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# Regulation of *Mst57Dc* Expression in Male Accessory Glands of *Drosophila melanogaster*

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***Mst57Dc* has been isolated as a male accessory gland transcript of *Drosophila melanogaster*. Its product is a secretory protein, which is phosphorylated by protein kinase A. In the present study, the expression pattern of *Mst57Dc* was analyzed. It is preferentially expressed in but not restricted to the male accessory glands. Other than in the accessory glands, it is slightly expressed in other body parts, including the head and female body. In the accessory glands, a high level of expression was detected right after eclosion when the titer of juvenile hormone III (JHIII) reaches a peak. Its accumulation was increased by mating, which has been known to act via JH. In $ap^{56f}$, a JH-deficient mutant, the level of *Mst57Dc* transcripts was about 60% of the wild type. Moreover a JH-responsive element like palindromic sequence and several sequence motifs were found in the 5′ and 3′ flanking regions of *Mst57Dc*. Taken together, JH is proposed as a regulator of *Mst57Dc* gene expression.**

**Keywords:** *Drosophila*; Gene Expression; JHRE; Juvenile Hormone; Male Accessory Gland; *Mst57Dc*.

## Introduction

Male accessory glands are secretory organs whose products are transferred to the female during mating. Approximately 40 secretory proteins have been identified in the glands which elicit physiological and behavioral responses in the mated females (Aigaki *et al*., 1991; Chen, 1984; Leopold, 1976), including increased egg laying (David, 1963) and decreased receptivity to further mating (Manning, 1967).

Previous studies on the development and physiology of accessory glands have showed that the expression of genes in this organ is regulated in an age- and mating-dependent manner (DiBenedetto *et al*., 1990; Monsma *et al*., 1990). As virgin males age past three days, the synthesis rate of accessory gland proteins decreases unless the flies mate. Mating stimulates the synthesis of accessory gland product (DiBenedetto *et al*., 1990; Monsma *et al*., 1990; Schmidt *et al*., 1985; Yamamoto *et al*., 1988). One of the accessory gland products, Acp95EF, has been reported as being up-regulated at eclosion and by mating (DiBenedetto *et al*., 1990).

Juvenile hormone (JH) is one of the candidate molecules that act as a regulator of accessory gland gene expression. In the insects, the hormones that govern growth and development have been known to control reproduction in adult life (Kim *et al*., 1999). It is secreted from the adult corpora allata, usually directs some aspect of oogenesis in the female (Riddiford and Truman, 1978), and is important in the maturation of accessory glands (Leopold, 1976; Riddiford and Truman, 1978) or reproductive behavior (Ringo *et al*., 1992) in the male. JHIII or methoprene, JH analogue, activates overall protein synthesis and transcription in the male accessory glands, and the glands of the JH-deficient mutant, apterous[4] ($ap^4$) flies, are smaller than those from wild-type flies of similar age, presumably this is the result of a decrease in seminal fluid proteins (Shemshedini *et al*., 1990; Yamamoto *et al*., 1988).

The product of *Mst57Dc* is one of the accessory gland proteins, which is phosphorylated by PKA *in vitro* (Cho *et al*., 1999). It is secreted to the lumen of the glands and transferred to the female during mating. Its accumulation is

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Abbreviations: JH, juvenile hormone; JHRE, juvenile hormone responsive element.

started only after eclosion and gradually increased during sexual maturation. The accumulation reaches a plateau about 3–5 d after eclosion, in which the glands become very swollen with the accumulated secretion. The protein remains abundant in the accessory gland secretion, even in 14-d-old virgin males.

In the present study, the regulation of *Mst57Dc* expression was observed. From the examination of expression patterns in developmental stage, after mating, and in the ap mutant, JH is proposed as a regulator of *Mst57Dc* gene expression.

## Materials and Methods

***Drosophila* culture** Flies were reared in half-pint glass bottles on standard medium (cornmeal, sugar, agar, and yeast) containing propionic acid as mold inhibitor and maintained at 70–80% humidity at 24 ± 1°C under a photoperiodic regime (12L : 12D).

Juvenile hormone deficient mutant, apterous$^{56f}$ (ap$^{56f}$) was used to measure the effect of JH on *Mst57Dc* gene expression. The allele ap$^{56f}$ is recessive and viable. Its wing and haltere are reduced to vestiges. Both sexes are fertile and long lived when homozygous (FlyBase Report).

**Aging and mating** To measure the level of *Mst57Dc* transcripts during adult life, 30 pairs of the accessory glands were isolated from 1-, 4-, 8-, and 14-d-old virgin male flies. To examine the change of *Mst57Dc* transcripts level after mating, a single 10-d-old virgin male and one female were placed together in a glass vial and allowed to mate. The accessory glands were dissected from the 30 mated male flies 4 h after the end of mating and used for total RNA extraction.

**Northern analyses** Total RNAs were isolated from the dissected accessory glands using UltraspecII RNA isolation system (BIOTECX). The extracted RNAs were electrophoresed on 1% agarose gels and blotted as described in Sambrook *et al.* (1989). As described in a previous study (Cho *et al*., 1999), the PCR product with primers, mst57-2 (5′GGAGTCCTGG-GATCTAACGG3′) and mst57-3 (5′TTACACTCCTTCCG-GTTTCCAAG3′) was used as the probe for Northern blotting. Ribosomal protein 49 (*rp49*) cDNA was used as RNA quantity control.

**RT-PCR** The eluted total RNA templates were co-reverse transcribed by 200 units of Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT, Promega) in 20 µl of the reaction mixture containing 50 pmol of random hexamer, 20 units of RNase inhibitor, 10 mM Tris·HCl, pH 8.3, 50 mM KCl, 5 mM $MgCl_2$ and 1 mM dNTP. The RT reaction was carried out at 37°C for 1 h followed by heat inactivation at 95°C for 5 min. Subsequently, 40 µl of PCR reaction mixtures containing 10 mM Tris·HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 10 pmol of *Mst57Dc* (same as that used in the Northern blot) and *rp49* primers and 2.5 units of *Taq* DNA polymerase (Promega) were made. PCR amplification was then carried out (denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 1 min).

## Results

**Distribution of *Mst57Dc* transcript** It has been reported that *Mst57Dc* is strictly expressed in the male accessory glands (Cho *et al*., 1999; Simmerl *et al*., 1995). Northern blot analysis showed that there was preferential accumulation of *Mst57Dc* transcript to the male accessory glands (Fig. 1). But RT-PCR analysis revealed that *Mst57Dc* transcripts are also present in small quantities in the brain and in the female body, although adult male accessory glands are the major expressing organs (Fig. 2).

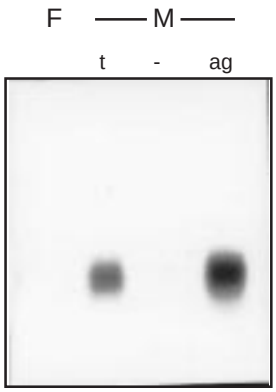


**Fig. 1.** Determination of *Mst57Dc* localization by Northern blot analysis. The *Mst57Dc* transcripts were only detected in the male accessory glands. F, female; M, male; t, total body; -, carcasses except the accessory glands; ag, the accessory glands.

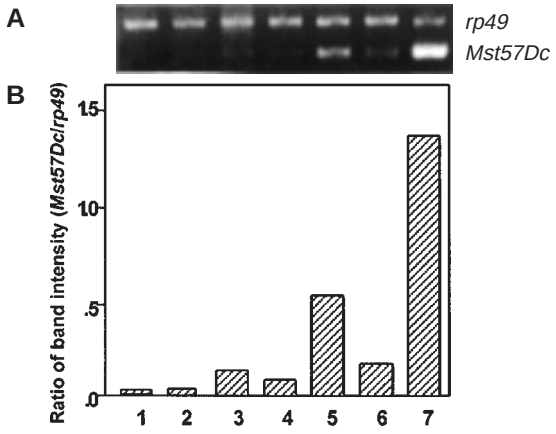


**Fig. 2.** Determination of *Mst57Dc* localization by RT-PCR. RT-PCR was performed with the total RNA of embryo (1), larvae (2), pupae (3), females (4), males (5), carcasses (male fly bodies excluding accessory glands) (6), and the accessory glands (7). **A.** PCR products from respective stages. The most of *Mst57Dc* transcripts are accumulated in the accessory glands. **B.** Relative band intensity of **A**.

**Increased amount of *Mst57Dc* transcript after eclosion in the accessory glands** Several other accessory gland transcripts have been known to be produced right after eclosion, and maintained during the gland maturation (Bertram *et al*., 1992; Chapman and Wolfner, 1988; DiBenedetto *et al*., 1990). To know whether *Mst57Dc* also shows the same expression pattern during gland maturation, we determined the level of *Mst57Dc* transcript in the glands of flies of different ages. The level of the transcript reaches a maximum at one day after eclosion and then decreased gradually during maturation (Fig. 3). The transcript expression profile is different from the amount of its product. Previously, we revealed that the accumulation of its products reaches a plateau at 3–5 d after eclosion (Cho *et al*., 1999). When virgin males are aged 14 d following eclosion, the level of *Mst57Dc* transcripts become reduced to 20% of that of young male flies (Fig. 3, lane 3).

**Stimulated *Mst57Dc* gene expression by mating** In the previous study, we characterized that the product of *Mst57Dc* is a secretory protein, which can be transferred to the female during mating like other accessory gland secretions (Cho *et al*., 1999). So, we expected that mating would up-regulate the expression of *Mst57Dc* to replenish the accessory glands. This was confirmed by the comparison of the *Mst57Dc* transcript amount in mated males to naive males. Mating induced the expression of *Mst57Dc* up to 1.7-fold level (Fig. 4). This is similar to previous observations on *mst316-lacZ* (DiBenedetto *et al*., 1990).

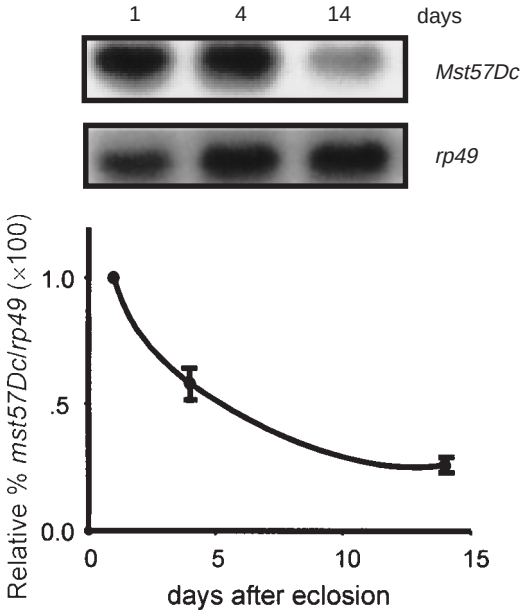


**Fig. 3.** Accumulation of *Mst57Dc* transcripts during maturation in the male accessory glands. Total RNAs extracted from the 30 male accessory glands of differently aged male flies were analyzed. The level of *Mst57Dc* is highest at 1 d after eclosion and reduced thereafter. Each value represents the mean ± standard error (S.E.) of three independent experiments. *rp49*, ribosomal protein 49.

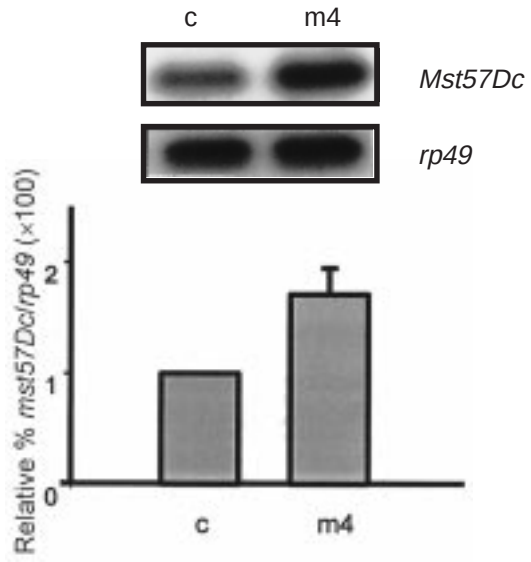


**Fig. 4.** Increase of *Mst57Dc* transcripts after mating. Total RNA from the 30 male accessory glands of virgin male (c) or mate males (at 4 h after mating, m4) were analyzed. Approximately 1.7-fold increased *Mst57Dc* accumulation was observed. Each bar represents the mean ± standard error (S.E.) of three independent experiments. *rp49*, ribosomal protein 49.

**Effect of juvenile hormone (JH) on expression of *Mst57Dc* transcript** Previous studies have been reported that JH mediates the mating-induced increase in transcription and translation of male accessory gland transcripts (Shemshedini *et al*., 1990; Yamamoto *et al*., 1988). Therefore, we tested whether the level of *Mst57Dc* transcripts is decreased in JH deficient mutant. To accomplish this, an apterous allele, $ap^{56f}$ was used. Accessory glands of $ap^{56f}$ were smaller than those of the wild type, and as shown in Fig. 5, the level of *Mst57Dc* transcripts was about 60% of wild type.

From the sequence analysis of 5′ flanking region of the *Mst57Dc* gene, we found an incomplete palindromic sequence (AGGTTATATAACC: at position +294 from the putative cap site), which shows similarity to juvenile hormone responsive element (JHRE: GAGGTTCGA-GACCTC). This sequence also has a similarity with known hormone responsive element, IR-1 (AGGTCAATGACCT). Besides, there are three sequence motifs (TATTCT (at positions −95 and +135), TTGAAAT (at positions −552, +176 and +764), and CATCAAA (at position +776)), which were found to be common in the 5′ flanking regions of the two JH target genes, juvenile hormone esterase-related protein and juvenile hormone esterase of

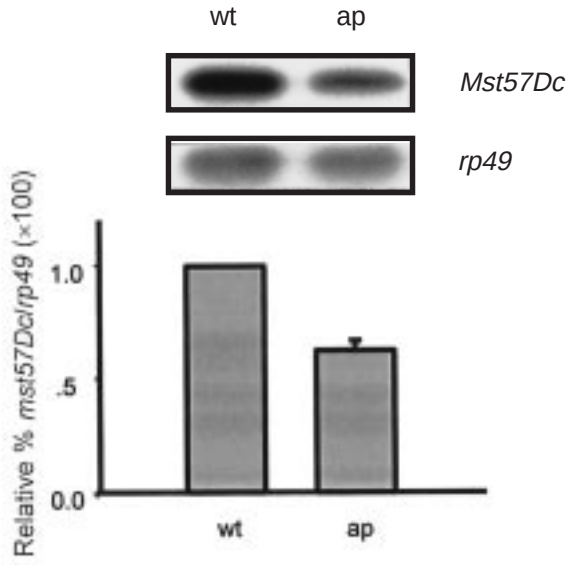


**Fig. 5.** Decrease of *Mst57Dc* transcripts in juvenile hormone deficient mutant, $ap^{56f}$. Only 60% of *Mst57Dc* transcripts were observed in the apterous mutant. Each bar represents the mean ± standard error of three independent experiments. *rp49*, ribosomal protein 49; wt, wild type fly; ap, $apterous^{56f}$ fly.

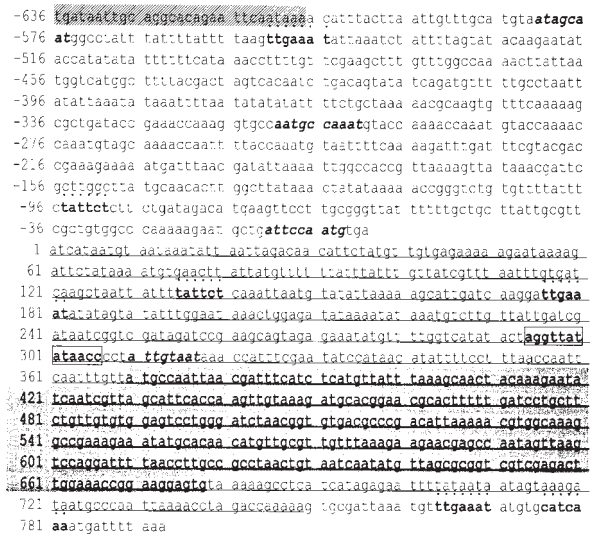


**Fig. 6.** Sequence motifs in the *Mst57Dc* gene and its 5′ flanking region. The genomic sequence presented here is a part of 3.7 kb genomic DNA of the *Mst57D* (Simmerl *et al*., 1995) modified by Cho *et al*. (1999). A palindromic sequence that shows similarity to juvenile hormone responsive element (JHRE), six sequence motifs found in juvenile hormone esterase-related protein (JHER) and juvenile hormone esterase (JHE), and five accessory gland-specific motifs were found. The putative transcripts of *Mst57Db* and *Mst57Dc* are marked in an oblique line and underlined, respectively. The coding region of *Mst57Dc* is in shaded type. A JHRE homologous palindromic sequence (AGGTTATATAACC) is presented in boxed and bold type letters. The sequence motifs found in JHER and JHE (TATTCT, TTGAAAT, and CATCAAA) are in dotted and bold type. The accessory gland-specific motifs (ATTGCAAT) are presented in bold-faced and italic type (GenBank accession number: AF142325).

*Trichoplusia*. Among the motifs, TTGAAAT and CATCAAA are located in the 3′ end of *Mst57Dc* gene (at +764 and +776, respectively). In addition, there are five copies of the accessory gland- or genital duct-specific consensus sequence (ATTGCAAT) in the 5′ upstream region of *Mst57Dc*. Four of them have been found by Simmerl *et al*. (1995), and one (at −311) is presented in this study. These possible regulatory elements are marked in Fig. 6.

## Discussion

*Mst57Dc* is an accessory gland transcript whose product is a PKA-dependent phosphoprotein (Cho *et al*., 1999; Simmerl *et al*., 1995). Its product is a secretory protein and transferred to the female during mating. Although its role in male reproduction remains to be established, the localization of its transcripts indicates that its role is not confined in the male accessory glands. The transcripts also exist in female and other male tissues except the accessory glands. Moreover, an EST clone (*Drosophila melanogaster* head cDNA clone GH27068) whose 5′ sequence is same as that of *Mst57Dc* was isolated from adult brain cDNA library by *Drosophila* genome project.

The age- and mating-dependent expression pattern of *Mst57Dc* shows good agreement with those of other transcripts that encode secretory proteins of the male accessory gland. Because male *D. melanogaster* are sexually mature about 12 h after eclosion (Ashburner, 1989), most of the accessory gland transcripts would be made by this time. Another cue that affects the expression of accessory gland transcript is a mating. Because about one third of seminal fluid proteins are transferred to the female during mating, overall protein synthesis and transcription are increased in the glands in order to refresh them.

Here, we propose JHIII as a regulator for the expression of *Mst57Dc*. Previous studies have shown that the expression of several insect proteins were increased by JH (Chinzei *et al*., 1982; Dhadialla *et al*., 1987; Graham *et al*., 1996; Miura *et al*., 1998; Noriega *et al*., 1997; Tittiger *et al*., 1999; Zhang *et al*., 1993). JH analogue, methopren and its derivatives can stimulate gene transcription in vertebrates by acting through the retinoic acid-responsive transcription factors, the retinoid X receptors (RXRs) (Harmon *et al*., 1995). Moreover, in *Drosophila*, the JH titer begins to increase just before eclosion and peaks just afterward (Bownes and Rembold, 1987), and overall accessory gland protein synthesis is stimulated by JHIII (Yamamoto *et al*., 1988).

Despite several target genes and their 5′ flanking sequences being isolated, the molecular mechanism of JH action is not well known. In the present study, we found a palindromic sequence that shows similarity to the putative JHRE of locust and the consensus element, IR-1. Although there is no evidence for the involvement of this sequence in JH action, a previous study about the binding protein in IR-1 makes it a reasonable candidate. That is, IR-1 has been shown to bind the receptor-family protein farnesoid receptor (FXR), which can mediate responsiveness to JH in transgenic mammalian cells (Forman *et al*., 1995).

Recently, two possible transcription factors that mediate JH action have been cloned and characterized in *Drosophila*. One of them is MET, a cytosolic JH receptor (Ashok, 1998). A previous study showed that MET existed in the male accessory glands of *D. melanogaster* and bound to the JH (Shemshedini *et al*., 1990). This protein shows homology with the basic helix-loop-helix/PER-ARNT-SIM (bHLH-PAS) family of transcriptional regulators. Another possible factor is a nuclear protein, ultraspiracle (USP). It is an invertebrate homologue of RXR, which regulates gene expression (Levin *et al*., 1992). It binds JHIII and JHIII acid with specificity, and can induce the JH-dependent transcription (Jones and Sharp, 1997). However, further study is required in order to characterize their role in *Mst57Dc* gene expression.

To summarize, we propose JH as a regulator of the gene expression of *Mst57Dc*. In addition, we found a putative JHRE and several motif-like elements for the first time in *Drosophila*. Further study on the regulatory elements might reveal the molecular mechanism of JH action in *Drosophila*.

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