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Evolutionary conservation of the mature oocyte proteome

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ABSTRACT

The proteome profiles of mature ovulated oocytes of the Cnidaria basal eumetazoan, the starlet sea anemone *Nematostella vectensis* was compared with published data of mammalian mouse mature oocytes. We identified 1837 proteins in *N. vectensis* oocytes including known oocyte- and germ-cell-specific markers, proteins associated with RNPs and vitellogenin, a major component of egg yolk proteins. Our findings suggest highly conserved enriched functional pathways in *N. vectensis* and the mouse mature oocytes. This study provides the first catalog of cnidarian oocyte proteins, revealing highly conserved ancient organization of life processes for over 500 million years of evolution.

Significance: The current study provides the first proteomic profile of an oocyte of a cnidarian organism the starlet sea anemone *N. vectensis* and gives new insights on the ancient origin of an oocyte proteome template. The comparative analysis with a chordate oocyte suggests that the oocyte proteome predates the divergence of the cnidarian and bilaterian lineages. In addition, the data generated in the study will serve as a valuable resource for further developmental and evolutionary studies.

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1. Introduction

A mature oocyte contains the full complement of maternal proteins and mRNAs required for fertilization and subsequent embryonic development. Because oocytes and early embryos are transcriptionally silent, developmental processes at these stages rely exclusively on maternal mRNAs and proteins [1–3]. Substantial research efforts have been devoted to unraveling of the molecular pathways governing gene expression and genome reprogramming and their contribution to cell differentiation, pluripotency and germ-cell formation in oocytes of eumetazoans [4,5]. Those studies pointed to conservation of functions as numerous genes expressed in oocytes were found to be conserved in oocytes of mouse, bovids, *Xenopus laevis* and *Ciona intestinalis* [4,5]. Comparative proteome profiling studies are still lacking at this stage, although information is available on proteome profiles of model and non-model

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organisms. These include oocyte proteomes of *Caenorhabditis elegans* [6], sea urchin [7], zebrafish [8,9] and the most intensively studied mouse [10–13]. Proteomic profiling offers a novel insight into facets of oocyte function which, owing to discrepancies between protein levels and the levels of corresponding transcripts, have not yet been revealed [14]. Such divergence between transcriptome and proteomic profiles in oocytes is particularly expected as oocytes contain translational silent mRNAs that are activated only after fertilization [3]. Moreover, oocytes of most oviparous eumetazoans accumulate yolk proteins that are not synthesized by the oocyte itself. In the present study we investigated evolutionarily conserved maternal proteins in oocytes by comparing the *Nematostella vectensis* oocyte proteomic profile with the published proteomic profiles of mouse oocytes at the metaphase II (MII) stage [11,13].

There is growing interest in cnidarians as representatives of ancestral basic eumetazoans because of their potential of offering important insights into early evolution [15]. As basal eumetazoans, diploblastic cnidarians form an outgroup of the bilaterian animals and are intermediate in complexity between sponges and bilaterians. An emerging developmental model system of cnidarians is the starlet sea anemone *N. vectensis* which has a published genome [16] and whose sexual reproduction can be induced and controlled in the laboratory. *N. vectensis* produce eggs whose ultrastructure during oogenesis exhibits features in common with other oviparous eumetazoans [17]. This raises the question of whether proteins present in this basal diploblastic model species have been retained through evolution suggesting conservation of oocyte-specific proteins. The expression of critical genes in bilaterian germ-cell specifications and genes that represent canonical pathways in bilaterian embryonic developmental have been described in *N. vectensis* [15,18].

In this study we generated the first catalog of cnidarian oocyte proteins of *N. vectensis* ovulated eggs. Ovulated oocytes, or eggs, are arrested at MII and are normally fertilized within a few hours. The terms “oocyte” and “egg” are used quite differently in various species, as mature ovulated oocytes in oviparous species are usually called “eggs”, whereas “oocytes” is the corresponding term in mammals. We identified 1837 proteins in mature ovulated oocytes of *N. vectensis* and show their putative similarity to oocyte of MII mouse (*Mus musculus*). Some of the identified proteins were associated with oocyte structure and function, while others were germ-cell-specific. We have gained fundamental insight into the proteome of oviparous *N. vectensis* eggs and highlighted putative proteins and functions in a basal eumetazoan that are shared with or differ from mammalian MII oocytes.

2. Materials and methods

2.1. Cultures of *N. vectensis*

N. vectensis were cultured in 12.5 ppt Red Sea salts at 18 °C and spawning was induced as previously described [19].

2.2. Collection, extraction and proteolysis of oocytes

Mature oocytes were isolated from their gelatinous egg sack using 3% cysteine (Fig. 1) [20] and washed in excess culture medium over 50 μ nylon mesh. Biological triplicates of 20 eggs (0.4 μg per oocyte) were collected from three different anemone groups and each sample contained mature oocytes from 4 to 6 egg sacks. The oocytes were extracted in 8 M urea, 400 mM ammonium bicarbonate and 10 mM DTT, and sonicated. 20 μg protein from each sample were reduced with 2.8 mM DTT (60 °C for 30 min), modified with 8.8 mM iodoacetamide in 400 mM ammonium bicarbonate (in the dark, room temperature for 30 min) and digested in 2 M urea, 25 mM ammonium bicarbonate with modified trypsin (Promega) at a 1:50 enzyme-to-substrate ratio, overnight at 37 °C. An additional second trypsinization was done for 4 h.

2.3. Mass spectrometry analysis

The tryptic peptides were desalted using C18 tips (Harvard Apparatus) dried and re-suspended in 0.1% formic acid. The peptides were resolved by reversed-phase chromatography on 0.075 × 200-mm homemade fused silica capillaries (J&W) packed with Reprosil reversed phase material (Dr. Maisch GmbH, Germany). The peptides were eluted with linear 214 min gradients of 5–35% and 8 min at 95% acetonitrile with 0.1% formic acid in water, at flow rates of 0.15 μl/min and the LC system was EASY nLC 1000 (Thermo). Mass spectrometry was performed by Q-Exactive mass spectrometer (Thermo-Fisher) in positive mode using repetitively full MS scan followed by higher-energy collisional dissociation (HCD) of the 10 most dominant ions selected from the first full MS scan. The mass spectrometry data from three biological repeats was analyzed using the MaxQuant software 1.3.0.5 [21] vs. the Nematostella section of the Uniprot database with 1% FDR (see below). The data was quantified by label free analysis using the same software. Label free analysis compares intensities across runs. Briefly, for every peptide, corresponding total signals from multiple runs were compared to determine peptide ratios. Pair-wise peptide ratios were only determined when the corresponding peak is detected in both LC–MS runs. A robust estimation of the protein ratio is calculated as the median of pair-wise peptide ratios interpolated with the square root of the ratio of summed-up intensities. The analysis is done after first applying a normalization procedure and recalibration of the retention times [21].

2.4. Bioinformatics analysis

2.4.1. Analysis of N. vectensis data

N. vectensis protein sequences and annotations, as well as their InterPro and PFAM domains, were retrieved from the UniProt database (date 25.2.2013). The dataset contained 24,999 proteins, out of which 1202 proteins had a meaningful annotation (i.e. not “predicted protein”). The sequences were submitted to the Blast2Go software, where blastp was run against the NCBI RefSeq protein database. A maximum of 20 hits with *e*-value $< 10^{-3}$ were retrieved for each query sequence. The annotations obtained from Uniprot and Blast2GO were used to construct a FASTA file which then served as a reference proteome in the analysis of the mass spectrometer data. The expressed proteins were tested

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