Analysis of the Guanidine Insoluble Centrosome Centromatrix in *Spisula solidissima* oocytes

by

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ABSTRACT

The centrosome is the major microtubule organizing center (MTOC) in animal cells. It is composed of two centrioles surrounded by an undefined pericentriolar material. Centrosomes isolated from *Spisula solidissima* oocytes retain microtubule nucleation potential (MNP) and nucleate and organize microtubules in defined media. Treatment of isolated centrosomes with 1.0 M KI removes MNP and 90% of centrosome protein, leaving a sedimentable, non-functional, KI-insoluble, centrosome remnant (KICR) that recovers MNP when incubated in cytoplasmic extracts of animal cells. We show that treatment of KICRs with 6.0 M Guanidine-HCl further dissociates proteins leaving a Guanidine-HCl insoluble centrosome remnant (GUCR) that also recovers MNP when incubated in cytoplasmic extracts. Trypsin treatment of KICRs or GUCRs abolishes their ability to recover MNP, indicating that proteins are required.

Electron microscopy revealed a loss of centrioles in both KICRs and GUCRs, both of which retained a centromatrix structure. In GUCRs, the area in the center of the centrosome, once occupied by a centriole, contained less electron dense material than did KICRs. Protein analysis indicates that GUCRs contain about 20-25 proteins, are less complex than KICRs, and are enriched in a number of proteins. While treatment of centrosomes with KI removes approximately 90% of the total centrosome protein, treatment with 6 M guanidine-HCl strips away even more. We determined that GUCRs represent only 6.7×10^{-6} % of total oocyte weight, and as little as 0.5% of centrosome weight. The identification of GUCR proteins is expected to lead to insights into conserved mechanisms governing centrosome assembly and function.

Mass spectrometry led to the identification of a peptide originating from a prominent 60 kDa GUCR protein whose sequence matched to translated contig TC379 in a clam EST database. The 60 kDa protein is enriched in centrosomes, KICRs, and GUCRs, and most highly enriched in GUCR fractions. This protein is referred to as "Guanidine Stable Protein" or GUSP.

Antibodies generated against peptides or expressed proteins from different regions of GUSP revealed the presence of GUSP in clam oocyte lysates, centrosomes, KICRs and GUCRs. Importantly, antibodies allowed the tracking of GUSP through various stages of biochemical purification. The analysis revealed that GUSP is sequentially enriched during purification steps such that it represents a greater amount of the fractionated protein as you move from clam oocyte lysate, to centrosomes, KICRs and finally to GUCRs. Immunofluorescence assays indicated that GUSP localizes to isolated centrosomes, KICRs and GUCRs *in vitro*. Unfortunately, several antibodies failed to provide clean localization in whole cells, including *Spisula* oocytes and mammalian HEK 239 cells

Based on the biochemical evidence, we propose that GUSP is a component of the centrosome-centromatrix. Future work will be aimed at understanding the mechanisms of the assembly of the GUCR centromatrix, localizing GUSP in whole cells, and defining the role of GUSP in centrosome function.