (arbitrary units); ++, signal intensity between 100–150; +, signal intensity below 50. Signal intensities were calculated with the NIH program. SP_{1-36A}, 36th amino acid cysteine is changed into an alanine; SP-AP, C-terminal part of SP has been fused to the N-terminal part of AP. Two different fragments of Acp26Aa were used. They generated the same result, hence, Acp26Aa refers to both fragments: 1–264 (full length) and 118–264. [Reproduced from ref. 64 with the permission of the publisher.]

belling of this brain structure with iodinated peptides. Involvement of the brain was also shown by the elegant experiments of Nakayama et al. [29]. Unfortunately, their methodological approach did not allow specification of the relevant brain structures.

Virgin and mated females are confronted with the same stimulus, the courting male, but they respond with opposite behaviours. How do these peptide pheromones induce this behavioural switch in the female? The foci for female post-mating behaviours, as mapped by the gynandromorph experiments mentioned above, probably represent neuronal circuits involved in the integration of sensory input and/or the execution of behaviours related to the sexual state. They could induce behavioural or physiological changes, as for example neuronal circuits mapped as sites of sex hormone action in rodents [69, 70]. At present, we cannot exclude that in *Drosophila* the Sps act on discrete neuronal circuits. However, binding to the proximal parts of peripheral nerves may indicate that they act differently. For example, they could modulate sensory input globally, rather than act at the foci mapped by gynandromorph experiments, where we did not detect increased labelling. In this way, the same stimuli could result in an altered behavioural response of the female, depending on the presence or absence of the Sps (e.g. engagement in mating versus rejection of courting males in virgin and mated females, respectively). This view is supported by an observation involving a behavioural change elicited by mating in the medfly *Ceratitis capitata* [71]. Virgin female medflies are attracted by male pheromones. After mating, they prefer the scent of mature guave fruit. This odour preference switch can be elicited by injecting extracts of accessory glands prepared from *C. capitata* males into virgin females. The responsible substance may be a SP-like molecule modulating the olfactory input in mated females. However, whether the *D. melanogaster* Sp pheromones modify sensory input or act on neuronal circuits awaits further molecular and neuronal characterization of the involved signalling mechanisms.

In the genital tract, the uterus and the oviduct are labelled (figs. 7G, 8). The values of the K_ds, the minimal concentrations needed to label these tissues, the AP-SP fragment labelling and the results of the affinity blots all suggest that the binding proteins in the female genital tract are molecularly different from the binding proteins characterized in the nervous system. Monsma et al. [72] have shown that accessory gland proteins can enter the haemolymph in *D. melanogaster* females. Thus, the identified binding sites in the genital tract could indicate the presence of a carrier protein. This view is supported by the above-mentioned results, especially by the observation that the binding protein of the genital tract is less demanding in terms of amino acid sequence specificity than the binding sites in the nervous system. At present, however, we cannot exclude the possibility that the genital tract may possess receptors of its own, eliciting, for example, ovulation and/or oviposition without the involvement of the nervous system.

In sum, we suggest that the binding in the nervous system reflects the localization of a receptor for the two peptides residing at the top of a signalling cascade eventually leading to the two post-mating responses. The binding in the genital tract locates the presence of a carrier protein responsible for the efficient transport of the peptides into the haemolymph from where they can reach their targets. This interpretation is consistent with all other findings reported in this review. Figure 8 summarizes the binding studies and the conclusions from other experiments mentioned above.

The role of sperm in the activity of Sps: the sperm effect

The post-mating responses last about 1 week after a mating of wild-type flies (= long-term effect) [7, 8, 73]. However, copulations with XO males, or with males lacking a germ line, induce the two responses only for about 1 day (= short-term effect) [73]. XO males and males without a germ line produce and transfer seminal fluid, but not sperm. Based on experiments with XO males, Manning [7] concluded that sperm were needed for the persistence of the post-mating responses. Hence, he dubbed his finding the 'sperm effect.' Injection of physiological amounts of SP or DUP99B (3 pmol/female) also elicits responses for about 1 day [32]. How are the Sps involved in the short- and long-term effects? Very likely, Sps contribute to the short-term effect, since XO males elicit both post-mating responses, but only short-term. In addition Ovulin elicits oviposition on day 1 [28]. Two hypotheses can be envisaged concerning a putative role of Sps and sperm in the long-term effect. (1) The Sp (and Ovulin) are only involved in the short-term effect. Sperm alone are responsible for the long-term effect (e. g. by stimulation of nerve endings in the female genital tract as observed in some Lepidoptera). (2) The Sps are involved in both effects. Sperm bind the two peptides but act only as a carrier. They have no activity of their own. Once arrived in the genital tract, sperm release the Sps which reach their targets via haemolymph.

Recent work in our laboratory supports the second hypothesis [S. Büsser, H. Liu, J. Peng and E. Kubli, unpublished data]. Males containing a deletion for the *DUP99B* gene elicit post-mating responses that are not significantly different from the responses induced by wild-type males. Since lack of DUP99B does not reduce the extent of the post-mating responses, its contribution must be minimal. This finding indicates that SP is the more important peptide. Support for this conclusion was also obtained by studying males lacking functional SP. Males containing a mutant *SP* gene (SP^0 males) were produced by the technique of targeted mutagenesis developed by Golic [74, 75]. After mating with an SP^0 male, oviposition is only slightly elevated on the first day (due to the presence of DUP99B and Ovulin), and the receptivity is strongly reduced only in the first 4 h after mating (due to the presence of DUP99B). Since sperm are transferred in normal quantities, the following conclusions can be drawn. (1) SP is the major agent eliciting the short-term and the long-term effect. Ovulin and DUP99B have only a minor effect on day 1. (2) Although sperm are needed for the long-term effect, they are very likely only the carrier for SP and have no effect on their own.

The latter conclusion is further supported by experiments studying the binding of the Sps to sperm with antibodies directed against specific epitopes of SP and DUP99B. Sperm were isolated from the seminal receptacles of mated females at various time intervals after mating and incubated with antibodies specific for SP or DUP99B. The N terminus of SP is responsible for binding to sperm [76]. Immediately after mating, SP binding is observed at the head and tail of sperm [S. Büsser, J. Peng and E. Kubli, unpublished data]. This pattern lasts about 5 h, but thereafter, the label on the tail is continuously lost. Five days after mating, SP is only found on the head of sperm. DUP99B also binds to sperm. Immediately after mating, binding of DUP99B is observed on the sperm head. But 5 h after mating, all the label has gone [J. Peng and E. Kubli, unpublished results]. This finding indicates that DUP99B is not involved in the long-term effect and is in accord with the results obtained with mutant males lacking DUP99B or SP (see above).

Taken together, we conclude that Ovulin and DUP99B play only minor roles in the short-term effect. Very likely, the short-term effect is mainly elicited by SP entering the haemolymph immediately after being transferred into the female. The long-term effect is probably induced by SP continuously released from sperm stored in the female genital tract. From there it would be transported into the haemolymph and reach its targets, e. g. the corpus allatum and the binding sites in the nervous system. After about 1 week, sperm are either lost or used up for fertilization and, as a consequence, the post-mating responses disappear and the female regains the 'virgin' state.

What is the ultimate function of SP binding to the tails of sperm? Multiple mating of females can result in fitness costs for both sexes [77]. *D. melanogaster* females caught from the wild contain sperm from four to six different males [78]. Hence, they reduce male fitness through sperm competition [79] and thus sexual conflict arises. As countermeasures, *Drosophila* males have evolved mechanisms that reduce the female lifespan [80] and the tendency to remate. Since the duration of the post-mating responses is dependent on the amount of SP transferred [18], it is in the males' interest to transfer as much SP as possible. The persistence of the long-term effect is very likely dependent on the amount of SP bound to the sperm tail. Hence, selection may have favoured long sperm tails carrying SP and other male substances increasing the reproductive fitness of the *D. melanogaster* males. This could explain the excessive length of the sperm tail in some *Drosophila* species. The 3-mm-long *D. bifurca* male, for example, produces sperm with a 58-mm-long tail [81]!

Conclusions and final remarks

Research on SP and DUP99B was initially started by a search for male peptides eliciting the two post-mating responses [14, 15]. Sequence analysis showed that the two

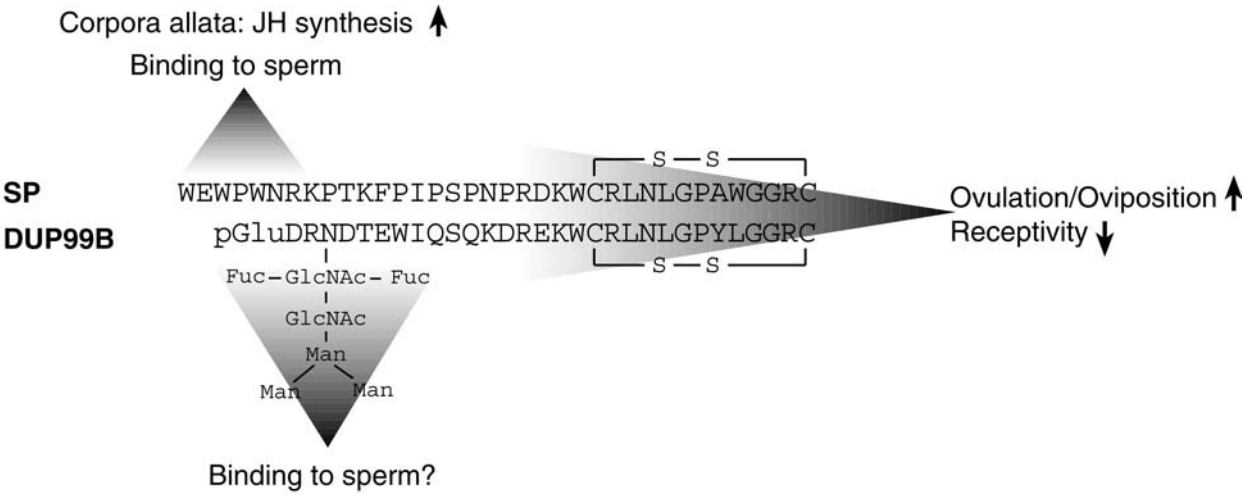

Figure 9. Compilation of known and assumed functions of DUP99B and SP. Results from in vitro and in vivo experiments. Some of the functions may be shared by the two peptides but based on different structures, some may be performed by both peptides with almost identical structures and some functions are unique to SP. SP is the more important peptide eliciting the two post-mating responses. Shaded, regions of functional importance. [Reproduced from ref. 15 with the permission of the publisher.]

isolated peptides share high amino acid similarity at the C-terminal ends of the peptides. Not surprisingly, this also turned out to be the essential part of the peptides responsible for eliciting the two post-mating responses. These findings suggested functional redundancy of the peptides. However, further experiments showed that the situation is more complex. Figure 9 summarizes our present knowledge about the roles of Sp. Some of these data have been obtained by in vitro experiments and so may not reflect the in vivo situation, because we do not know whether the two peptides reach the same targets in vivo. Taken together, the experimental evidence shows that some functions are unique to one peptide, and some functions may be shared by the two peptides but are based on different molecular structures. Finally, some functions may be performed by both peptides with almost identical structures. However, at least with respect to the post-mating responses, the major contribution is provided by SP.

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