Biol Res 26: 407-410 (1993)

Prof Dr Luis Izquierdo (1928-1992): His contributions to Developmental Biology

CLAUDIO BARROS[1] and GERALD SCHATTEN[2]

[1]Laboratory of Embryology, Faculty of Biological Sciences, Pontifical Catholic University of Chile, Santiago, Chile.
[2]Department of Zoology, University of Wisconsin, Madison, USA

Prof Luis Izquierdo death has left the Chilean scientific community mourning the loss of a prominent scientist. His main line of research was associated with the early development of the mammalian embryo, with special emphasis on embryonic regulation and membrane regionalization of alkaline phosphatase and 5'nucleotidase as markers of the compaction and differentiation.

Key words: *Developmental biology, obituary, scientific contributions.*

The untimely death of Prof Luis Izquierdo, last December, leaves us mourning the loss of a prominent scientist, renowned educator, dedicated statesman within scientific and academic institutions, and beloved friend. He was born in Santiago, Chile, on the 4th of July of 1928. He was a life-long defender and supporter of Chilean science, medicine and intellectual pursuits.

Professor Izquierdo obtained his Medical Doctor's degree in 1953 and soon afterwards, with the aid of a Rockefeller Foundation Fellowship, went to the Free University of Brussels to work under the direction of Prof Albert Dalcq. Upon his return to Chile in 1955, he was offered a faculty position at the Pontifical Catholic University of Chile with the aim of organizing teaching and research in Developmental Biology (1). In 1964, he moved to the University of Chile, where he and other professors founded the Faculty of Sciences, the first one in Chile.

During his training with Dalcq, Izquierdo began his studies on the early development of the rat (9,10). Using methylene blue as a vital stain, he found that at the eight cell stage the distribution, as well as the size, of metachromatic grains in each blastomere cytoplasm, made possible to predict the positional fate of these embryonic cells. Izquierdo later extended this work to electron microscopy, and showed that on each cell of the eight cell stage conceptus there is a polarization of the cytoplasm, as evidenced by the presence of a column of multivesicular bodies running from the nucleus to the periphery (19). In subsequent work, his group found plasma membrane regionalization, as manifested by alkaline phosphatase (ALP) and 5'nucleotidase (5'NUC), which precedes cytoplasmic polarization (23). They also described a fragment of a sperm tail in the egg cytoplasm. This description came soon after it was shown that fertilization was a gamete membrane fusion phenomenon (6,33). Later on, they addressed the question of the gene activity during early mouse development (5,16,18). In their conclusions, they noted the different pattern of RNA metabolism during the early development of mammals, as compared to that of echinoderms.

The study of embryonic regulation (*i.e.*, the process by which normal development is restored after the embryo is subjected to developmental disturbances) has been a major enigma for classical embryologists. Prof Izquierdo recognized that the possibility of studying this problem in mammals required adequate *in vitro* culture methods.

Correspondence to: Dr Claudio Barros, Laboratorio de Embriología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago 1, Chile. Fax: (56-2) 222-5515

He had an extraordinary ability to overcome technical difficulties and the lack of adequate CO_2 incubators; the shortage of financial resources was conveniently solved by using desiccators, water baths and other very simple pieces of equipment. He studied mouse embryo's ability to regulate its development by destroying with a puncture blastomeres in preimplantation conceptus. The results revealed that the percentage of blastocysts formed after destroying one half of the conceptus decreases as development proceeds. Embryonic regulation expressed after blastomere isolation or after blastomere destruction shows that the remaining parts of the conceptus can replace the lost one or damaged parts of the embryo (7,24). In subsequent work, preimplantation conceptus were centrifuged at 50,000 and 90,000 g. In these experiments, the cytoplasm becomes stratified and within 40 minutes stratification disappeared, except for the lipid droplets at the centripetal pole. Normal blastocysts developed from this centrifuged conceptus (33). The use of cytochalasin D, while delaying the time for recovery, did not prevent it.

According to Izquierdo, embryonic regulation requires the existence of a developmental clock that specifies an ordered expression of genes, or processing of their products, signaling blastulation. Based on a series of observations on fusion of half or double embryos, he concluded that blastocyst formation did not depend upon the number of cells nor upon the number of cell cycles (7,29). As a result of these observations, he proposed the existence of a developmental clock that signals the time for blastocyst formation. His work also showed that using increasing concentrations of LiCl as part of the culture medium, the relative cell number diminished accordingly, but the onset of blastulation was not affected (2,14).

Izquierdo was also interested in the study of the regionalization of membrane components using 5'NUC and ALP as labels, in order to understand cell differentiation during early mammalian development (8,15,17,27). The reaction products of these enzymes can be detected starting from the four cell stage and become located at the surface area of cell contact, but are absent on the free surfaces of the blastomeres (8,11,12,13,15,17,20,21,23). He proposed a model in which discrete patches of cell membrane are formed at the apposed surfaces of adjoining blastomeres, thus generating a regionalization necessary for compaction and for the peripheral sealing required for blastocyst differentiation. In fact, blastomeres adhere during compaction by their labeled surfaces, and zonular tight junctions are established precisely at the transition from the labeled to the unlabeled regions around peripheral blastomeres.

However, the reaction product of 5'NUC or ALP would appear at the two cell stage if the two cell preimplantation stages were delayed with cytoskeleton inhibition (22). Reaction product could also be induced to appear by artificial contact with other embryos or with lectins. Cycloheximide would not prevent the appearance of the reaction, suggesting that it is not the result of protein synthesis (30). This evidence led Izquierdo to conclude that the first evidence for an spatial order in mammalian preimplantation conceptus could be the appearance of membrane regionalization, because it arises epigenetically as a consequence of cell to cell contact. It may constitute the spatial basis of embryonic regulation and of the normal spatial differentiation of early mouse conceptus. This idea was later supported by the production of monoclonal antibodies raised against surface antigens, that are shared by preimplantation mouse embryos and F9-teratocarcinoma cells (4). The hypothesis proposed assumed the existence of true stage specific antigens on the surface of embryonic cells, which are not expressed in other developmental stages or differentiated tissues. The results showed that inner cells mass and trophoblast differentiation could be coded by antigens present from oogenesis (trophoblast) and by antigens arising during cleavage (inner cell mass) (3).

Cell compaction during early mammalian development reflects the first sign that precedes differentiation and the formation of the blastocyst. Microvilli present at the contact surface between blastomeres disappear during compaction. Moreover, blastomeres isolated from 8 or 16 cell-stage conceptus

can regenerate their microvilli (31). In subsequent work, microvilli with abundant microfilaments connecting the cortical cytoskeleton of neighboring cells were found to be present around the contact surfaces (26). During compaction, an increase in cell adhesion was observed. In his last work, using confocal microscopy, Izquierdo demonstrated that when compaction was inhibited by various treatments, a concomitant loss of localization of cell adhesion and cytoskeleton molecules was observed. When compaction was later permitted, the normal localization of these molecules was recovered (28). At the time of his death, he had addressed the question of the regulation of protein synthesis during compaction, using one- and two-dimensional gel electrophoresis of embryos incubated with drugs that affect DNA replication (aphidicolin) or compaction (CCD, EGTA, Con-A, α-lactalbumin). The work showed that cycles of DNA replication are involved in regulating the pattern of protein synthesis, while changes induced in cell form or contacts during compaction do not alter the pattern significantly (25).

Professor Izquierdo is well remembered by the students and fellows he mentored, many of whom now hold prominent academic and professional positions. Among the many characteristics which we miss are his quick wit, the sparkling glint in his eye when encountering a novel scientific theory, the breadth of his interest and knowledge in intellectual and artistic pursuits, and his charm and charisma. We are really saddened by Professor Luis Izquierdo's tragic passing away.

In a broad sense, Prof Izquierdo also contributed significantly to the development and promotion of science in Chile. At the time of his death, he was President of the National Science Research Council and he was also member of the Editorial Board of Biological Research. This volume dedicated to his memory is just a small tribute to all his efforts to increase the level of scientific research in Chile.

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