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Picking the right tool for the job – phosphor-proteomics of egg activation

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Abstract

Eggs are the rarest cell in the human body, yet their study is essential for the fields of fertility, reproduction, and fetal health. Guo et al use a “surrogate” animal to discover the phosphoproteomic pathways involved in egg activation. With datasets of several thousand phosphosites on 2500 different proteins, these investigators have defined new pathways, connections to pathways, and priorities in searches for how eggs are activated at fertilization. These results in a sea urchin are now transposable to mammals for testing on a per candidate strategy.

Large cells, easy to manipulate, amazing signal transduction, complete control over activation of the cell, and no transcription or translation occurring during this short timeframe of cell activation. The time frame for activation of these cells is on the order of minutes maybe 10 in a mammal, less than one in others. Further, the cell has many screens to share – phosphorylation, calcium fluxes, lipid modifications, and many implied activities primed for the key experimentalist and observer.

Sounds too good to be true, right? Unfortunately, you are right - at least in most cases. These cells of amazing characteristic for the experimentalist are eggs, the rarest of cells in the human, the mouse, and other mammals. Humans are born with only about 500,000 oocytes, ovulate 1–2 oocytes every 28 days, or 8–15 oocytes on average upon hormonal stimulation - and are very hard to get! Superovulation in mice may yield on average 10–50 oocytes depending on the strain (Luo et al., 2011; Pfeiffer et al., 2015), whereas in rhesus monkeys the yield is highly variable—from a few to over 100 (Nusser et al., 2001), and abattoir animals (cows and pigs) have about 12 oocytes per ovary (Hamano and Kuwayama, 1993). And just when you thought things could not get any worse, in the case of human eggs at least, most research is performed on discarded oocytes, which may be of poor quality, unfertilizable, or incapable of meiotic maturation. Therefore, the findings from these cells are highly variable, and may have limited applicability to fertilizable, mature oocytes.

Sea urchins, however, release millions of eggs, all having completed meiosis and stored in a haploid, suspended animation state (at least in terms of transcription, translation, and many other metabolic events) until that is, when the sperm flips the switch to active the egg and the beginning of embryonic development. It is at this time that Guo et al have focused their efforts on defining changes in the phosphoproteome that occur within the first five minutes of sperm contact. While a sea urchin is not a human, the eggs certainly overlap in many of

their activities. How many and which ones? That is a tough question to answer since we know so little about the activation of mammalian eggs in general and the phosphoproteome even less. The major focus of research in egg activation in mammals takes advantage of the single cell analysis in calcium activation. Specific dyes of defined fluorescence activity are prized as metrics for examining calcium dynamics in single cells. The mechanism of calcium release in a human is likely also significantly different than in a sea urchin. In mammals a specialized phospholipase C subtype (zeta) appears to be donated by the first fusing sperm, somehow is activated in the egg cytoplasm, and there it initiates an essential series of calcium transients that includes release from the endoplasmic reticulum into the cytoplasm and then re-uptake into the cytoplasm. This dynamic repeats for many hours, the profile of which is specific for different mammals, and each profile types essential for the species. The difference in sea urchins is what flips the switch. It is not PLCzeta, the genome of echinoderms does not appear to have such an enzyme, although sea urchins – as all eggs studied – do exhibit defined and repeatable calcium fluxes from the ER, into the cytoplasm, and back again into the ER.

Fortunately, these calcium dynamics are visible on a cell-by-cell basis and 5 or 10 mammalian oocytes can reveal important mechanisms. Phosphoproteomics however is quite a different challenge. Many investigators of mammalian oocytes use candidate approaches of phosphoproteins to successfully map pathways and intersections in the egg activation scheme. Yet, discovery based approaches and screens are hard to imagine in these few eggs. This is where the impact of the Guo et al., 2015 work will likely be felt the strongest.

Guo et al took sea urchin eggs and with synchronous activation, compared the global phosphoproteome of the egg to time points post-fertilization. The non-biased, and quantitative Mass Spectroscopy reads were then normalized to the egg to reveal phosphopeptides that were increased or decreased in the first five minutes of egg activation. The investigators identified over 6000 sites of phosphorylation on 2500 phosphoproteins. Approximately 15% of these sites were differentially present during egg activation, implying important regulatory steps involved in the egg activation phenomenon. Remarkably though, as a result of their high-throughput methodology and the quality of the reference genomic resources, Guo et al discovered new phospho-signaling pathways involved in egg activation, pathways identified in other cell types, but heretofore not known to be involved in egg activation steps. The investigators tested candidate phosphor-kinases found in the screen to validate their approaches (e.g. ppMAPK, p90RSK, pCaMKII) and established a phosphoproteome network based on timing, abundance, and correlation with other phosphosite appearances in a gene set enrichment analysis. While these computational connections between various enzymes and pathways are only predictions, the value of these predictions is that now candidate approaches can be taken to test the models. Such tests can be accomplished on these large populations of sea urchin eggs with a large mass for biochemical approaches, but they can also be tested in eggs of other animals on a per cell basis using for example, antibodies to P-specific proteins and perturbations to such systems with small molecule inhibitors and RNAi approaches.

The impact of this work should be substantial. The available datasets [ProteomeXchange with identifier PXD002239 (<http://proteomecentral.proteomexchange.org/dataset/>)]

[PXD002239](#)]] have thousands of phopho-sites of general interest to those interested in phopho-pathways and their evolution in various animals and cells types, but are of particular interest to anyone studying fertilization, gamete biology, and reproduction. The human in vitro fertilization (IVF) clinic is replete with eggs that look perfectly fertilizable, yet with IVF or even with intracytoplasmic sperm injection (ICSI), when the egg is not fertilizable, one may now turn to a more sophisticated molecular explanation and not just to another cycle of expensive ART.

What is next? For me it would be oocyte maturation. Perhaps the investigators can make use of the synchrony of closely related sea star oocytes to study the signal transduction changes that occur during meiotic maturation stimulated by the maturation hormone, 1-Methyladenine (1-MA). Sea stars store their millions of oocytes not having completed meiosis as in sea urchins, but in prophase 1 of meiosis, with large germinal vesicles containing the 4-copies of each chromosome. Upon exposure to the normal meiotic activating hormone 1MA, an investigator can test and explore the many signaling pathways involved in this meiotic event. Many similar events of maturation as may occur in mammals appear to occur in sea stars, so these investigators, their effective collection of approaches, and the closely related sea star seem primed for next generation study. It is clear from other work that during meiosis that calcium fluxes are stimulated following 1-MA treatment, as well as phosphorylation and dephosphorylation, G-protein fluxes, and again, a lack of transcription and translational changes to complicate the mix. Thus, the investigators have a large wave to continue to ride, and having set up the important investigational criteria, can now help the field of both phosphoproteomics and reproductive biology chip away at yet another important mystery in the process of egg activation.

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Figure.