Membrane changes in neural target cells studied with fluorescent lectin probes

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SUMMARY

The competent ectoderm of Pleurodeles waltl comprises two cell layers with characteristic differences in their morphology, their composition and the molecular arrangement of the various constituents.

The use of labelled lectin probes for observations of ectoderm tissue in vitro with u.v. microscopy (epi-illumination) and the quantification of the results show the following:

1) Differences in labelling according to the nature of the lectins (SBA, PSA, LCA and Con A). These differences provide information on the nature of the carbohydrates which are present at this stage and on the number of receptors.

2) Differences in fluorescence intensity of the surfaces studied. The internal surface of the ectoderm is labelled more densely than the external surface.

3) Rearrangement of the lectin receptors with a new molecular configuration, stressing the fluidity of the membrane (by the mobility of the receptors throughout the membrane) and its importance for the occurrence of neural induction.

4) Existence of membrane glycoconjugate turnover.

5) A difference in behavioural characteristics between the internal and the external surfaces with respect to the lectins and the formation of an extracellular matrix on the internal surface alone. The extracellular matrix seems to have a role in morphogenetic movements.

INTRODUCTION

It has been shown that the presumptive ectoderm cells of gastrulae exhibit characteristic changes during development (Tarin, 1971; Monroy, Baccetti & Denis-donini, 1976; Kohonen & Paranko, 1978; Grunz & Staubach, 1979). As we have already reported (Duprat, Gualandris & Rouge, 1982), the molecular organization of the plasma membrane of the target cell seems to play a crucial role at the gastrula stage in the process of neural induction. The plasma membrane must possess a special organization and possibly the process of induction itself results from special molecular reorganizations in the same membrane (Takata, Yamamoto & Ozawa, 1981).

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Therefore since many important developmental phenomena must operate through cell surfaces, the aim of this work is to study certain cell surface properties of the responding tissues in neural determination.

Lectins are a group of proteins (essentially glycoproteins) which are able to bind specifically to carbohydrate moieties on the cell surface. Such marker probes have been employed in amphibians (Johnson & Smith, 1976; Nosek, 1978; Tencer, 1978; Boucaut et al. 1979; Barbieri, Sanchez & Delpino, 1980; Takata, Yamamoto & Ozawa, 1981) using fluorescent-labelled lectins to visualize and study the dynamics of cell surface glycoconjugates in morphogenesis.

We have previously reported the important roles played by cell surface glycoconjugates and their topography in the process of neural induction. The present experiments were designed to elucidate the nature and turnover of the glycoconjugate molecules present on target cell surfaces of *Pleurodeles waltl*, which might be involved in this process. We used fluorescent-labelled lectins which were specific for N-AC-α-D-galactosamine, α-D-galactose (SBA); α-D-mannose and α-D-glucose (PSA, LCA and Con A).

**MATERIALS AND METHODS**

*Pleurodeles waltl* gastrulae (stage 8) were used as donors of presumptive ectoderm and were staged according to the table of development established by Gallien & Durocher (1957).

After removal of the jelly coat and the vitelline membrane, the ectoderm (target tissue) was microsurgically excised in Holtfreter solution including penicillin (100 i.u. ml⁻¹); streptomycin (100 μg ml⁻¹) and buffered with 5 mM-Tris; pH 8.0.

These ectoblast explants were incubated for various lengths of time (1, 3, 15 & 30 min) at 20 °C in Holtfreter medium containing lectin (50 μg ml⁻¹) (Gualandris & Duprat, 1981; Duprat et al. 1982). They were then washed thoroughly with Holtfreter solution and observed *in vitro*.

1. *Preparation of lectins*

CH-Sepharose 4B (which is formed by covalent linkage of 6-amino hexanoic acid to CNBr-activated Sepharose 4B) and Sephadex G100 were products of Pharmacia, Uppsaal, Sweden; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl was obtained from Merck, Darmstadt, West Germany. Fluorescein isothiocyanate (FITC, isomer I) and purified bovine serum albumin were obtained from Sigma, St Louis, U.S.A. Acrylamide, bis-acrylamide, T.E.M.E.D. and Coomassie brilliant blue R were from BioRad, Richmond, U.S.A. Ultragel ACA 202, di-chlorotriazinyl-fluorescein (D.T.A.F.) and tetramethyl-rhodamin-isothiocyanate (TRITC) were supplied by IBF, Villeneuve-la-Garenne, France. N, N-dimethyl-formamide was purchased from Fluka, Buchs, Switzerland, and
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Jack-bean meal from Serva, Heidelberg West Germany. All other reagents were commercial preparations of Merck (pro analysis grade).

Soy-bean lectin: SBA

Soy-bean lectin (SBA) was purified by affinity chromatography on Sepharose-N-caproylgalactosamine prepared according to Allen & Neuberger (1975), and frozen at −30°C until analysis but for no more than 2 months.

Pisum sativum lectin: PSA

Garden-pea lectin (PSA) was isolated by affinity chromatography on Sephadex G100 (Agrawal & Goldstein, 1967) and then stored frozen at below −30°C.

These methods have been published (Duprat et al. 1982).

Lens culinaris lectin: LCA

Preparation as for PSA, but with lentil meal.

Canavalia ensiformis lectin: Con A

Preparation as for PSA, but with Jack-bean meal.

2. Purity of lectins

The purity of the SBA, PSA, LCA and Con A preparations (Fig. 1) was checked by polyacrylamide gel electrophoresis according to Davis (1964), at a constant current of 2 mA/gel. Protein fractions were fixed and stained with Coomassie brilliant blue according to Chrambach, Reisfeld, Wyckoff & Zaccari (1967).

The protein content of the SBA, PSA, LCA and Con A preparations was estimated by the microbiuret procedure of Goa (1953) using a bovine serum albumin standard.

3. Lectin labelling

As previously described (Duprat et al. 1982), fluorescent-labelled lectins (FITC-SBA, FITC-PSA, FITC-LCA) were prepared according to the slightly modified procedure of The & Feltkamp (1970).

Labelling of SBA, PSA and LCA by TRITC was performed under the same conditions as FITC labelling, but using a fluorochrome solution of twice the concentration, since TRITC labelling is less effective than FITC labelling. However, labelling of Con A was conducted using a modified procedure, DTAF instead of FITC was used. TRITC or DTAF were dissolved in N, N-dimethylformamide prior to coupling; 40 μg of TRITC or 20 μg of DTAF per mg of protein were added to lectin solubilized in 0.1 M-carbonate buffer pH 9.0 under continuous stirring. The reaction was continued in the dark, at 4°C for 4 h. The
Fig. 1. Purity of lectins by polyacrylamide gel electrophoresis: At alkaline pH PSA shows two main bands corresponding to the isolectins previously described (Entlicher, Kostir & Kocourek, 1970). In the same conditions SBA also shows two bands while at acid pH only a single band is obtained. Clearly the above data are not correlated with the polypeptide compositions of the lectins since SBA is a tetramer of almost identical subunits, while PSA is a tetramer consisting of two types of markedly different subunits (Lis & Sharon, 1981). Con A gives a single band under the same conditions, however gels show Con A with probably degradation products (Liener, 1976). LCA, which comprises two isolectins (Howard & Sage, 1969) gives two closely related bands on electrophoresis.

labelled lectins were separated from free DTAF or TRITC by filtration through a column of Ultra gel ACA 202 using 0.05 M-Tris, 0.15 M-NaCl pH 7.6 as eluent. Fig. 2A, 2B, illustrate these two described methods to separate labelled lectins from free fluorochromes.

The labelled lectins were subsequently concentrated by precipitation with 70% saturated ammonium sulphate. After extensive dialysis against 0.05 M-Tris, 0.15 M-NaCl pH 7.6 the lectin solutions were stored frozen and always used within two months in order to prevent any possible molecular aggregation or release of free fluorochrome.

As in the case of unlabelled lectins, the purity of the labelled lectins was checked by polyacrylamide gel electrophoresis and result was identical to that shown Fig. 1.

4. Fluorescence microscopy

A Leitz Dialux epifluorescence microscope with an incident source HBO 50
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Fig. 2A. Isolation of TRITC-labelled by affinity chromatography on Sephadex G 75 column. Arrow 1: 0.05 M-Tris, 0.15 M-NaCl pH 7.6 buffer. Arrow 2: identical buffer + 0.1 M-glucose. The first peak corresponds to free TRITC and the second to the labelled LCA released by addition of glucose to the elution buffer, the same pattern is observed for FITC-LCA, FITC-PSA and TRITC-PSA.

Fig. 2B. Isolation of FITC-labelled SBA by chromatography on a ACA 202 column. 1st peak: labelled lectin. 2nd peak: free fluorochrome. The same pattern is observed for TRITC-Con A, TRITC-SBA and DTAF-Con A.

Orthomat with filter I2 (BP 450-490, LP 515), was used to examine cells for FITC labelling and with filter M2 (BP 546/14., LP 580) in order to examine cells for TRITC labelling. Fluorescence micrographs were made on Ektachrome 160 ASA film with a Wild MPS 45 camera.
5. **Fluorescent lectin inhibition**

The binding of each fluorescent lectin was prevented when its inhibitory sugar was co-present in the incubation solution, or when the labelling lectin was pre-incubated in the suitable inhibiting sugar, at the concentration of 0.1M (SBA, α-D-gal; PSA, LCA and Con A, α-D-man).

6. **Quantitative results of fluorescence**

Quantification of the fluorescence intensity was performed with a Leitz microscope coupled to an MP V2 type photomultiplier. The measurements in Table 1 were made at 0.6 kV (S = 1) and at 0.5 kV (S = 1) in Table 2.

The explant was scanned through a window of 178μm diameter (covering 18 to 20 cells at a time) and the fluorescence of each explant surface was measured at 20 to 25 spots so as to cover its whole area. Each experiment used at least three explants. These series of measurement supplied a set of intensity values (arbitrary units) from which the means were determined.

The test of Kolmogorov-Smirnov adapted for the study of two samples of non-normal distribution was used to interpret the results (Siegel, 1956).

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**RESULTS**

*In situ*, the presumptive ectoderm of *P. waltl* consists of a double cell layer (Fig. 3). Intact ectoderms were used immediately after their dissection from gastrulae and observation with bright-field microscope revealed that the external side comprises small highly pigmented polyhedral cells (Fig. 4), while the internal side is formed of larger spherical cells and is only very slightly pigmented (Fig. 5).

**I. Reorganization of lectin receptors**

1. **Control observations**

Explants not treated by fluorescent lectins did not show any autofluorescence. Lectin binding is rapid: explants exhibit fluorescence after an incubation of 1 min. The same pattern of fluorescence was also found when the explants were treated for longer times (3 min or 15 min). Whether the lectins were labelled with

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**Fig. 3.** Histological section (7.5μm; U.N.N.A. colouration) of presumptive ectoderm of gastrula (stage 8a) of *Pleurodeles waltl*. Two cell layers are visible. Bar = 10μm.

**Fig. 4.** External surface of presumptive ectoderm of early gastrula stage (stage 8a). The cells of this layer are small and polyhedral with strong pigmentation. Bar = 10μm.

**Fig. 5.** Internal surface of presumptive ectoderm of early gastrula stage (8a). The cells are round and slightly pigmented. Bar = 10μm.
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FITC or TRITC their behaviour with respect to the explants was identical. In our experimental conditions lectin binding was stable, being still observed several hours after fixation. Lectins used at the concentration 50 μg ml⁻¹ did not exert toxic effects on the explants: treated cells lived as long as the controls, and no nuclear or cytoplasmic anomalies or cytolysis or abnormal behaviour were observed in these cells (observations on living cells for 3 or 4 weeks and ultrastructural studies).

2. Fixation of lectins on the two explant surfaces

Ectoderm explants were treated in vitro with fluorescent lectins in order to check the binding of the lectins at the cell surface, to investigate the modifications brought about in the membrane topography of cells aggregated in a tissue explant and to compare the behaviour of the outer and the inner surfaces of the target tissue.

After lectin binding a reorganization of the topographical structure of the membrane takes place with a rearrangement of the various receptor sites on both surfaces of the explant. The ectoderm explants differ morphologically on their internal and external surfaces.

**External surface.** Lectin binding results in a wide fluorescent band at the periphery of cells. The fluorescent moieties are evenly spread over the cells (Fig. 6A) but are apparently restricted to spaces between cells. After binding, the ectoderm explants show a rearrangement of bound lectins. The redistribution of fluorescent-lectin binding results in small patches, which are rapidly brought together at one pole of the cell in the form of a cap (Fig. 6B, 6C).

This aggregation of fluorescence from the diffuse band on the cell periphery into clusters of various sizes, is rapid. Lectin binding is almost instantaneous and receptor migration occurs in less than 15 min. In some cases the fluorescence pattern shows caps formed in less than 1 min.

**Internal surface.** First, there is fluorescence in a thin intense band at the periphery of the cells (Fig. 7A). Then, the fluorescence develops into a fine network on each cell with a spoke-like structure which seems to converge at a central point (Fig. 7B, 7C). Just above the level of the cell surface a large spreading network of tangled filaments which densely bind lectins is formed (Fig. 8). This filamentous material remains stuck to the support when the cells are detached, indicating its extracellular location.

Since, it is the inner surface of the ectoderm which undergoes neural induction in the embryo, could this extracellular matrix play a role in cell induction? No network of extracellular material was ever observed on the outer surface of the

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Fig. 6. External surface of ectoderm incubated in FITC-SBA for 3 min. (A) immediate observation: homogeneous distribution of lectin around the cells. (B) after 2–3 min, each cell presents several clusters of bound FITC-SBA. (C) after 10 min, capping phenomenon indicating surface modifications. Bar = 10 μm.
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Fig 6A–C
ectoderm. To clarify this point we associated in vitro the natural inductor tissue (blastoporal lip) onto the outer surface of the isolated ectoderm according to the methodology previously described (Gualandris & Duprat, 1981). Induction occurred in this experiment (25/25 positive cases) as for a blastoporal lip association onto the inner surface of the explant. We concluded that the extracellular matrix on the inner surface of the ectoderm does not play an essential role in neural determination.

Thus our experiments indicate that 1) the capping phenomenon, only described on isolated cells, can also occur when the cells are aggregated in a tissue; 2) the molecular reorganization with respect to lectin binding is different for the external and the internal sides of the ectoderm.

Table 1 shows comparative studies of the fluorescence intensity with the different lectins (SBA, PSA, LCA and Con A) on the external and the internal surfaces of ectoderms.

In all cases 1) the lectin is rapidly bound and the fluorescent patterns differ as described above, but each surface acts in the same way towards the various lectins; 2) the lectin binds more densely to the inner than to the outer surface. The experiments also pointed out that the order of intensity of fluorescence is: Con A < LCA < PSA < SBA. Moreover the ratio of external side/internal side fluorescence is constant (=1/2).
Table 1. Comparative study of the fluorescence intensity (in arbitrary units), with different lectins, of the internal and the external surfaces of treated ectoblast

<table>
<thead>
<tr>
<th>Lectins</th>
<th>Fluorescence of lectins in solution (50 μg ml⁻¹)</th>
<th>Fluorescence of the external surface</th>
<th>Fluorescence of the internal surface</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>SBA</td>
<td>100-52 ± 0-398 *100</td>
<td>20</td>
<td>46-92 ± 1-928 *45-76 ± 1-91</td>
</tr>
<tr>
<td>PSA</td>
<td>20-99 ± 0-436 *100</td>
<td>11</td>
<td>6-54 ± 0-295 *31-15 ± 1-40</td>
</tr>
<tr>
<td>LCA</td>
<td>52-02 ± 0-890 *100</td>
<td>10</td>
<td>6-74 ± 0-053 *12-95 ± 0-10</td>
</tr>
<tr>
<td>CON A</td>
<td>54-64 ± 0-810 *100</td>
<td>10</td>
<td>2-26 ± 0-010 *4-13</td>
</tr>
</tbody>
</table>

No. = Numbers of measurements.
* = The actual values observed are in roman print; bold print corresponds to values expressed as a "100" of the fluorescence of the lectin solution used.

The changes in the fluorescence pattern described above occur rapidly (in less than 15 min at 20 °C) and the pattern is not further modified with time. It is still present several hours after lectin binding, indicating that, under our experimental conditions, the binding of lectins to the cell receptors is not labile. It should however be noted that the intensity of fluorescence decreases with time (4 h, 6 h, 24 h). This phenomenon will be discussed in relation to glycoconjugate turnover.

II. Turnover of membrane glycoconjugates

Experiments using labelled SBA, PSA and LCA, were performed to observe the kinetics of changes in the explants, in vitro, over 24 h and more. The different studied explants were placed in the same conditions of temperature, pH and darkness.

Experiment 1. When the explants, incubated in unlabelled lectin for 3 min, were incubated in fluorescent lectin: no fluorescence was observed on the explant surfaces. All the lectin-binding sites were saturated with the first incubation.

Experiment 2. If the explants were incubated in lectin solution containing the corresponding inhibitory sugar (SBA: α-D-galactose, PSA: α-D-mannose) no fluorescence was observed. This indicates that the reaction was specific because the fluorescence was abolished in the presence of the inhibiting sugar. The fluorescence normally observed results in specific binding of labelled lectins.

Experiment 3. Immediately after incubation in the fluorescent lectin solution
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Table 2. Quantitative analysis of the fluorescence intensity against time, on treated ectoblast.

- **FITC-SBA treatment** -

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fluorescence values (mean ± s.d.)</th>
<th>No. (Number of measurements)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A)</strong> Lectin solution (50 µg ml⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate observation</td>
<td>23.99 ± 0.102</td>
<td>19</td>
</tr>
<tr>
<td>Observ. after 24 h</td>
<td>25.29 ± 0.293</td>
<td>44</td>
</tr>
<tr>
<td><strong>B)</strong> Experiment 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 min treatment</td>
<td>5.01 ± 0.143</td>
<td>69</td>
</tr>
<tr>
<td><strong>C)</strong> Experiment 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 min treatment + 24 h normal medium</td>
<td>2.89 ± 0.052</td>
<td>70</td>
</tr>
<tr>
<td><strong>D)</strong> Experiment 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 min treatment + 24 h normal medium + 3 min treatment</td>
<td>9.30 ± 0.143</td>
<td>72</td>
</tr>
<tr>
<td><strong>E)</strong> Experiment 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h treatment</td>
<td>19.22 ± 0.533</td>
<td>73</td>
</tr>
</tbody>
</table>

The Kolmogorov-Smirnov test pointed out significant differences between all these experiments at *P* < 0.01 (see Results). Similar results were obtained with PSA and LCA.

(3 min) the explants were washed and examined: fluorescence was intense (Table 2B).

**Experiment 4.** When explants were treated in the same way as for experiment 3 and then placed in normal Holtfreter medium for 24 h, the fluorescence intensity was seen to decrease but not to totally disappear; glycoconjugate reorganization was still observed (Table 2C).

**Experiment 5.** Similar explants, incubated in fluorescent lectin for 3 min then placed for 24 h in normal medium, were incubated a second time in the fluorescent lectin solution for 3 min: the fluorescent intensity was high (Table 2D).

**Experiment 6.** Explants continually incubated for 24 h in fluorescent lectin solution presented a very high fluorescence intensity, probably resulting from the addition of the normal fluorescence to the accumulation of released material (Table 2E).

Under our experimental conditions the labelled lectins were unaltered after 24 h storage, and the fluorochromes did not lose any of their activity (Table 2A).
In experiment 4, the low fluorescence reflects the disappearance of lectin-binding receptors. In experiment 5, the fluorescence is again intense; this seems to result from the renewal of the receptors. The new receptors then bind the lectin when it is introduced into the culture medium.

**DISCUSSION**

It is now well established that lectin binding to the cell surface brings about a rearrangement of the membrane constituents which bind the lectin (Inbar & Sachs, 1973; Nicolson, 1973, 1974, 1976; Garrido *et al.* 1974; Zalik & Cook, 1976; Sharon, 1977). The use of lectins conjugated with fluorochromes demonstrated the presence of lectin receptors at the surface of *Pleurodeles* cells. It enabled their nature to be determined and their mobility in the membrane to be analysed.

We took the precaution of isolating, purifying and checking the labelling of all the lectins used. Great variations were noticed in commercially available batches of lectins with lack of homogeneity and presence of contaminating elements (Riikola & Weber, 1981, 1982). It appears, that our lectin batches were of good quality, giving no artifactual effects on the materials studied.

Comparative studies of internal and external surfaces of the ectoderm by direct labelling of the explants were carried out. In this way the fluorescent lectins show that lectins bind more densely to the inner surface. Moreover the differences in lectin binding between the two surfaces indicates that the inner surface possesses receptors for SBA and PSA as well as for LCA and Con A, implying that the surface glycoconjugates include N-Ac-α-D-galactosamine, α-D-galactose, α-D-mannose and α-D-glucose receptors.

The order of binding intensity on the outer surface was SBA > PSA > LCA > Con A; but Con A was not always found in detectable amounts. So, the outer surface seems to possess receptors of the α-D-galactose type, the others being less frequent or less accessible to the lectins. The results obtained with PSA and LCA, both of which bind preferentially to the same sugar: α-D-mannose, suggest a difference in their affinity for their receptors. Indeed the fluorescence observed with PSA was more intense than that obtained with LCA even though the number of moles of fluorochrome bound per mole of lectin was identical.

It is interesting to point out that the embryonic cells react with pea lectin, indicating the presence on the cells of N-glycan complexes (N-Ac-glucosamine, mannose, fucose receptors) which until now had only been detected in adult tissue (Kornfeld, Reitman & Kornfeld, 1981).

Direct labelling would seem to indicate the absence of certain lectin receptors at the outer cell surface of the explants. Several comments can be made: either the carbohydrates are indeed absent, or they are present but not accessible, or not visualized. As far as the availability of the receptor sites is concerned, Moscona (1971) showed that the receptors of certain lectins are present at the surface of embryonic cells in a masked form.
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Our results are in agreement with those obtained in *Xenopus laevis* using various lectins (WGA, SBA, RCA, PSA, Con A) on chemically fixed embryos (Nosek, 1978). The experiments bring to light differences in lectin binding between the internal and the external surfaces especially in the ectoderm layer at the animal pole of the gastrula. But the PSA and Con A receptors do not seem to be as numerous in *Pleurodeles waltl* as in *Xenopus laevis*.

The fact that we observed a variation in the time of appearance of stable membrane reorganization patterns can be explained by differences between eggs in a same batch and/or differences in the exact age of the gastrula studied (stage 8a, 8b). Johnson & Smith (1977) working with *Xenopus laevis* showed that the rate of binding-site redistribution varies according to the exact stage of development of the gastrulae; a rise in the number of clusters or caps occurs with increasing age of the gastrulae.

On the inner surface we report two major observations. First, binding of lectins into the membrane demonstrating the presence of glycoconjugates and their relative mobility. The fluorescence patterns on each cell develop to form a spoke-like structure. Second, a filamentous network is formed which could be composed of extracellular material. This fibrous layer lies over the cells and can be separated from them; this material remains stuck to the support when the cells are detached.

The extracellular matrix, first reported *in vivo* in amphibians by Tarin (1971) at the interface between the chordamesoderm and the ectoderm during gastrulation, was studied by Johnson (1977) who showed the presence of extracellular materials that stain with toluidine blue and lanthanum nitrate. Similarly, Kosher & Searles (1973) found that mucopolysaccharide sulphates were synthesized and accumulated at the cell surface during gastrulation. Recently, in *Ambystoma maculatum* gastrulae, Nakatsuji, Gould & Johnson (1982) described a network of fine extracellular fibrils that covers the inner surface of the ectodermal layer and seems to serve as a guiding substratum for migrating mesodermal cells. Boucaut & Darribere (1983) in *Pleurodeles*, Bride (1982) in *Xenopus* and Nakatsuji & Johnson (1983a,b) demonstrated the presence of fibronectins at the chordamesoderm-ectoderm contact during invagination of the blastoporal lip at the gastrula stage.

**CONCLUSIONS**

So, using fluorescent lectin probes, it was observed that (1) the inner and the outer surfaces of the ectoderm present different behavioural characteristics; (2) the extracellular matrix we observed on the inner surface of the ectoderm (containing N-Ac-α-D-galactosamine, α-D-galactose, α-D-mannose, α-D-glucose glycoconjugate molecules) does not seem to play an essential role in neural induction. This matrix is thought to be involved in the morphogenetic movements of gastrulation.
Experiments are currently underway to attempt to accurately produce a time
course for glycoconjugate turnover in *Pleurodeles* and to assess the role of the
target cell plasmalemma structure in the neural induction process both *in vivo*
and *in vitro*.

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RESUME

L'ectoblaste compétent de *Pleurodeles waltl* est constitué de deux couches de
cellules qui présentent des caractéristiques différentes dans leur morphologie,
leur composition et l'arrangement moléculaire des différents constituants.

Des lectines couplées à des fluorochromes (FITC ou TRITC) ont été utilisées
*in vivo* et l'intensité de fluorescence quantifiée. Ces observations montrent qu'il
existe:

1) des différences dans le marquage des explants selon la nature des lectines
étudiées (SBA, PSA, LCA ou Con A). Ces différences fournissent des indications
sur la nature des glucides présents à ce stade et leur quantité.

2) des différences d'intensité de fluorescence selon la face externe ou interne
de l'explant; la surface interne étant toujours plus intensément fluorescente que
l'externe;

3) un remaniement des récepteurs lectiniques avec mise en place d'une con-
figuration moléculaire nouvelle, mettant en évidence une certaine fluidité de la
membrane. Ceci est à relier avec des résultats précédents (*JEEM, 70, 171-187,
1982*) et montre l'importance de la membrane des cellules ectoblastiques dans le
déroulement de l'induction neurogène.

4) un renouvellement des glycoconjugués membranaires;

5) des différences comportementales des deux faces de l'explant, avec la
formation d'une matrice extra-cellulaire uniquement sur la face interne. Cette
matrice n'est pas nécessaire au déroulement de l'induction neurogène et semble
donc être impliquée dans les mouvements morphogénétiques de la gastrulation.

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