GLYCOGEN METABOLISM AND THE NUCLEAR ENVELOPE–ANNULATE LAMELLA SYSTEM IN THE EARLY CHICK EMBRYO

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SUMMARY
The intracellular sites of glycogen degradation in the mid to late uterine chick embryo were determined by cytochemical localization of glucose-6-phosphatase at the ultrastructural level. Enzyme activity was found between the two membranes of the nuclear envelope, in the annulate lamellae and in specialized glycogen-containing membrane scrolls. Annulate lamellae and glycogen scrolls were most frequent during the stages of intensive glycogen degradation. Annulate lamellae appear to be formed from the nuclear envelope. During the early post-laying stages annulate lamellae disappeared and were replaced by endoplasmic reticulum that appeared initially in scroll-like formations.

INTRODUCTION
Glycogen appears to be the primary energy source of early embryonic development (e.g. see Gusseck & Hedrick, 1972; Vernier & Sire, 1976). In the chick, although the oocyte accumulates large quantities of glycogen during oogenesis (Greenfield, 1966; Paulson & Rosenberg, 1972), this glycogen is utilized completely by the time the uncleaved egg reaches the magnum. There is, however, a second accumulation of glycogen based on yolk degradation, which begins before cleavage, reaching a maximum in the mid-uterine embryo (Eyal-Giladi, Raveh, Feinstein & Friedlander, 1979). This glycogen is synthesized in special cytoplasmic vesicles, which break down in mid-uterine embryos, releasing free clusters of glycogen particles into the cytoplasm. Almost all this intracellular glycogen is utilized during the second half of uterine development, beginning at the time of symmetrization of the embryo. Glycogen utilization is first evident at the future posterior side of the blastoderm, from where it spreads anteriorly (Kochav, Ginsburg & Eyal-Giladi, 1980). Degradation of glycogen takes place within glycogen scrolls and cytoplasmic vesicles that contain floccular material (FLOVs) (Eyal-Giladi et al. 1979).

Glucose-6-phosphatase (G6Pase) has been shown to be involved in utilization of embryonic glycogen in the rat (Devos & Hers, 1974), and salmon embryo (Vernier

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G6Pase is strongly bound to the microsomal membranes in rat hepatocytes (Hers, Berthet, Berthet & De Duve, 1951; Duttera, Byrne & Ganoza, 1968). It has been localized in situ to the cisternae of the endoplasmic reticulum (ER), and the nuclear envelope of differentiating rat (Leskes, Siekevitz & Palade, 1971a,b) and salmon embryo (Vernier & Sire, 1976) hepatocytes. However, although cells of the uterine chick embryo show very active glycogen metabolism they do not contain ER. Furthermore, ER is very sparse in the early incubated embryo before primitive streak formation (Raveh, Friedlander & Eyal-Giladi, 1971; Bellairs, Lorenz & Dunlap, 1978).

In the present study we show that G6Pase activity is located in the nuclear envelope, the cytoplasmic annulate lamellae and in membranes of specialized scroll-like structures that contain glycogen. These membrane structures become particularly abundant during the stages of intensive glycogen degradation.

**MATERIALS AND METHODS**

Uterine and early laid eggs were collected and processed for light and electron microscopy as described by Eyal-Giladi et al. (1979). The uterine stages of the chick embryo are designated by Roman numerals referred to as E.G & K (Eyal-Giladi & Kochav, 1976). For cytochemical localization of G6Pase whole embryos were treated according to the method of Vernier & Sire (1976). Control embryos were treated as described by Yokoyama, Okada, Tokue & Aso (1975).

**RESULTS**

**Intracellular localization of glycogen**

In mid to late uterine embryos (stages VI—VII E.G & K) glycogen particles were frequently found enclosed in membranous scroll-like structures suspended by stalks inside FLOVs (Fig. 1; Eyal-Giladi et al. 1979). In addition, glycogen particles were found in vesicles associated with stacks of annulate lamellae and within FLOVs (Fig. 2). Occasionally glycogen particles and floccular material were found within the perinuclear lumen and sometimes a direct continuity between the intramembranous lumen of the nuclear envelope and FLOVs was found.

**Nuclear envelope – annulate lamellae – FLOV interrelation**

In the above mid to late uterine embryos (stages VI—VIII E.G & K) the nuclear envelope, which was previously smooth, became extremely undulated and dilated. Stacks of annulate lamellae were found parallel to it, sometimes showing continuity.

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**Fig. 1.** Stage VII E.G & K mid-uterine embryo showing a cross-section through a glycogen scroll suspended by a stalk within a FLOV (f). Rows of glycogen particles are enclosed between two membranes. ×20000.

**Fig. 2.** Stage VII E.G & K embryo as above showing a stack of annulate lamellae (al) between the nucleus and a FLOV (g). Cytoplasmic vesicles containing glycogen are in direct continuity with a FLOV that contains glycogen (g). ne, nuclear envelope. ×24000.
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Figs 1 and 2
with protrusions of the external membrane of the nuclear envelope (Fig. 3). FLOVs were found in direct continuity with the terminal cisternae of stacks of annulate lamellae, from which they appear to arise (Fig. 4\text{A,B}).

**Intracellular localization of G6Pase activity**

Embryos at the above stages incubated with glucose 6-phosphate and lead ions for the localization of G6Pase activity, showed the enzyme between the two membranes of the nuclear envelope (Figs 5\text{A,B}, 6), in glycogen scrolls suspended within FLOVs (Fig. 5\text{A,B}) and in stacks of annulate lamellae (Figs 6, 7). Control sections incubated with the reaction mixture in the presence of 10mM-NaF did not show any activity over any of these membranes (Fig. 8).

**Temporal correlation between abundance of annulate lamellae and glycogen degradation**

Stacks of annulate lamellae were encountered frequently in embryos of the above stages (VI–VIII), during which glycogen was previously shown to be utilized (Eyal-Giladi et al. 1979; Kochav et al. 1980). At the time of laying (stage X E.G & K) the embryos no longer contained glycogen and the annulate lamellae were exceedingly rare. The latter were seldom found in unincubated and early incubated (up to stage XII E.G & K) embryos and never found in embryos with a fully developed hypoblast or at the primitive streak stage (Raveh et al. 1971).

**Inverse relation between annulate lamellae and ER at different developmental stages**

We did not find ER in uterine embryos. After the disappearance of the glycogen during the late uterine stages, the cells of the stage X E.G. & K embryo of a freshly laid egg became packed with ribosomes and rough ER appeared for the first time. Characteristically, the early rough ER showed a scroll-like formation (Fig. 9); later stage XII E.G & K embryos showed loose elements of rough ER, which appear to be remnants of these scrolls.

**DISCUSSION**

We have localized G6Pase activity to the membrane structures that are associated with glycogen, i.e. the nuclear envelope, annulate lamellae and glycogen scrolls, indicating that these structures are involved in glycogen degradation. This is the first localization of enzymic activity to the annulate lamellae. Further support for the role of the annulate lamellae in glycogen degradation is indicated by the appearance of vesicles filled with floccular material, which are in open communication with the annulate lamellae, as well as by the finding that the latter were especially abundant at the stages of active glycogen degradation during the transformation of a non-polar stage VI E.G & K blastodisc into a stage X blastoderm with a fixed posterior–anterior polarity. These stages have previously been shown to be important in
Fig. 3. Stack of annulate lamellae associated with an evagination of the outer membrane of the nuclear envelope (ne) showing a continuity at one point with the outer membrane (arrow). \(\times 24000\).

Fig. 4. A. Stack of annulate lamellae showing formation of FLOV (arrows) by dilation of the terminal cisternae. B. FLOVs of various sizes (arrows), the smallest being pinched off from the annulate lamellae. \(\times 20000\).
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Fig. 5. A, B. Localization of G6Pase activity to the intramembrane cistern of the nuclear envelope (ne), to a glycogen scroll (gs), and to the membrane of a FLOV (f). ×20 000.

Fig. 6. Annulated glycogen scroll sectioned transversely, heavily labelled with lead phosphate deposit indicative of G6Pase activity. ×20 000.

Fig. 7. Annulate lamellae in longitudinal section showing G6Pase activity. ×20 000.

Fig. 8. Control blastoderm incubated in the reaction mixture in the presence of 10 mM NaF. The nuclear envelope (ne) and the annulate lamellae are not labelled. ×20 000. Granular inclusions appear in the mitochondria of both control and treated embryos but are not demonstrative of G6Pase activity.
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Fig. 9. Longitudinal section of a scroll of ER found in an embryo of a freshly laid egg (stage XII E.G & K), which lacks glycogen particles and contains mitochondria and ribosomes between the leaves of the scroll. Compare with earlier scroll shown in Fig. 1. ×20,000.

nucleolar ontogenesis and onset of embryonic ribosomal synthesis (Raveh, Friedlander & Eyal-Giladi, 1976).

Localization of G6Pase activity to the membranes of the glycogen scrolls found in the FLOVs confirms our previous conclusions, which were based on morphological evidence, that these structures are involved in glycogen degradation (Eyal-Giladi et al. 1979).

The annulate lamellae may present a mechanism for increasing the nuclear membrane surface and thus increasing some additional activities of this membrane. This could be particularly important in cells that are poor in ER, as it has been suggested that the nuclear envelope can fulfil the minimal endomembrane functions (Franke, Scheer, Krohne & Jarasch, 1981). Our localization of G6Pase activity to the annulate lamellae is thus consistent with the morphological evidence on their continuity with the nuclear envelope. There is no indication that the pore complexes are involved in glycogen degradation, as we found that glucose 6-phosphatase is located between the pores in both the annulate lamellae and the nuclear envelope. Moreover, the enzymes that have been localized to the pore complexes seem to be involved in transport of macromolecules between the nucleus and the cytoplasm.
In the mid-uterine chick embryo the nuclear envelope seems to be involved in the formation of annulate lamellae, as shown earlier in other organisms (e.g. see Steinkamp & Hoefer, 1974; Smith & Berlin, 1977; Thomopoulos & Kastritis, 1983; reviewed by Kessel, 1983). It shows increased blebbing activity during the stages of annulate lamella generation, stacks of lamellae accumulate preferentially adjacent to the nuclear envelope and occasionally continuity is found between the outer nuclear membrane and the membrane of the annulate lamella.

Our cytochemical results indicate a functional continuity between the nuclear envelope and the annulate lamellae. In addition, gradual replacement of lamellae by ER after the formation of functional embryonic nucleoli (Raveh et al. 1976) and the generation of the first rough ER as scroll-like formations in the blastoderm of a freshly laid egg suggest a transformation of glycogen scrolls, and possibly also annulate lamellae, into ER. Thus our data support the hypothesis of a membrane continuum with interchangeability of the membranous elements (Porter, 1961; Franke et al. 1981). This idea is also supported by numerous reports of enzyme activities common to both the nuclear membrane and the ER (Jarasch et al. 1979; reviewed by Franke et al. 1981), and by reports of a direct continuity between annulate lamellae and ER (Jasper, 1976; Kessel, 1983).

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REFERENCES


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