Characterization of the sperm factor responsible for initiating [Ca2+] i oscillations during fertilization in mammalian eggs

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Abstract

During fertilization the sperm activates the mammalian egg by eliciting $[Ca^{2+}]_i$ oscillations. However, how the sperm triggers $[Ca^{2+}]_i$ oscillations in mammalian eggs remains unknown. To test the possibility that a factors) from the sperm is able to elicit $[Ca^{2+}]_i$ oscillations, sperm fractions (factors) were prepared from different species and injected into mammalian oocytes or eggs. The results show that injection of sperm factor from either porcine or human sperm triggered long-lasting $[Ca^{2+}]_i$ oscillations in mouse oocytes and bovine eggs in a pattern similar to the physiological responses observed in each of these species, implying that the sperm factor is functionally conserved among mammalian species. In addition, sperm factor-induced $[Ca^{2+}]_i$ oscillations appeared to be mediated by the IP3 receptor, while the ryanodine receptor may be involved in the modulation of these oscillations. Furthermore, $[Ca^{2+}]_i$ oscillations induced by sperm factor are capable of initiating normal egg activation and parthenogenetic development. ^ In order to isolate and characterize the unknown active molecule(s) in sperm factor, different tissues or cell extracts were screened for the presence of sperm factor like activity. It appears that the $[Ca^{2+}]_i$ oscillation-inducing activity is sperm/testis specific. In addition, the results showed that the active component contains a protein moeity and that the same single active component of porcine sperm factor is present in both soluble and in less soluble sperm compartments. We also demonstrated that gpd/oscillin, a proposed active component of mammalian sperm factor, is not responsible for the $[Ca^{2+}]_i$ oscillation-inducing activity as shown by the lack of effects on Ca^{2+} responses or absence in active fractions following immunodepletion or a combination of fractionation techniques. Similarly, our results also showed that neither PLCγ1, PLCγ2 nor tr-c-Kit is likely to be the active component of sperm factor. ^ Although we were unable to identify a specific candidate molecule, these experiments have led to the identification of three polypeptides in the final active fraction after sequential chromatographic steps. Our results also show that the active component has an isoelectric point of 6.5–7.0 and a relative molecular weight ranging from 29–68 kDa. These polypeptides will be sequenced and used to raise monoclonal or polyclonal antibodies, which will be used to screen a testis cDNA library. This last step is expected to lead to the isolation of the gene encoding sperm factor. ^

Subject Area

Cellular biology

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