
A PRELIMINARY REPORT ON EARLY CELL TYPE-SPECIFIC ANTIBODIES IN XENOPUS LAEVIS

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In recent years much work has been done to find biochemical markers of developmental stages. Amphibian embryos are a focus of attention1–3 in these studies. Such markers are temporally or regionally restricted in early amphibian development4,5. There are still very few cell type markers for early amphibian embryos. The aim of the present investigation was to obtain monoclonal antibodies against early germ layer-specific antigens. The monoclonal antibody 3D7 recognizes an antigen that generally appears to be localized close to the cell membrane and also all over ectodermal cells in the early development of Xenopus laevis.

Embryos obtained via artificial fertilization by standard procedures were staged according to Nieuwkoop and Faber6. Embryos were dejellied in 2% cysteine hydrochloride (Sigma), pH 8.0, and then cultured in MMR (pH 7.8).

Monoclonal antibodies were raised against a crude cell homogenate from late blastula stage of X. laevis embryos. Immune mouse splenocytes were fused with Sp2/O-Ag14 myeloma cells7 using published procedures8. Hybridomas that gave positive reaction were subcloned twice by limiting dilution. Screening of hybridoma supernatants was by ELISA using a cell homogenate from embryos at blastula stage. Colour development was measured at 492 nm in a Titerpak Multiscan. Hybridomas that gave positive reaction were further tested by immunocytochemistry. Monoclonal antibody 3D7 was used in the study.

Embryos were fixed in 0.1% glutaraldehyde/2% paraformaldehyde for 6 h at room temperature. After fixation embryos were washed in PBS, dehydrated with ethanol, and embedded in paraffin. Sections were cut on a Spencer 820 (Lameris, Utrecht) microtome, collected and dried at 37°C for 24 h. Sections were deparaffained with xylol and ethanol. Incubation with 3D7 ascites fluid (1:1000 dilution) for 60 min at 37°C was followed by 3 washes in PBS before incubation with a 1:50 dilution of goat anti-mouse IgM(Fc)–FITC (fluorescein isothiocyanate, Nordic) for 60 min at 37°C. Slides were washed with PBS, mounted in PBS–glycerol (1:1), and examined under a Leitz fluorescence microscope.

In control experiments, cryostat sections of unfixed or methanol-fixed embryos were used. Antibody localization was the same, but the quality of sections was poor and the background immunofluorescence high. Of all fixation procedures tried, the glutaraldehyde/formaldehyde fixation combined with paraffin embedding gave the best signal-to-background ratio.

The temporal and spatial pattern of 3D7 immunoreactivity analysed by immunocytochemistry shows many interesting features. In the unfertilized egg, the antigen is not localized but evenly distributed over the cytoplasm (figure 1, a). By 30 min post-fertilization, however, a striking redistribution of the antigen occurs (figure 1, b); the antigen becomes localized in a broad band in the periphery of the egg. In the cleavage stages, the antigen remains localized in the outer periphery of the cytoplasm (figure 1, c and d). When more cell layers develop, this outer-peripheral localization is restricted to the peripheral cell layer (figure 1, d). The intracellular localization is also restricted to the peripheral cell layers in the late blastula and early gastrula stages (figure 2, a). When the embryo gastrulates, the antigen become localized in the blastopore region,
both dorsally and ventrally (figure 2, b). Staining was found in the cells lining the blastopore and in the extracellular space between the two cell layers in the presumptive archenteron. No antigen was detected between the involuting mesoderm and the overlying ectoderm, the area in which both fibronectin and laminin accumulate during gastrulation. During neural stages, however, the antigen is found only in the ectoderm, the presumptive epidermis (figure 2, c). After stage 20 the antigen disappears completely. Up to the neurula stage the antigen is present both in the intracellular and extracellular matrix. The cell membranes are also stained and staining is observed between the individual cells (figure 1, b–d; figure 2, d).

There are various processes related to germ layer differentiation and pattern formation, which can only be followed with the help of cell marking experiments using appropriate antibodies. The present work describes the appearance and distribution of an antigen recognized by the monoclonal antibody 3D7 for *X. laevis*.

The most notable facts concerning the localization of the antigen are:

i) The antigen is localized in a broad, bright, peripheral band immediately after fertilization.

ii) The localization changes dramatically during the earliest embryonic stages. Intracellular localization is restricted to the periphery of the embryo; extracellularly, it is found between all cells in the embryo. During gastrulation, the antigen also becomes localized in the blastopore region, inside the cells and between the cell layers that line the presumptive archenteron.
Figure 2. Distribution of antigen recognized by monoclonal antibody 3D7 in sections of early *Xenopus laevis* embryos. a, Stage 7 embryo; b, stage 8 embryo; c, stage 9 embryo; d, peripheral cell layer staining. (a, b, c, × 6.3; d, × 40).

The cause of the cyclic band pattern remains unclear. Biochemical characterization of the antigen is being carried out.

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