Migration of the Chick Primordial Germ Cells From the Intracoelomically Transplanted Germinal Crescent Into the Genital Ridge

In normal development of an avian embryo, primordial germ cells (PGC) are originally located extraembryonally, in the germinal crescent, and reach the genital ridges by vascular route. There is an increasing amount of data showing that the genital ridges attract primordial germ cells, and that this attraction is chemotactic in character and is not species specific. SIMON[1] obtained colonization of chick genital ridges by duck primordial germ cells in embryonic parabioses in vitro; REYNAUD[2] succeeded in producing settlement of turkey primordial germ cells in the genital ridges of a chick embryo after injecting a suspension of PGC into the blood vessels. As DUBOIS[3,4] has shown, PGC may also pass directly from the germinal crescent to the genital ridge, if both these structures are put in contact and cultured for a certain time in vitro. Using the above observation as a basis, an attempt was undertaken to provide the embryo in ovo with 'alien' primordial germ cells by introducing the germinal crescent into the coelomic cavity. This method, like REYNAUD's method, would make it possible to trace the fate of PGC for a far longer period than is possible under conditions of culture in vitro.

Embryos of the Leghorn strain in stages from the primitive streak to 7 somites (about 24 h of incubation), were used as donors, and 25–30 somite embryos (about 66 h of incubation) as hosts. HAMBURGER's[5] method, modified by HARA[6], of intra-coelomic grafting was used. The transplants were excised in Locke's fluid deprived of Ca and Mg; this caused the germ layers to separate at the edges and to a certain extent facilitated attachment of the transplant to the host. The small size of the coelomic cavity of the young embryo did not permit introducing the whole germinal crescent, but efforts were made to ensure that the transplant included at least the whole breadth of germinal crescent. Hosts were sacrificed 0–30 h after operation, and posterior parts fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 6 μ and stained with hematoxylin and eosine.

The material consisted of 36 grafts. The flat transplants curl at first and then round out into vesicles, in which the entoderm is always located on the external side. The transplants have a tendency to attach themselves at the ends, often creating U-shaped formations. Contact between transplant and host may be established very quickly, even within the first 3 h after operation.

Contact between the crescent and the genital ridge was observed in 18 cases only (Table). The numbers of primordial germ cells in the transplants were very small, even in transplants fixed immediately after operation. This is probably due to the fact that they contained only part of the germinal crescent and were taken from relatively young donors. In 4 cases, however, it proved possible to observe the primordial germ cells at the moment of crossing the boundary between the transplant and the host (Figures 1 and 2). Unfortunately the presence of the host's own primordial germ cells in the genital ridge made it impossible to identify the PGC of the transplant which might have penetrated previously into the ridge. It is noteworthy that penetration of PGC into the genital ridge was observed in one graft as old as 24 h (Figure 2). The host had by then been incubated for 90 h and had reached the stage when normally the migration of PGC has long since been completed. Evidently the genital ridges, as REYNAUD[2] has already pointed out, are able to attract primordial germ cells for a far longer time than has hitherto been assumed to be the case.

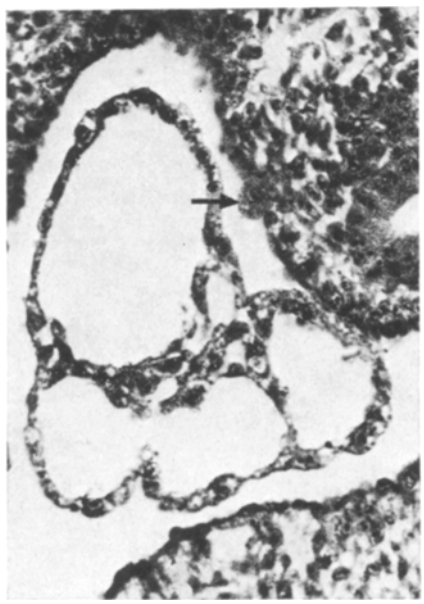

Fig. 1. Germinal crescent after 5 h of intracoelomic development. Primordial germ cell (arrow) at the very moment of penetration into the genital ridge. × 400.

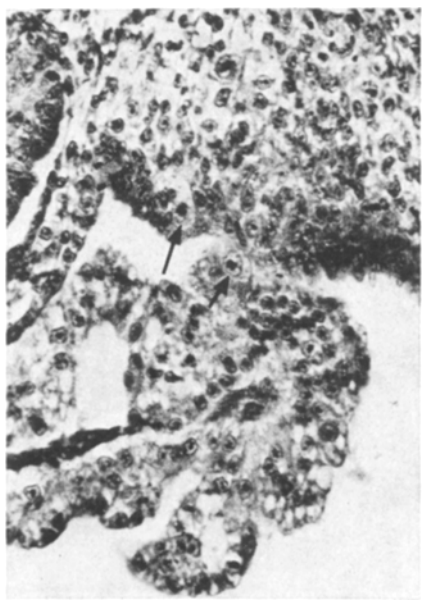

Fig. 2. Part of a germinal crescent after 24 h of intracoelomic development, showing migrating primordial germ cell (short arrow). Primordial germ cell lying in genital ridge (long arrow) probably originates also from the graft. × 400.

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Numbers of primordial germ cells in grafts attached to the genital ridge

Number	Age of graft (h)	Number of PGC
1	0	2
2	0	8
3	0	5
4	1	11
5[a]	1	10
6	2	44
7	2	4
8[a]	3	13
9	4	4
10[a]	5	22
11	6	8
12	9	110
13	19	17
14	21	1
15	24	0
16	24	7
17[a]	24	7
18	30	0

[a] Immigration of primordial germ cells into the genital ridge was observed in these grafts.

Intra-coelomic transplantation of the germinal crescent can thus be applied as a method for introducing a small number of 'alien' primordial germ cells into the genital ridge. It would, however, be necessary to have sterile hosts, or use 2 species differing greatly as to the size of primordial germ cells, in order to be able to trace the fate of these cells in the developing gonad[7].

Résumé. Lorsque le croissant germinal est introduit in ovo dans la cavité coelomique de la région gonadique, les cellules germinales primordiales peuvent sortir du greffon et émigrer vers l'épithélium germinatif.

TERESA ROGULSKA

Department of Embryology, University of Warsaw, Warszawa 64 (Poland), 28 January 1969.

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Connection Between a Mitochondrion and Endoplasmic Reticulum in Liver

The concept of continuity between various membrane-bound organelles has long appealed to biologists. Such possible interconnections have been freely illustrated diagrammatically, but in only a few instances, documented with electron micrographs. Direct continuity of the nuclear envelope with the endoplasmic reticulum and between profiles of rough and smooth endoplasmic reticulum is perhaps the best documented[1]. Recently, morphologic evidence has been presented which suggests direct connections between outer mitochondrial membranes and the sarcoplasmic reticulum of skeletal[2,3] and cardiac[4] myocytes. This communication concerns the familiar physical proximity of endoplasmic reticulum and mitochondria in hepatocytes, and reports an observed instance of continuity between these organelles in rhesus liver.

Results from a study which compared dose-distribution and morphologic effects of 32 and 55 Mev protons on rhesus liver were previously reported[5]. We are now describing a connection between organelles located in the irradiated tissue that lay proximal to the area of the Bragg peak. Tissue was fixed either in collidine-buffered OsO_4[6] or cacodylate-buffered glutaraldehyde[7]. The glutaraldehyde-fixed specimens were postfixed in OsO_4 after being put through several changes of cacodylate buffer during a 24–72-h period. All steps prior to dehydration were carried out at 4 °C. Tissue was rapidly dehydrated in ethanol, infiltrated with propylene oxide followed by 50% epoxy in propylene oxide, and finally brought through 3 changes of 100% epoxy resin (a mixture of Epon and Araldite)[8]. Sections were mounted on 200- and 300-mesh bare copper grids. They were then stained with 0.5% uranyl acetate[9] for 15 sec and with 0.4% lead citrate[10] for 30 sec and studied at 100 Kv with an RCA electron microscope, model EMU 3H.

A pattern consisting of a curved profile of endoplasmic reticulum partially encircling a mitochondrion was repeatedly seen in thin sections of hepatocytes in both control and irradiated liver. Sacular, dilated ends of these profiles of endoplasmic reticulum were close to the outer mitochondrial membranes. When viewed at low magnification, these 2 organelles frequently appeared connected. In all but one instance, further study of these apparent points of merger between organelles revealed juxtaposition of organelles but either distinct separation by a thin rim of cytoplasm or uninterpretable obliquity of the membranes.

However, one such association of a profile of endoplasmic reticulum and a mitochondrion in an irradiated hepatocyte exhibited continuity of the membranes and internal spaces of these organelles (Figure). Fortuitously, the membranes were oriented normal to the plane of section at the site of transition from reticular membrane to mitochondrial membrane.

The mitochondrion requires many enzymes in accomplishing oxidative phosphorylation. The proteinaceous enzymic molecules are probably synthetized in the rough

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