doi: 10.1387/ijdb.092873eb

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

SUPPLEMENTARY MATERIAL

corresponding to:

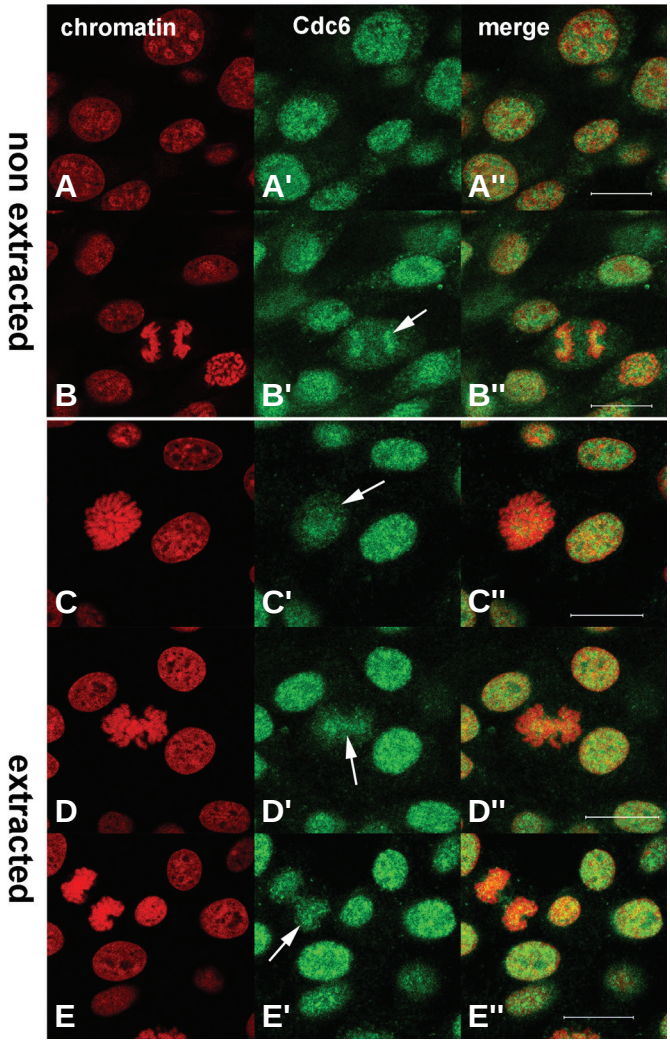
Factors engaged in reactivation of DNA replication in the nuclei of growing mouse oocytes introduced into the cytoplasm of parthenogenetic one-cell embryos

EWA BORSUK and RENATA CZOLOWSKA

Immunodetection of Cdc6 in somatic cells

The specificity of the anti-Cdc6 was verified by immunostaining of NIH3T3 somatic cells grown in culture, submitted or not to the extraction procedure. NIH3T3 cells, which were the generous gift from dr. Miaczynska (International Institute of Molecular and Cell Biology, Warsaw, Poland), were grown in Dulbecco's modified EaglE's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Biochrom, Berlin, Germany) and antibiotics (Invitrogen, Eugene, USA) in the presence of 5% CO_2 under humidified atmosphere. Cells were seeded on 22 mm glass cover slides, each placed in a 35 mm culture dish. After 48 h of culture cells confluent in 80-90% were used for extraction procedure and/or immunostaining. The extraction was performed according to the method described by Todorov *et al.* (1995). Extracted and non-extracted cells were fixed in 4% PFA in PBS_A for 20 min. Non-extracted cells were than permeabilized in 0.2% Triton X-100 for 20-30 min. Cells were blocked in 2% BSA in PBS_A overnight. The Cdc6 protein was detected by mouse monoclonal anti-Cdc6 antibody (Molecular Probes, Eugene, USA) used in the same concentration as for the oocytes and hybrid cells. The Vectashield mounting medium with propidium iodide (Vector Laboratories Inc., Burlingame, CA, USA) was used to prepare the slides for microscopy and stain the chromatin.

In NIH3T3 cells the Cdc6 protein was detected in both the soluble and the insoluble form. Nonextracted cells stained for total Cdc6 showed uniform signal in the nucleus and weak, diffuse signal in the cytoplasm of interphase cells (Supplementary Fig. S1. A', B'). In dividing cells increased signal for total Cdc6 was observed on the telophasic groups of chromatin and weak staining was present in the cytoplasm (Supplementary Fig. S1. B'). The insoluble form of the protein co-localized with the chromatin during interphase (Supplementary Fig. S1. C', D', E'), but was not detected in the cytoplasm of these cells. During mitosis the distribution of insoluble Cdc6 differed in different stages of the division. At metaphase and anaphase this staining was very faint, while at telophase it was stronger and concentrated on the condensed groups of chromatin (Supplementary Fig. S1. D', E'; arrows).



Supplementary Fig. S1. Distribution of Cdc6 protein in the nuclei of non-extracted and extracted NIH3T3. (A-E) *Chromatin stained with propidium iodide (colored in red), total Cdc6* **(A', B')** *and insoluble form of Cdc6 protein* **(C'- E')** *(colored in green).* **(A" – E")** *Merge of chromatin and Cdc6. Bar 20 μm. In non-extracted cells, soluble and insoluble forms of Cdc6 were detected in the nuclei of cells in interphase or on groups of telophase chromatin (B', arrow). A weak cytoplasmic signal of Cdc6 is present in both interphase and mitotic cells. In extracted cells, a clear signal is visible in interphase nuclei and none in the cytoplasm. In the prophase of mitosis, a weak signal is observed around the group of chromosomes (C', arrow). It becomes more concentrated on the two groups of chromatin in anaphase (D', arrow) and telophase (E', arrow).*