ORC 1 is Required for the First Round of DNA Synthesis in the Zygote

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The origin recognition complex (ORC) is a six-subunit complex that identifies origins of replication. It was originally discovered in yeast but is also crucial for mammalian cells. ORC subunit 1 (ORC1) is the final subunit that binds to the complex, and the first to be releases as each origin is fired during replication. We have previously demonstrated that one of the other ORC subunits, ORC4, that is part of the complex that binds to origin before ORC1 is absolutely required for zygotic DNA synthesis. However, previous studies have shown that certain cancer cells can replicate DNA in the absence of ORC1, and it was possible that the zygote might replicate DNA without ORC1. We crossed knock-in mice with loxP sites flanking exons with *Zp3-Cre* mice to delete *Orc1* exons 9-14 during oogenesis to generate *Orc1*-CKO mice that had oocytes that did not contain *Orc1*. These *Orc1*-CKO oocytes were parthenogenetically activated and tested for DNA replication with EdU (5-Ethyl-2'-deoxyuridine) incorporation. We found that 55% of parthenogenetically activated oocytes had positive EdU staining. All parthenogenotes progressed to the 2-cell stage, but only 37% of 2-cell embryos were EdU positive. The large majority of these embryos were arrested at the 2-cell stage, but some developed to 3-cell embryos before arrest. When *Orc1*-CKO oocytes were injected with wild-type sperm, the DNA replication was similar; 55% and 57% of 1 cell zygote and 2-cell embryos were EdU positive, respectively. However, some of these ICSI generated embryos progressed to the blastocyst stage; and only 19% reached blastocyst stage. These data suggest that ORC1 is required for complete DNA replication in the zygote, and clearly required for the progression beyond the 2-cell stage. It also suggests that ICSI can partially rescue ORC1 deletion in the oocyte by providing a new, paternal, *Orc1* gene.