# LIVE CONFOCAL ANALYSIS OF FERTILIZATION AND EARLY DEVELOPMENT

Uyen Tram and William Sullivan

Department of Biology, Sinsheimer Labs, University of California, Santa Cruz, CA 95064

Embryonic development is a dynamic event and is best studied in live animals in real time. Much of our knowledge of the early events of embryogenesis, however, comes from immunofluourescent analysis of fixed embryos.[1,2] While these studies provide an enormous amount of information about the organization of different structures during development, they can give only a static glimpse of a very dynamic event. More recently real-time fluorescent studies of living embryos have become much more routine and have given new insights to how different structures and organelles (chromosomes, centrosomes, cytoskeleton, etc.) are coordinately regulated.[3,4,5] This is in large part due to the development of commercially available fluorescent probes, GFP technology, and newly developed sensitive fluorescent microscopes. For example, live confocal fluorescent analysis proved essential in determining the primary defect in mutations that disrupt early nuclear divisions in *Drosophila melanogaster*.[6] For organisms in which GPF transgenics is not available, fluorescent probes that label DNA, microtubules, and actin are available for microinjection.

One of the most intriguing issues of early development to which this new technology can be applied is the inheritance of centrosomes during parthenogenesis. The centrosome is the cell's main microtubule organizing center and is assembled at fertilization using components from both the sperm and egg. Previous analyses of microtubule organization in fixed embryos showed that cytoplasmic microtubule-based asters were a common feature in parthenogenetic eggs.[7,8] It was difficult, however, to determine conclusively what relationship, if any, these cytoplasmic asters had to the embryonic centrosome. To determine how centrosomes are inherited in eggs that develop in the absence of fertilization, we developed a protocol for live analysis of embryos from the haplodiploid parthenogenetic wasp *Nasonia vitripennis*; unfertilized *Nasonia* eggs develop as males while fertilized eggs develop as females.[9] By modifying available techniques in *Drosophila,* we were able to microinject a mixture of rhodamine labeled tubulin and the DNA binding dye Oligreen into embryos and follow microtubule and chromosome dynamics simultaneously in real time. We show that centrosomes are inherited differently in unfertilized and fertilized eggs. Numerous cytoplasmic microtubule asters appear in the egg cytoplasm at the end of meiosis in both fertilized and unfertilized eggs. In unfertilized eggs, exactly two asters become associated with the female pronucleus and these two function as the zygotic centrosome; the free asters degenerate. In fertilized eggs, the sperm organizes two centrosomes which then function as the zygotic centrosome. The maternal asters do not associate with either the female or male pronuclei and degenerate, as in unfertilized eggs. Centrosome inheritance thus occurs reciprocally: unfertilized male embryos inherit maternal centrosomes while fertilized female embryos inherit paternally derived centrosomes. With live analysis, we are able to definitively show that centrosomes in parthenogenetically developing embryos are inherited from a maternal pool of cytoplasmic asters. Additionally, live analysis

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revealed that the number of centrosomes, whether inherited maternally or paternally, is precisely regulated. Each zygotic nucleus becomes endowed with exactly two centrosomes and once this happens, additional centrosomes are not captured and eventually disappear. This suggests an unsuspected mechanism of regulating centrosome number, which may operate in mammalian cells as well.

**References:**

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