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# 2DE identification of proteins exhibiting turnover and phosphorylation dynamics during sea urchin egg activation

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**Abstract**

The animal egg is a unique quiescent cell, prepackaged with maternal mRNAs and proteins that have functions in early development. Rapid, transient signaling at fertilization alters egg physiology, resulting in $Ca^{2+}$ release from the endoplasmic reticulum (ER) and cytoplasmic alkalinization. These events trigger the zygote developmental program through initiation of DNA synthesis and entry into mitosis. Post-translational modifications of maternal proteins are responsible for the spatio-temporal regulation that orchestrates egg activation. We used functional proteomics to identify the candidate maternal proteins involved in egg activation and early development. As the first step of this analysis, we present the data on the baseline maternal proteome, in particular, on proteins exhibiting changes in abundance and in phosphorylation state upon egg activation. We identify 94 proteins that were stable, reproducibly displayed a shift in isoelectric point, or changed in relative abundance at specific times after activation. The identities of these proteins were determined by quadrupole time-of-flight tandem mass spectrometry. The set of the most dynamic proteins appear to be enriched in intermediary metabolism proteins, cytoskeletal proteins, gamete associated proteins and proteins that have $Ca^{2+}$ mediated activities.
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## Introduction

The cellular events occurring within seconds to minutes post fertilization are collectively termed egg activation and include changes in intracellular physiology, signaling through kinases and secondary messengers, remodeling of the cytoskeleton and a dramatic increase in the overall metabolic rate of the egg (reviewed in Epel, 1997; Runft et al., 2002; Whitaker, 2006). Reverse genetics approaches have been used to identify and characterize several maternal proteins that mediate these events. In mammals, fish, amphibians and echinoderms, the identification of egg proteins by assessing specific functional activities has revealed key signaling pathways that regulate the initiation of development (Runft et al., 2002; Whitaker, 2006). In addition, a number of forward genetic screens and genome-wide RNAi screens performed in nematodes, zebrafish and *Drosophila* have led to the identification of a broader repertoire of genes associated with the female sterile phenotype (Dosch et al., 2004; Fitch and Wakimoto, 1998; Geldziler et al., 2004; Kamath et al., 2003; Labbé et al., 2006; Ohsako et al., 2003; Perotti et al., 2001; Piano et al., 2000; Szabad et al., 1989). In most of these forward genetic model systems the fertilization event is difficult to control and quantify, thus connection between the female sterile phenotype and a specific egg activation event remains tentative. A more direct and unbiased approach to the problem of protein factors that mediate egg activation is to study a model with tractable biochemistry and *in vitro* fertilization (Sato et al., 2002). One such a model is the sea urchin *Strongylocentrotus purpuratus*. Visualization of gamete interaction and egg activation events is routine in sea urchins, and large quantities of synchronously fertilizing gametes can be obtained. While echinoderms are not yet amenable to forward genetic screens, the recent completion of the *S. purpuratus* genome sequence

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(Sodergren et al., 2006) facilitates application of many genome-scale technologies to the study of egg activation.

Proteomic approaches, which seek to identify various proteins and their modified forms in the biological sample and quantify the abundance of each variant, are especially relevant for studying cells such as eggs, where regulatory mechanisms are driven by post-transcriptional events, including mobilization and post-translational modifications (PTM) of existing, maternal proteins. Furthermore, in sea urchins, very little detectable transcription or translation occurs in the minutes following fertilization (Davidson, 1982; Epel, 1967); therefore, physical and spatial modulation of existing proteins such as phosphorylation, degradation and changes in protein solubility and availability is responsible for successful activation. Identification of these proteins also provides further validation of existing tiling array data, EST database sequencing and current genome annotation (Sodergren et al., 2006).

Recently, several large scale screens have been conducted in *C. elegans*, zebrafish and *Drosophila* to identify the global changes in the mRNA population as oocytes mature and then transition from egg to embryo (DeRenzo and Seydoux, 2004; Schier, 2007; Stitzel and Seydoux, 2007). However, only a few studies (see below) have used a proteomics based approach to visualize how maternal proteins are changing at fertilization.

Approximately 350 proteins have been detected in the mature pig oocyte (Ellenderova et al., 2004), and global proteome analysis has identified approximately 500 protein spots in the samples of mouse oocytes, including 32 spots that were less abundant at the morula stage mouse embryos than in the oocyte (Coonrod et al., 2002; Sasaki et al., 1999). A similar study in the plant *Solanum chocoense* detected 619 proteins in the unfertilized oocyte (Vyetrogon et al., 2007). Using SYPRO Ruby we recently estimated the number of proteins and their abundance in a soluble fraction of sea urchin eggs and early embryos. Approximately 600 maternal proteins were reproducibly detected in the egg and by 2 min post fertilization. In the next few minutes, this number was reduced to 464, then increased at 15 min (587), and reached a steady level (567 proteins) at 30 min (Roux et al., 2006). These data indicate rapid and significant changes in levels of total protein abundance during sea urchin egg activation and early development, at least in this subset of relatively abundant proteins.

Protein phosphorylation is an important regulatory mechanism of egg activation in all species that have been studied (Whitaker, 2006; Kinsey, 1997a; Sato et al., 1998) and the sea

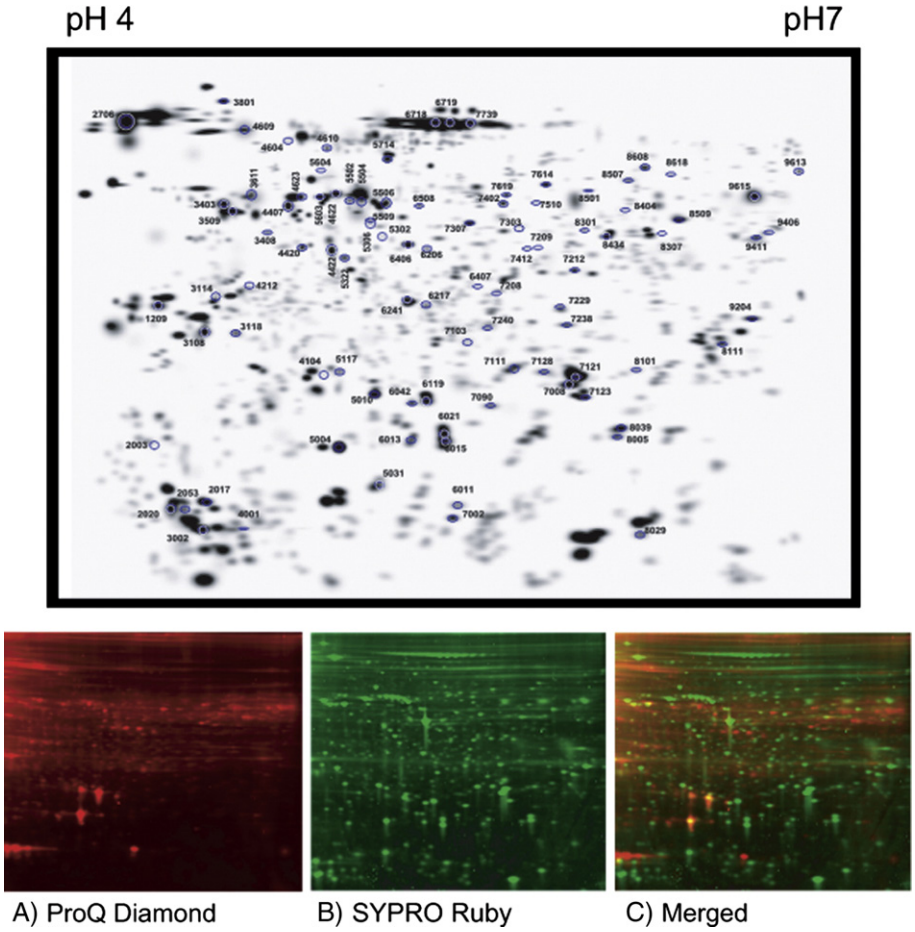


Fig. 1. Multiplexed 2DE analysis of sea urchin egg and zygote proteins. Top panel: Image of the PDQuest synthetic master gel representing all spots identified within a user-defined match set (spots detected from all time points are included, UF, F2 and F30). Circled spots were those picked for Q-ToF MS/MS identification. The numbering corresponds to the Spot ID (PDQuest spot # assignment) shown in Table 1. Lower panels: Images of a 2DE gel (UF) stained with (A) ProQ Diamond phospho-stain and (B) SYPRO Ruby total protein stain. The merged image is shown in panel C.