Permeable embryonic cells of sea urchins as a model for studying nucleus-cytoplasm interactions[1]

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Summary. Sea urchin embryonic cells were made permeable by treating them with glycerol solution for the purpose of exchanging cytoplasmic components. When proteoglycans prepared from embryos of advanced stages of development permeated into these glycerinated cells, these substances accumulated rapidly in the nucleus, being bound with chromatin.

We have reported previously that sulfated proteoglycans play crucial roles in cell differentiation during early stages of the development of sea urchins, acting as a cytoplasmic factor capable of modifying gene activity in the nucleus. This proposition was based on the following facts: a) developmental arrest was induced in sea urchin embryos at the blastula stage, when they were treated with several kinds of inhibitors of proteoglycan synthesis[3]. This inhibition was cancelled by the simultaneous addition of proteoglycans obtained from normal embryos at the post-gastrular stage[4]; b) sea urchin eggs microinjected with proteoglycans ceased to develop at a stage corresponding to the stage at which proteoglycans were extracted[4], and proteoglycans exhibited some stage-specificity in electrophoretic patterns in SDS-polyacrylamide gels[5]; c) the chromatin structures were loosened microscopically on treating the isolated nuclei with proteoglycan or the polysaccharide moiety of proteoglycan[5], and at the same time, an increase in hyperchromicity was observed at the lower melting temperature in thermal denaturation profiles of the treated chromatin[6]; d) RNA synthesis in vitro in nuclei isolated from pre-gastrular embryos was enhanced by adding post-gastrular proteoglycans[5].

In order to obtain further information on the mode of action of proteoglycans as related to the mechanism of gene activation needed for the progression of development, we attempted to introduce heterogenous cytoplasm into permeabilized embryonic cells of sea urchins by means of glycerol treatment. Attempts to permeabilize the eukaryotic cells by using several solvents other than glycerol have been reported[7-13].

The glycerinated cells (referred to as permeable cell models) were prepared according to the gravitation method developed by ourselves[14] with several improvements. Embryos of the sea urchins (*Hemicentrotus pulcherrimus* and *Strongylocentrotus nudus*) at any stage of development, which were deprived of fertilization membrane by treating with 1 mM 3-amino-1,2,4-triazole[15] (Wako Pure Chemicals, Tokyo), were placed on the top of a vertical glycerol gradient (continuous from 10 to 50%) containing 150 mM KCl, 50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol and 1 mM phenylmethyl sulfonyl fluoride. They were allowed to sink slowly by their own weight through the gradient at 0 °C, during which time they were gradually glycerinated without severe damage to the cytoplasmic or nuclear structures. The time required for an egg to pass through the entire gradient (about 25 cm in distance) is about 8 h. The permeable cell models can be directly treated with test reagents, if the reagents are made in 50% glycerol. If necessary, the cell models can gradually be returned to aqueous solutions, although they become fragile to mechanical agitation.

The accomplishment of permeabilization in cell models was checked by the penetration through the cell membrane of Blue Dextran (BD) 2000 (Pharmacia, Uppsala), which is a dextran of high molecular weight (2,000,000 average) conjugated with blue dye. When cell models were incubated with BD solution, they were infiltrated with BD until an equilibrated state was achieved after 1 h (fig. 1a). Living cells were not infiltrated at all after incubation for 3 h.

The stoichiometry of the amount of BD which infiltrated into cell models was examined using unfertilized egg cell models (for convenience of calculation). Since the final concentration of BD solution was 20 $\mu g/mm^3$ (2%) and the total volume of the soaked egg cells was 0.90 $mm^3/10^3$ eggs (calculated on the assumption that the average diameter of

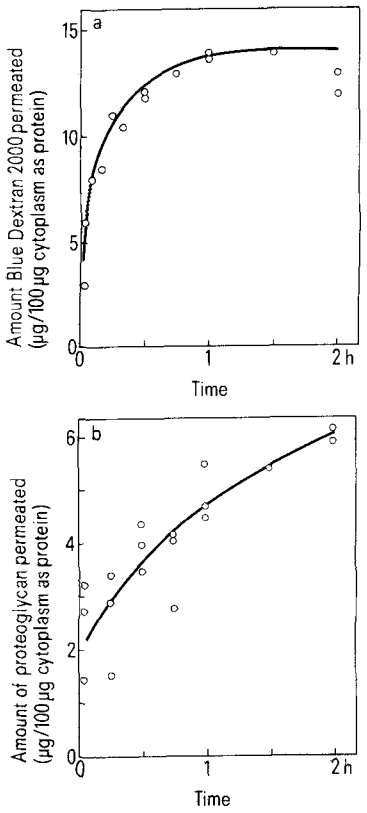

Figure 1. *a* Permeation of Blue Dextran (BD) 2000 into glycerinated cell models (64-cell stage). To 2 ml of 2.5% BD solution was added 0.25 ml of cell model suspension. The dye and cell models were in 50% glycerol containing 150 mM KCl, 50 mM Tris-HCl (pH 7.4) and 10 mM $MgCl_2$. The mixture was incubated at 22 °C with gentle shaking, and after 2 quick washes with 50% glycerol solution the cell models infiltrated with BD were extracted with 5 ml of cold 5% trichloroacetic acid. The clear supernatant was obtained by centrifugation at 10,000 × g for 20 min. The absorbance at 610 nm was measured with BD solution of known concentration serving as the standard. *b* Permeation of fluorescein-labeled proteoglycans into glycerinated cell models (64-cell stage). To 1.5 ml of proteoglycan solution (obtained from gastrulae) was added 0.5 ml of cell model suspension. The final concentration of proteoglycan was 1.86 mg/ml mixture. The conditions of incubation were the same as in the case of BD. After incubation, the cell models were washed quickly with the glycerol solution 3 times by gentle centrifugation. The cell models infiltrated with fluorescein-labeled proteoglycan were homogenized, and an aliquot (1.5 ml) was digested with Pronase E (1 mg) at 37 °C overnight. The digest was diluted with an equal volume of water and centrifuged at 22,500 × g for 30 min. The fluorescence intensity was measured by a fluorophotometer (590 nm for excitation and 515 nm for emission).

egg cell models was 120 μm), the amount of BD enclosed in the egg cell models was found to be 22.8 $\mu g/10^3$ eggs. This value coincided closely with the experimentally determined value of 22.5 $\mu g/10^3$ eggs.

Next, fluorescein-labeled proteoglycan (prepared from embryos at the gastrula stage by means of Na-pyrophosphate extraction and DEAE-Sephadex chromatography as previously described[5]) was infiltrated into cell models (8–16-cell stage) by incubation. An equilibrated state was not achieved during incubation for 2 h, and cell models seemed to accumulate the proteoglycans only gradually (fig. 1b).

Examining the distribution of dye-labeled proteoglycans in the cell models under a fluorescence-microscope, it was found that this substance was steadily concentrated into the nucleus (fig. 2), suggesting that the proteoglycans introduced into the cell models have an intense affinity with the nuclear structures. The accumulation of dye-labeled proteoglycans in the nucleus was also ascertained by examining the localization of fluorescein in subcellular structures after fractionation of cell models; accordingly, 73.3% of dye was recovered in the sedimentable portion at 900 × g (nuclei were predominant), 14.0% in the sediment at 105,000 × g (microsomal fraction) and 9.8% in the unsedimentable portion at 105,000 × g (cytosol).

The heterogenous combination of nucleus and cytoplasm can be achieved by means of transplantation of the nucleus[16,17], cell fusion[18,19], and so-called cybridization[20], and these procedures have made important contributions to elucidating the mechanism of nucleus-cytoplasm interactions. Complementary to these techniques, the use of glycerol-treated cell models has the added merit that the essential reactions between nucleus and cytoplasm are observed separately from other complicated reactions occurring in living cells. The situation is comparable to the utilization of glycerinated cell models in the study of cell motility[21].

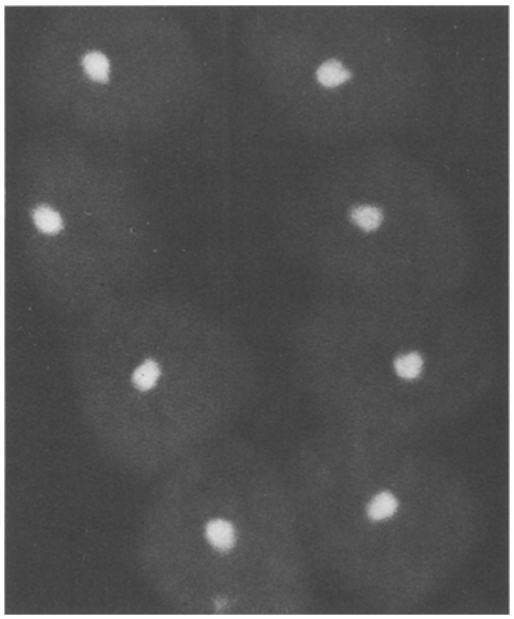

Figure 2. Cell models (8-cell stage) incubated with fluorescein-labeled proteoglycans for 1 h. Accumulation of proteoglycan into the nucleus is very noticeable. The condition of incubation was the same as in figure 1b. (× 500).

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Species-specificity of paragonial substances as an isolating mechanism in *Drosophila*[1]

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Summary. In 2 closely-related species of *Drosophila*, the oviposition stimulating effects of the substances produced in the male accessory glands (paragonia) are species-specific. A unique reproductive isolating mechanism due to this specificity is discovered.

In many insects, substances produced in the male accessory glands and transmitted to the female during copulation are known to stimulate oviposition[2]. If the effects of these substances were species-specific, they should function as reproductive isolating mechanisms by preventing the wastage of female gametes following erroneous heterospecific mating[3]. However, there has been little evidence for this type of isolating mechanism despite the extensive variations of accessory gland substances among species[4,5].

Drosophila suzukii and its close relative, *D. pulchrella*, were