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Regulatory region of the vitellogenin receptor gene sufficient for high-level, germ line cell-specific ovarian expression in transgenic *Aedes aegypti* mosquitoes

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Abstract

Vitellogenin receptor (VgR) is responsible for the receptor-mediated endocytosis of vitellogenin (Vg) in the egg formation of an oviparous animal, including insects. Little is known about regulation of VgR gene expression. We analyzed the upstream region of the *VgR* gene from *Aedes aegypti* (*AaVgR*) to identify regulatory elements responsible for its expression in germ cell-specific ovarian expression. Experiments with genetic transformation using the transgene containing the 1.5-Kb upstream portion of the *AaVgR* gene fused with DsRed and the *piggy*Bac vector showed that this regulatory region is sufficient for correct female and ovary-specific expression of the transgene. This 1.5-Kb upstream region contained binding sites for the ecdysone regulatory hierarchy early gene products E74 and BR-C, as well as transcription factors determining correct tissue- and stage-specific expression of GATA and HNF3/fkh. In situ hybridization demonstrated that in the ovaries of transgenic females DsRed mRNA was present in ovarian germ cells and nurse cells of mature ovarian follicles, together with VgR mRNA. In contrast, DsRed mRNA was absent in the oocyte that had a high level of endogenous VgR mRNA. Although the 1.5-Kb upstream region was sufficient to drive a high-level germ line cell-specific expression of the reporter, additional signals were required for translocation of exogenous mRNA from nurse cells into the oocyte.

Keywords: Transgenesis; Transposable element; Oocyte; Receptor-mediated endocytosis; DsRed

1. Introduction

In oviparous animals, accumulation of resources for sustaining embryo development occurs via receptor-mediated endocytosis (RME) during egg maturation. This process involves massive production of yolk protein precursors (YPP) by extra-ovarian tissues, such as gut, fat body, or liver, and subsequent YPPs accumulation via RME in developing oocytes (Telfer, 2002; Winter, 2002). In insects, YPPs are synthesized and secreted by the fat body, the multifunctional tissue, which during reproductive cycles is becoming extraodinary specialized for massive production of YPPs, (Giorgi et al., 2005). Vitellogenin (Vg) is a large phosphoglycolipoprotein, which in its mature form consists of either multiple apo-proteins in hemimetabolous insects, or just two in holometabolous insects (Tufail et al., 2005). Vg is a precursor of vitellin (Vn), which in most insects is the major YPPs (Telfer, 2002; Masuda et al., 2005). Accordingly, several types of YPPs are accumulated by insect oocytes; however, Vn, the storage product of Vg is the egg's protein major component (Snigirevskaya and Raikhel, 2005).

The presence of a specific vitellogenin receptor (VgR) in the oocyte membrane has been demonstrated in a number of insect species through studies of Vg uptake by cultured ovaries (reviewed in Raikhel and Dhadialla, 1992). Several VgRs have been characterized biochemically (Osir and Law, 1986; Indrasith et al., 1990; Dhadialla et al., 1992; Ferenz, 1993; Sappington et al., 1996). Molecularly, VgR has first been characterized from the mosquito *Aedes aegypti* and has been designated AaVgR (Sappington et al., 1996). Remarkably, it has been found to be structurally similar to the previously identified *Drosophila* yolk protein receptor (Schonbaum et al., 1995), despite the fact that the

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substrate of this receptor, *Drosophila* yolk protein, bears no homology to the Vgs of other organisms (Bownes et al., 1988). More recently, VgRs from several other insects have been cloned, including the fire ant, *Solenopsis invicta* (Chen et al., 2004b) and the American cockroach, *Periplaneta americana* (Tufail and Takeda, 2005). All insect VgRs and *Drosophila* YpR have similar modular structure and belong to the family of low-density lipophorin receptors (LDLR) (Sappington and Raikhel, 1998, 2005).

Little is known about the regulation of the *VgR* gene expression in insect oocytes. The gene *AaVgR* has been cloned and characterized (Cho and Raikhel, 2001). The gene has been shown to be expressed early in development in the germ cell line in germarium, and later in the nurse cells with VgR mRNA accumulating in the developing oocytes. Translation of VgR mRNA occurs during previtellogenic development of the female mosquito coinciding with the elevated levels of juvenile hormone (Sappington et al., 1995, 1996; Cho and Raikhel, 2001).

Here, we report the sex- and tissue-specific expression of the *DsRed* reporter gene using 1.5 kb of the 5′ upstream region of the *AaVgR*. We show that this region of the *AaVgR* gene is necessary and sufficient for high-level specific germ line-specific ovarian expression. This is the first report of germ line cell-specific transformation in mosquitoes.

2. Materials and methods

2.1. Insects

Mosquitoes, *A. aegypti*, wild-type Rockefeller/UGAL strain, designated thereafter as *wt,* were maintained in laboratory culture as described by Hays and Raikhel (1990).

2.2. In vitro transcription/translation

The TNT system (Promega) was used for in vitro transcription/translation of the different cDNAs in rabbit reticulocyte lysate, utilizing the appropriate promoter. To monitor the in vitro reactions, synthesized proteins were labeled with [^{35}S]methionine (1200 Ci/mmol) from NEN radio-chemicals, and the radio-labeled products were visualized by electrophoresis and auto-radiography.

2.3. Electrophoretic mobility shift assays (EMSA)

EMSA were carried out as described in the manufacturer's manual (Promega). DNA probes for EMSA were made by annealing together complementary oligonucleotides, end-labeled by T4 polynucleotide kinase using [γ-^{32}P]ATP (NEN). Oligonucleotide used to generate the different DNA probes were as illustrated in Fig. 1B (only sense strands are shown).

2.4. Plasmids

The pBac[3 × P3-EGFP afm] transformation vector was used to transform the mosquitoes. This vector has an artificial eye-specific promoter (3 × P3) in front of a TATA sequence and the *EGFP* gene (Horn and Wimmer, 2000). The 5′ flanking region of *Aa*VgR was linked to the DsRed gene to test the function of the putative promoter. About 1.5 kb of the 5′– upstream region of *AaVgR*, which contains 500 bp of *Aa*VgR 5′ UTR, was amplified by PCR with ProofStart polymerase (Qiagen Co.) using the forward primer (5′–GGGGTACCGGCGCGCCGTGAA TACAGCATTATCAATG–3′) and the reverse primer (5′–CATGGGCCCCAAAAAAAGAAATGCCCTACAA AG–3′). The genomic clone pVgRG36-*Sal*7.0 was used as a template for PCR. The PCR product was digested with *Kpn* I and *Apa* I and then inserted into the reporter vector, pDsRed1-N1/*Kpn*I + *Apa*I. To insert 5′ flanking region and DsRed together, the insert was transferred into the intermediate vector pSLfa1180fa and then introduced into the pBac[3 × P3-EGFPafm] plasmid at the *Asc* I-unique cloning site.

2.5. Micro-injection of mosquito embryos

Embryos were collected for micro-injection about 90–270 min post-blood meal (PBM). The pre-blastoderm embryos were injected as described by Kokoza et al. (2001a). Injected embryos were heat shocked 16–20 h after DNA micro-injection and maintained in a rearing chamber with 27 °C temperature and 85% humidity. G_0 adults were crossed with virgin *wt* females or males, and the resulting G_1 progeny was screened for eye EGFP fluorescence.

2.6. Microscopy

The observations and screening for EGFP expression were performed using a fluorescence microscope (Nikon SMZ 800) equipped with a GFP-B filter (GFP Band Pass, cat # C/6445, Ex 470/40 DM 495 BA 525/50) and a GFP-L filter (GFP Long Pass, cat # C/6601, Ex 470/40 DM 495 BA 500 (LP)).

2.7. Northern hybridization analysis

Total RNA was extracted from the ovaries, fat bodies, guts, or whole bodies of mosquitoes using the protocol of the company (Qiagen Co.). For Northern blot analysis, total RNA was separated on a 1.2% formaldehyde-agarose gel and transferred onto a nylon membrane (Amersham Co.). The filter was hybridized with [^{32}P] α-dATP -labeled cDNA probe, washed, and exposed to the phospho image screen (Sambrook et al., 1989).