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Fates and targets of male accessory gland proteins in mated female *Drosophila melanogaster*

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Abstract

Male accessory gland proteins (Acps) in *Drosophila* are components of the seminal fluid and are transferred to females during copulation. In mated females, Acps enhance egg production, augment sperm storage, induce refractory mating behaviors, and affect the female's longevity. To address the functions of eight previously uncharacterized Acps and further analyze five others, we determined the tissues to which they target after transfer to females. Each Acp has multiple targets and is unique in its pattern of localization. Within the reproductive tract, Acps target to the uterus, oviduct, sperm storage organs, ovary and oocytes. Some Acps also leave the reproductive tract, to enter the hemolymph. Some Acps are detected on the surface of eggs laid by mated females but were not detectable within those eggs. Our results can help to identify the likely functions of these Acps as well as to create models for the mechanism of action of Acps.

Keywords: Reproduction; Acp; Ovary; Spermathecae; Seminal receptacle; Mating plug; Sperm storage

1. Introduction

The accessory gland is a secretory tissue of the *Drosophila* adult male reproductive system. It produces and secretes a complex mixture of proteins that form components of the seminal fluid, which is transferred along with sperm to the female during copulation (Chen, 1984; Monsma and Wolfner, 1988). In mated females, accessory gland proteins (Acps) induce physiological and behavioral changes. They enhance the female's egg production, increase her rate of ovulation, reduce her sexual receptivity, assist in the female's storage of sperm and also contribute to the reduced life span of the mated female (reviewed in Wolfner, 2002; Gillott, 2003; Ravi Ram and Ramesh, 2003; Chapman and Davies, 2004; see also Wolfner et al., 2004; Wigby and Chapman, 2005).

Acp genes were identified in differential hybridization screens, functional assays and an accessory gland EST screen (Schäfer, 1986; DiBenedetto et al., 1987; Monsma and Wolfner, 1988; Chen et al., 1988; Simmerl et al., 1995; Wolfner et al., 1997; Swanson et al., 2001). As many as 55 of the estimated 70–106 Acps have been identified so far (Swanson et al., 2001; Holloway and Begun, 2004; Mueller et al., 2005). Many of these fall into several predicted protein classes including protease inhibitors, proteases, lipases, antimicrobial proteins, CRISPs (Cysteine Rich Secretory Proteins), lectins and peptides or prohormone-like molecules (Coleman et al., 1995; Wolfner et al., 1997; Monsma and Wolfner, 1988; Chen et al., 1988; Lung et al., 2002; Mueller et al., 2004). Though coding sequences are available for these putative Acps, functions are known or predicted only for four specific Acps thus far, based on genetic tests (Herndon and Wolfner, 1995; Neubaum and Wolfner, 1999; Heifetz et al., 2000; Lung et al., 2002; Chapman et al., 2003; Liu and Kubli, 2003; Wigby and Chapman, 2005). To make predictions of likely functions of other Acps and to determine probable mechanisms of Acp

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action, it is important to determine where individual Acps target in females after mating.

Previously some targets of five Acps (Acp26Aa (ovulin), Acp36DE, Acp62F, Acp70A (sex peptide) and Acp76A) in mated females had been reported. In at least three cases, for which functional data are available (Acp26Aa, Acp36DE and Acp70A), there is a correlation between the localization/target in the mated female and the function of a given Acp: Acp26Aa, an ovulation hormone, localizes at the base of the ovary and also enters the hemolymph of the mated female from where it has the potential to reach neuro-endocrine targets (Monsma et al., 1990; Herndon and Wolfner, 1995; Heifetz et al., 2000). Acp36DE, which is essential for sperm storage, localizes in the sperm storage organs, the anterior mating plug and at a barrier to sperm movement in the lower oviduct (Bertram et al., 1996; Neubaum and Wolfner, 1999; Bloch Qazi and Wolfner, 2003). Acp70A, which has apparent binding sites in the reproductive tract or in peripheral as well as afferent nerves, is known to stimulate the rate of egg production and also affects the receptivity as well as longevity of the mated female (Ottiger et al., 2000; Aigaki et al., 1991; Chen et al., 1988; Wigby and Chapman, 2005). Localization has also assisted in suggesting a function for Acp62F, which is toxic upon ectopic expression. Since it enters the hemolymph, its toxicity has been suggested to contribute to the decreased life span of the mated female, and because it enters the sperm storage organs, it has been suggested to also participate in sperm storage (Lung et al., 2002).

Reasoning from the above examples, an Acp involved in regulating egg laying, life span or receptivity might enter the hemolymph and ultimately target to the brain, endocrine system or, for an egg-laying hormone, the musculature of the reproductive tract. In contrast, an Acp involved in sperm storage may remain in the mated female's genital tract, localizing in or near the sperm storage organs or sperm mass; some Acps that regulate sperm storage could potentially also enter the hemolymph and act from there, for example by affecting contractions that cause the sperm to enter storage. Using this as a basis, in the present study we investigated the targets for 13 Acps (eight new Acps and also included a more comprehensive investigation of five previously characterized Acps whose targeting had previously been examined only in a limited set of tissues) in order to understand their potential role in the mated female. Our results generated a targeting map of these Acps in the mated female.

2. Materials and methods

2.1. Acps and antibodies

Eight previously uncharacterized Acps [CG1656, CG6289, CG8137, CG9334, CG11864, CG11598, CG14560 and CG17575 Swanson et al., 2001; Mueller et al., 2005] were analyzed. Sequences encoding each Acp (full length, with signal sequence, confirmed by DNA sequencing (Cornell Bioresource Center)) were cloned into the protein expression vectors pDEST15, pDEST17, pBAD using the Gateway entry cloning system (Invitrogen). These expression clones generated fusion proteins of Acps with N-terminal GST, 6XHis or Thioredoxin tags, respectively. For each Acp fusion, gel mobility with predicted molecular weight was verified. In general, Acps as GST fusion proteins were used to generate antisera and either 6XHis or Thioredoxin fusion proteins were used for antibody purification. Please see the web supplement (see Appendix A) for details regarding the predicted molecular weights of Acps, different Acp fusion types used for generation and subsequent purification of Acp antibodies. Fusion proteins were either gel purified (as in Monsma and Wolfner, 1988) or column purified using glutathione beads following the procedure of Guan and Dixon (1991), and polyclonal antibodies were generated in rabbits (Cocalico Biologicals Inc.). Acp antibodies were affinity purified from their respective antisera as described in Bertram et al. (1996). Antibody specificity was verified by cross-reacting with only the appropriate fusion protein and by detection of male accessory gland specific, male biased or male-specific bands of predicted size on western blots of fly proteins.

2.2. Flies

The Canton-S strain of *D. melanogaster* was used for all our experiments. To confirm whether the synthesis of these Acps is male accessory gland specific, samples were analyzed from spermless males (progeny of *tudor* mothers (*tud*^{1} *bw sp*/*tud*^{1} *bw sp*), crossed to Canton-S males; Boswell and Mahowald, 1985) and *Prd* males (*prd*$^{2.45}$ *prd*-GsbN + PrdC/*SM1*, *ry*; Xue et al., 2001) lacking Acps. All flies were maintained on yeast-glucose medium at room temperature (22 ± 1 °C) and 12:12 light dark cycle.

2.3. Sample preparation and western blotting

Virgin females and males were collected within 3–4 h of eclosion and aged for 3–5 days. Flies were either mated or kept as unmated controls. Matings typically lasted about 20 min, and flies were observed to ensure that there were no interrupted or partial matings. Female reproductive tracts were dissected out and used for sample preparation immediately after mating, except as noted. For the time course analysis, the mated female reproductive tract samples were prepared at 0, 1, 2, 3 or 4 h after the end of mating. Samples from virgin females were included as negative control. For analyzing tissue targets, samples from the uterus, the two types of sperm