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# Transcriptome and gene expression profile of ovarian follicle tissue of the triatomine bug *Rhodnius prolixus*

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ABSTRACT

Insect oocytes grow in close association with the ovarian follicular epithelium (OFE), which escorts the oocyte during oogenesis and is responsible for synthesis and secretion of the eggshell. We describe a transcriptome of OFE of the triatomine bug *Rhodnius prolixus*, a vector of Chagas disease, to increase our knowledge of the role of FE in egg development. Random clones were sequenced from a cDNA library of different stages of follicle development. The transcriptome showed high commitment to transcription, protein synthesis, and secretion. The most abundant cDNA was a secreted (S) small, proline-rich protein with maximal expression in the vitellogenic follicle, suggesting a role in oocyte maturation. We also found Rp45, a chorion protein already described, and a putative chitin-associated cuticle protein that was an eggshell component candidate. Six transcripts coding for proteins related to the unfolded-protein response (UPR) by were chosen and their expression analyzed. Surprisingly, transcripts related to UPR showed higher expression during early stages of development and downregulation during late stages, when transcripts coding for S proteins participating in chorion formation were highly expressed. Several transcripts with potential roles in oogenesis and embryo development are also discussed. We propose that intense protein synthesis at the FE results in reticulum stress (RS) and that lowering expression of a set of genes related to cell survival should lead to degeneration of follicular cells at oocyte maturation. This paradoxical suppression of UPR suggests that ovarian follicles may represent an interesting model for studying control of RS and cell survival in professional S cell types.



## 1. Introduction

Making viable eggs that will survive in the environment independently of the maternal body is a key step in the reproduction process of all oviparous animals, including insects. In their way from oocyte precursor to the mature egg—a process frequently completed in just a few hours, these cells accumulate large amounts of reserves and increase about a thousand-fold in size, followed by isolation from the external world by formation of the eggshell. Egg development in insect ovaries is divided into three main phases: pre-vitellogenic growth, vitellogenic growth (also called vitellogenesis), and choriogenesis. Pre-vitellogenic growth occurs in the anterior end of the ovariole, in the case of the hemipteran telotrophic ovary, in a lanceolate structure called tropharium (Atella et al., 2005). Despite their astonishing growth rates, oocytes themselves show very modest synthetic activity (Wallace et al., 1972), and their development is supported mainly

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by other cells either from the ovary or from extra-ovarian tissues (i.e., fat body).

Early growth of oocytes takes place in close association with other cell types such as the surrounding follicular epithelium (FE) and nurse cells. These are linked to oocytes by means of cytoplasmic projections/bridges called trophic cords that are used to provide both proteins and RNA. Trophic cords are retained until the early phases of vitellogenic growth (Telfer et al., 1982). During vitellogenesis, oocytes rapidly accumulate large amounts of lipids, glycogen, RNA, and yolk proteins. Vitellin, the most common yolk protein, derives from a hemolymphatic lipoprotein called vitellogenin (Vtg), which in most cases is synthesized in the fat body and is taken up by oocytes by receptor-mediated endocytosis. Synthesis of Vtg by ovarian FE (OFE) cells has been reported in cycloraphan dipteran ovaries (Postlethwait et al., 1980) as well as in the triatomine *Rhodnius prolixus* (Melo et al., 2000). Also, non-vitellin yolk proteins such as the 30-kDa protein from lepidopteran eggs are synthesized by FE. Upon completion of vitellogenesis, fully grown eggs are separated from the external world by the chorion, a protective proteinaceous layer synthesized and secreted by follicle cells at the very end of oocyte development inside the ovary. After choriogenesis, FE cells degenerate and die, exhibiting several features characteristic of programmed cell death, in a process that is believed to be important to prevent blockage of the ovarioles and to support proper egg development (Nezis et al., 2006, 2002).

The endoplasmic reticulum (ER) is the gate to the exocytotic pathway, where newly synthesized secretory (S) proteins must fold and acquire their correct functional tri-dimensional structure. In recent years, it has been recognized that frequently a large portion of nascent polypeptide chains fail to fold appropriately, eventually leading to accumulation of misfolded proteins in the ER—what is called reticulum stress (RS) (Banhegyi et al., 2007). Eukaryotic cells respond to ER stress by increasing transcription of genes for ER resident chaperones and proteins that help either to accelerate flux in the secretory pathway or to promote destruction of misfolded proteins (Kohno et al., 1993; Mori et al., 1992; Romisch, 2005). Collectively, this process has been termed the unfolded-protein response (UPR). To eliminate organelles or unhealthy cells, autophagy or apoptotic cell death take place whenever the UPR does not occur properly or is not sufficient to deal with the ER stress challenge (Lai et al., 2007).

To provide insights into the molecular framework involved in development of the insect ovary, we describe here a transcriptome of the most abundant transcripts found in the follicle of the triatomine *R. prolixus*, a vector of *Trypanosoma cruzi*, the causative agent of Chagas disease. Several messages related to oocyte and embryo development were identified, and expression of selected transcripts during follicle development was studied. We propose that failure in triggering UPR during choriogenesis leads to death of FE cells as part of the developmental program of the insect egg.

## 2. Materials and methods

### 2.1. Insects and tissues

*R. prolixus* were from a colony maintained at 28 °C and 70% relative humidity. Insects used here were adult mated females having their second blood meal after their imaginal molt. Ovarioles were dissected free of tracheae and ovarian sheath. Ovarian follicles at vitellogenic stages (0.5–2.0 mm) or initiating choriogenesis (about 2 mm) were opened with tweezers and the oocyte content discarded. Due to their reduced size, pre-vitellogenic follicles were separated from tropharium and used both for library and quantitative real-time PCR (qPCR). Tropharium samples were used only for qPCR and were not included in the cDNA library.

### 2.2. cDNA library and sequencing

mRNA was isolated from using the Micro FastTrack mRNA isolation kit (Invitrogen, Carlsbad, CA), and the PCR-based cDNA library was made following the instructions for the SMART cDNA library construction kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions. cDNA from follicle cells isolated as described above were fractionated in three size intervals using a gel filtration column, ligated separately to the λ TriplEx 2 vector, packaged with packing extract Gigapack III Gold (Stratagene, La Jolla, CA), and then joined in a single pool. The obtained phage library was mixed with log phase XL-1 Blue cells (Stratagene) and plated in agar medium containing IPTG and X-gal for blue/white screening. A sample of individual white lysis plates was chosen randomly and the cloned insert amplified using PT2F1 (5′-AAG TAC TCT AGC AAT TGT GAG C-3′, upstream) and PT2R1 (5′ CTC TTC GCT ATT ACG CCA GCT G-3′, downstream) primers and sequenced in a DNA sequencing instrument ABI 377 (Applied Biosystems, Foster City, CA) using the BigDye Terminator 3.0 kit (Applied Biosystems) and the primer PT2F3 (5′-TCT CGG GAA GCG CGC CAT TGT-3′, downstream a PT2F1) as described elsewhere (Valenzuela et al., 2002).

### 2.3. Bioinformatics

The sequences obtained were cleaned from vector and primer sequences and assembled into contigs using CAP3 software (Huang and Madan, 1999) mastered by an in-house bioinformatics program (Ribeiro et al., 2007). The protein coding potential of the contigs and singletons obtained was examined using the blastx algorithm (Altschul and Gish, 1996) to search the non-redundant protein (NR) and Gene Ontology (GO) (Ashburner et al., 2000) databases. As the translation mRNA of proteins that are coded by mitochondrial DNA involves the use of an alternate genetic code, we have also compared the transcripts with a database created from mitochondrial DNA sequences. Essentially, these analyses were performed using a program pipeline already described in the literature (Valenzuela et al., 2003). To get hints on the possible biologic role of the proteins obtained from the translated contigs, rpsblast was used to search for conserved protein domains in the Pfam (Bateman et al., 2000), SMART (Schultz et al., 2000), COG (Tatusov et al., 2003), and conserved domains (CDD) (Marchler-Bauer et al., 2002) databases. This information is particularly important, because we kept in our analysis open-reading frames that lack the initial methionine (5′-truncated), stop codon (3′-truncated), or both (fragment) and not only full-length coding sequences. Although the BLAST result against GO gives insight to a putative function of the transcripts, in the process of manual annotation the transcripts were classified into one of the following fifteen classes: protein synthesis, protein export machinery, transcription machinery, transcription factor, proteasome machinery, extracellular matrix (ECM)/S protein/ovarian function, protein modification machinery, energy metabolism, signal transduction, nuclear regulation, transporter, cytoskeletal/cell adhesion, intermediate metabolism, unknown (U), and unknown conserved.

To confirm that the expressed sequence tags (ESTs) derived from the *Rhodnius* genome, these were also BLASTed against the *R. prolixus* genome. Supercontigs from the *R. prolixus* assembled genome (release 1.0.1, from January 2009) were downloaded from http://genome.wustl.edu/genomes/view/rhodnius_prolixus/. The FASTA files of these supercontigs were broken into 50-kb fragments with 10 kb from previous sequence, and a database was created. This procedure was taken because BLAST does not work well if subjects in the database are very long sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastDocs).