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A DNA-Inducible Kinase Activity Undergoes a Change in Cellular Localization During Development in *Arbacia*

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DNA-dependent phosphorylation has been detected in various cell types from a wide range of organisms. The enzyme responsible for this activity in human cell lines, DNA-dependent protein kinase (DNA-PK), has been well characterized. Human DNA-PK is composed of two subunits—a regulatory subunit (Ku) and a catalytic subunit (p460, previously known as p350) and has been shown to have roles in transcription, recombination and repair of DNA (1–4).

A DNA-dependent kinase activity has previously been found in crude extracts from fertilized sea urchin eggs (5, 6). *In vitro* kinase assays have shown that this activity is present in cytoplasmic extracts of fertilized *Arbacia* eggs only up to the 4-cell stage (6). In this report we further examine the cellular localization of DNA-dependent kinase activity in urchin development.

We performed *in vitro* DNA-dependent kinase assays using a specific peptide derived from p53—a known substrate of human DNA-PK (7). This enabled us to compare the relative levels of activity in both cytoplasmic and whole cell extracts from different stages of development. As in the assays performed with α -casein as a substrate (6), we were unable to induce the kinase activity in extracts from unfertilized eggs, although a high level of DNA-inducible activity is present in both cytoplasmic and whole cell extracts of fertilized eggs (Fig. 1a). This activity gradually decreases in cytoplasmic extracts of 2- and 4-cell stages (up to 103 min post-fertilization) and is not detectable in cytoplasmic extracts from the blastula stage onward. Sonicated whole-cell extracts from all stages post-fertilization did, however, contain high levels of inducible kinase activity. This result suggests that the cytoplasmic activity in the fertilized egg becomes localized to the nuclear fraction in embryos at later stages.

A monoclonal antibody (mAb 42-26) raised against p460, the catalytic subunit of human DNA-PK (2), was used to probe western blots of cytoplasmic extracts from eggs and developing embryos. The serum recognizes a protein of ~ 460 kDa in both unfertilized eggs and early embryos of all stages as well as protein of similar size in human HeLa cell extracts (Fig. 1b). This protein is thought to represent the urchin homolog of the catalytic subunit of DNA-PK.

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Lastly, we used the anti-human p460 monoclonal serum to immunostain *Arbacia* eggs and embryos (Fig. 1c). The urchin p460-related protein appears to be exclusively cytoplasmic in unfertilized eggs and then undergoes a change in localization to the nuclei in cleaving embryos.

These results confirm the presence of a DNA-dependent protein kinase activity in fertilized urchin eggs. *In vitro* kinase assays reveal that the localization of this activity changes from the cytoplasm in fertilized eggs to the nuclei in later stages of development. A protein related to human p460 is present in all stages of *Arbacia* development and may represent the catalytic subunit of urchin DNA-PK. The change in localization of this p460-related protein from the cytoplasm to the nuclei after fertilization is consistent with the change in localization of DNA-inducible kinase activity in specific *in vitro* kinase assays. Although the role of the DNA-PK activity in *Arbacia* development is not yet known, we suggest that the enzyme is activated upon fertilization and may be required during the early rapid cell cycles in embryogenesis.

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Figure 1.

(a) DNA-dependent kinase activity of cytoplasmic and sonicated whole cell extracts from different stages of *Arbacia* development. Kinase reactions were set up exactly as in (6) except that a specific peptide derived from p53 (EPPLSQEAFADLWKK) was used as substrate (8). Reaction products were spotted onto p81 phosphocellulose paper, which was washed successively with 30% acetic acid, 15% acetic acid, and then 5% acetone. The ^{32}P incorporated on the dried filters was quantitated by scintillation counting, (b) Immunoblot analysis of extracts made from eggs and embryos of *Arbacia* (prepared as in (7)). Extracts were run on a 7.5% SDS gel, transferred to nitrocellulose, and processed for immunoblotting using a monoclonal antibody (mAb 42-26) against human p460 and an anti-mouse alkaline phosphatase-conjugated secondary antibody. The position of molecular weight markers in kDa are indicated on the left, (c) *In situ* whole-mount immunostaining of *Arbacia* eggs and embryos using mAb 42-26. Unfertilized eggs (A and B), 2- to 4-cell embryos and morula (C

and D) were immobilized on poly-L-lysine coverslips and fixed in 3.7% formaldehyde, Q. 05% NP-40 in PBS for 8 min. After incubation in blocking solution (10% goat serum/1% BSA in PBS) for 30 min at room temperature, the coverslips were treated with either mouse pre-immune IgG (A and C) or with mAb 42-26 anti-p460 (B and D), each at 1:50 in the block solution for 30 min at room temperature. After washing in PBS, the coverslips were incubated with a fluorescein-conjugated goat anti-mouse secondary antibody (1:64; Sigma) for 30 min and then washed again in PBS before being viewed with immunofluorescence. Scale bars: A and B, 27 μ m; C and D, 14 μ m.