The fine structure of the developing retina in
Xenopus laevis

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SUMMARY

The fine structure of the developing retinal cells in Xenopus laevis was studied from stages 26 to 36. At all stages examined the cells contained large numbers of free ribosomes, polysomes, small mitochondria, lipid and yolk droplets and scanty granular reticulum. A basal lamina covered the smooth internal margin of the optic vesicle and also the external aspect of the germinal pigment epithelial cells.

At all stages examined zonulae adhaerentes occurred between adjacent cells at the outer aspect of the optic vesicle and maculae adhaerentes diminutae were occasionally observed. A third type of intercellular junction, characterized by a narrow gap of 3–9 nm, occurred throughout the retina up to stage 30 but only at the periphery beyond this stage. It is suggested that the disappearance of these junctions from the central portion of the retina may be correlated with retinal cell specification* which is known to occur at stage 30–31. These junctions may represent sites for the cell to cell transfer of small molecules which are required for cell differentiation. Since new cells are continually being added to the retina from the ciliary margin beyond stage 30 the persistence of junctions in this region may explain how these new cells also become specified.*

INTRODUCTION

At a certain stage of embryonic development the cells of the retina undergo changes which result in a precise specification* of their projection on to the optic tectum (Stone, 1960). It has been shown by Jacobson (1968a, b) that in Xenopus laevis these changes occur at embryonic stage 30–31, as tabulated by Nieuwkoop & Faber (1956). He rotated the eyes of the Xenopus embryos at various stages from 28 to 35 and later mapped the retinotectal projections after metamorphosis. Those animals in which the eye had been rotated 180° before stage 30 showed normal projections. When the eye was rotated after stage 31 the result was complete inversion of the tectal projections across both the

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* The term retinal specification, marked throughout by an asterisk, is used here to refer to the condition in which each individual retinal ganglion cell first acquires the positional information that later predisposes it to connect at a specific location in the optic tectum.
nasotemporal and dorsoventral axes. Jacobson (1968b) correlated the period of specification* with the cessation of DNA synthesis in the central retinal cells using autoradiography. However, Straznicky & Gaze (1971), using a similar technique, later showed that DNA synthesis occurs after the period of specification* in the cells at the periphery of the retina. In this region cell division takes place well beyond stage 31, in order to increase the dimensions of the developing retina.

The purpose of the present study was to examine the fine structure of the developing retinal cells of *Xenopus* in order to determine whether the period of specification* is accompanied by any morphological changes. A similar study by Fisher & Jacobson (1970) was unable to correlate any fine structural changes with cell specification*. However, in the present work particular attention was paid to various intercellular junctions which were apparently overlooked by the previous workers.

**MATERIALS AND METHODS**

Embryos of *Xenopus laevis* were staged according to the normal tables of Neiuwkoop & Faber (1956) and those at stages 26–36 were prepared for electron microscopy. Jelly coats and vitelline membranes were removed with watchmakers’ forceps and the denuded embryos fixed in either (a) ice-cold 1% osmium tetroxide buffered at pH 7.5 with acetate veronal for 1 h (Palade, 1952), or (b) ice-cold 3% glutaraldehyde buffered at pH 7.4 with sodium cacodylate (Sabatini, Bensch & Barnett, 1963) for 2 h followed by post-fixation in osmium for 1 h. After fixation the embryos were dehydrated in a graded series of ethyl alcohols and embedded in Araldite (Glauert & Glauert, 1958).

Sections 1µm thick were cut and stained with 1% toluidine blue in 1% borax for light microscopy in order to locate the optic vesicle. Thin sections were cut with glass knives on an LKB Ultrotome III ultramicrotome, mounted on uncoated copper grids and double stained with alcoholic uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) before examination in a Philips EM 300 electron microscope.

**OBSERVATIONS**

*General appearances of retinal cells*

At stage 28 the optic vesicle is 4–5 cells in thickness and each cell is elongated in shape with an ovoid nucleus (Fig. 1). A single layer of flattened cells (the germinal pigment epithelium) occurs on the outer aspect of the optic vesicle. A thin basal lamina is present on the inner aspect of the optic vesicle (Fig. 3) and also on the outer aspect of the germinal pigment epithelium (Fig. 2). The internal margin of the optic vesicle is relatively smooth in profile and microvilli and cilia were not observed.
A low-power electron micrograph of the wall of the optic vesicle at stage 28. Part of the developing lens is visible in the top left-hand corner of the field (l). The germinal pigment epithelium (p) has become detached from the outer aspect of the optic vesicle at the bottom right of the field. × 2400.
A basal lamina (bl) covers the outer aspect of the germinal pigment epithelium (p), which is characterized by the presence of numerous membrane-limited dense granules (g). The arrows indicate zonulae adhaerentes between adjacent retinal cells. × 24000.
Developing retina of Xenopus

The germinal retinal cells contain large numbers of free ribosomes, polysomes and small mitochondria (Fig. 2), many of which possess dense intramitochondrial granules. In addition, large electron-dense yolk droplets and paler staining lipid droplets are frequently encountered (Fig. 1). A few randomly orientated cytoplasmic microtubules were observed in most of the germinal cells. Very few membranes of granular endoplasmic reticulum occur in the early stages but these increase markedly in number from stage 32 onwards. Vesicles of smooth endoplasmic reticulum are quite numerous although Golgi complexes were only occasionally observed. In contrast, Golgi membranes are well developed in the germinal pigment epithelial cells which also contain numerous spherical or oval dense granules separated from a limiting membrane by a clear space (Fig. 2).

Intercellular junctions

Three types of intercellular junction were observed in the developing retina of Xenopus laevis. The first type is similar to the zonula adhaerens described in various epithelial tissues (Farquhar & Palade, 1963) and occurs between adjacent cells at the outer border of the optic vesicle (Figs. 2, 4). These junctions were present at all stages examined. In the region of a junction the apposed plasma membranes are separated by an intercellular space which varies in width from 10 to 20 nm and contains a material of moderate electron density (Fig. 4). There is also an increased density of adjacent cytoplasm for distances varying from 0.1 μm to over 1.0 μm.

The second type of junction is similar in cross-section to the zonulae adhaerentes although the examination of serial sections indicated that it has the shape of a macula approximately 0.5 μm in diameter (Fig. 5). Although rather infrequent, junctions of this type were observed in the developing retina at all stages examined and are similar to the maculae adhaerentes diminutae described by Hay (1968) in various embryonic tissues.

The third type of intercellular junction in the developing retina also has a macula structure with a diameter of about 1.0 μm (Fig. 6). At these junctions the irregular intercellular space abruptly narrows to a small gap which varies between 3 and 9 nm in width. The apposing membranes are unusually electron-dense and appear very straight over the region of the junction. A material of medium electron-density fills the space between the cells and frequently an array of regularly spaced electron-densities occurs in the intercellular space. There is no increased density of adjacent cytoplasm although sometimes small mitochondria occur in the immediate vicinity. Junctions of this type are usually observed singly although sometimes they are accompanied by the macula adhaerens diminuta type of junction (Fig. 7).

This third type of junction occurs at random throughout the developing retina from stage 26 (the earliest stage examined) to stage 30 although the majority are observed close to the inner aspect of the optic vesicle. At stage 31
and beyond junctions of this type are no longer observed in the central region of the retina although a few occur at the periphery from stage 32 to stage 36 (the latest stage examined).

DISCUSSION

The present study has shown that during the early stages of development all the retinal cells of *Xenopus laevis* are similar in fine structure and exhibit features which are typical of immature cells. After stage 32 there is a gradual increase in the amount of granular reticulum as observed by Fisher & Jacobson (1970) which is possibly indicative of cell differentiation.

Junctions of the zonula adhaerens type are present between the cells at the external margin and possibly serve to maintain the shape of the developing optic vesicle. Punctate junctional regions, the maculae adhaerentes diminutae, with a similar cross-sectional appearance to the zonulae adhaerentes are randomly distributed throughout the retina at all stages examined and may also play a part in cell adhesion (Hay, 1968). However, the most significant observation of the present study concerns the presence of a third type of junction which resembles the gap junction described in the vertebrate brain by Brightman & Reese (1969). This type of junction was observed throughout the retina up to the time of specification* but only at the extreme periphery of the retina at stage 32 and beyond. The disappearance of these junctions from the central portion of the retina at the exact time of specification* leads one to propose that the two events may be correlated. Similar temporary cell attachments have been observed in a variety of embryonic tissues by numerous workers (Lowenstein, 1967; Furshpan & Potter, 1968) and it has been suggested that they may represent sites for the cell to cell transfer of small molecules or ions which would then act as a messenger for the initiation of cell differentiation.

If such an hypothesis is correct it could also explain the persistence of this type of junction at the poles of the retina after the period of specification*. Autoradiographic studies (Straznicky & Gaze, 1971) have shown that cells are continually being added to the developing retina of *Xenopus* from the ciliary...

**Figures 3-7**

Fig. 3. A basal lamina (bl) covers the inner aspect of the optic vesicle. Note the absence of specialized junctions between retinal cells in this position. ×72000.

Fig. 4. A zonula adhaerens between two adjacent cells at the outer aspect of the optic vesicle at stage 32. ×72000.

Fig. 5. A pair of maculae adhaerentes diminutae between adjacent cells at stage 32. ×72000.

Fig. 6. An intercellular junction between adjacent cells of the optic vesicle at stage 28. Note the increased density of apposed membranes. ×72000.

Fig. 7. A similar junction to that shown in Fig. 6 flanked on either side by maculae adhaerentes diminutae. ×72000.
margin. These additional cells must also become specified* and this may be achieved by the formation of intercellular junctions between the new cells and those retinal cells which are already specified.*

REFERENCES


(Manuscript received 1 May 1972)