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Gradual Loss of a DNA-Inducible Protein Kinase Activity From the Cytoplasmic Extracts of *Arbacia* Embryos

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The DNA-dependent protein kinase is a serine/threonine protein kinase that requires nicked double-stranded (ds) DNA for its activity (1). It plays a crucial role in DNA repair (2), but its role in development is not clear. DNA-activated protein phosphorylation has been reported in extracts from cultured human cells and the oocytes or the embryos of several marine invertebrates (3). Herein, we show that the cytoplasmic extracts of sea urchin (*Arbacia punctulata*) eggs contain a kinase that is inducible by exogenous dsDNA only after fertilization and is not detectable in cytoplasmic extracts prepared from blastulae, gastrulae, and plutei.

Homogenate-catalyzed DNA-dependent phosphorylation assays performed in cytoplasmic extracts of eggs and embryos demonstrate that unfertilized eggs lack the DNA-inducible kinase activity (Fig. 1). However, the enzyme activity is seen in extracts from fertilized eggs. A gradual decline in the cytoplasmic enzyme activity is observed through 50 (first cleavage) and 103 (second cleavage) minutes post-fertilization. Cytoplasmic extracts prepared from the blastulae, gastrulae, and plutei also lack the DNA-inducible phosphorylation activity. Unfortunately, we could not perform a meaningful enzyme assay in whole cell extracts because cell sonication generates nicked nuclear DNA. For this reason, the control reaction in the absence of DNA could not be performed in these extracts since α -casein is a non-specific substrate for DNA-dependent protein kinase and can be phosphorylated by other kinases. Therefore, a specific substrate is needed to assay for the specific enzyme activity in whole cell extracts.

These results demonstrate the presence of a DNA-dependent protein kinase in the cytoplasm of eggs and embryos of sea urchin. The rapid appearance of activity in the cytoplasmic extracts from fertilized eggs could be due to either the synthesis of the enzyme itself or the activation of preexisting inactive enzyme by post-translational modification. However, we can not rule out the possibility that the enzyme is nuclear in unfertilized eggs and is released after fertilization.

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Literature Cited

1. Carter T, Vancurova I, Sun I, Lou W, DeLeon S. Mol. Cell. Biol. 1990; 10:6460–6471. [PubMed: 2247066]
2. Finnie NJ, Gottlieb TM, Blunt T, Jeggo PA, Jackson SP. Proc. Natl. Acad. Sci. USA. 1995; 92:320–324. [PubMed: 7816841]
3. Walker AI, Hunt T, Jackson RJ, Anderson CW. EMBO J. 1985; 4:139–145. [PubMed: 4018025]
4. Ballinger DG, Bray SJ, Hunt T. Dev. Biol. 1984; 101:192–200. [PubMed: 6319208]

Figure 1.

Autoradiogram shows variation in DNA-dependent protein phosphorylation activity during embryonic development. Cytoplasmic extracts from eggs and embryos were prepared as already reported (4). A 15 μ l reaction required 10 μ l of cytoplasmic extract, 75 ng of sonicated calf thymus DNA, 15 μ g dephosphorylated α -casein (Sigma) as substrate, 2 mM $MgCl_2$, and 130 μ M ATP. The reaction was carried out at 15°C, and started by adding 10 μ Ci of [γ - ^{32}P]ATP (6000 Ci/mmol) (NEN, Du Pont). The phosphorylation reaction (1 μ l) was analyzed on a 10% polyacrylamide gel (SDS-PAGE). E—extract only (without any exogenous substrate); C—extract with exogenous α -casein added as an exogenous substrate. Arrows indicate the position of phosphorylated casein. Absence and presence of dsDNA in the extract are indicated by – and +, respectively.