Flexibility and patterning in early mammalian development: a dilemma for embryologists.

Mammals reproduce only sexually by fertilization of an egg-cell by a spermatozoon. Although both germ cells contribute equally to the genome of the resulting offspring, the egg is by far the more important partner: being much larger than the spermatozoon it contributes practically all the cytoplasm with all organelles (except a sperm-introduced centriole) and the cell membrane. The egg is a polarized cell, which means that its structural and molecular constituents are spatially arranged along the so called animal-vegetal polar axis in a unique way that insures development of an embryo, and finally of an individual, according to the stable and repeatable – from generation to generation – scenario. Another superiority of the egg-cell over the spermatozoon is that it is able to initiate development by itself (either spontaneously or as result of experimental intervention) and in some non-mammalian animals completes it effectively, i.e. gives rise to parthenogenetic progeny. The fate of unsuccessful spermatozoa is as a rule miserable – they pass away unnoticed.

The above arguments make it obvious why the egg is the most precious cell for the species in question and the most intriguing cell for embryologists. I have spent my scientific life on looking with the never-ending admiration on mouse eggs, mouse embryos and mice that developed from embryos that I had earlier subjected to various experimental treatments. My research has been always 'curiosity driven': at first I did not think of and probably even did not expect any practical applications of my embryological adventures. But with the passing time when more and more scientists became interested in mammalian gametes and embryos it became evident for me that experimental embryology can produce results that may turn out to be useful in animal breeding and human biotherapy.

Trying to recall the most important event that influenced and directed my research in experimental embryology, I came to the conclusion that it was a fortuitous observation of a two-cell mouse embryo in which one cell was accidentally destroyed. Immediately, I asked myself a question: would the remaining cell form a normal, though half-sized embryo, or would it form a handicapped and non-viable embryo? I transplanted the damaged embryo to the oviduct of a foster mother and in few days I knew the answer: at least up to the stage when embryos embed in the uterus a damaged embryo can develop normally. After many months of experimentation I produced a number of normal, adult fertile mice which developed from embryos in which I had intentionally destroyed one of the first two cells. When I learned that a part of the embryo can develop into a complete mouse, the next question I asked was whether two early embryos aggregated together would develop into one normal mouse or into a monstrous foetus (individual?)? The first option turned out to be true and we called thus produced animals – chimaeras. These two experiments – the first carried out in Poland and the second in Great Britain – proved that the early development of a mammalian embryo is extremely flexible, or – as the embryologists say – the embryos have great regulative capabilities. Further studies carried out upon my return to Poland have shown that up to the stage when the embryo is built of several cells, the fate of cells is not yet determined. At this stage cells have two developmental pathways to choose: either to become predecessors of the foetus and later the animal, or to contribute to the foetal membranes, the auxiliary structures that are discarded at birth. It was suggested that the decision as to the choice of one of these two ways depends on the position occupied by cells in the aggregation:

these situated inside will form the foetus, those located outside will built the auxiliary membranes. This idea is known as the 'outside-inside' hypothesis. During the next years many experimental embryologists have provided dozens of examples of great developmental flexibility of early embryos in several mammalian species. It has been shown that also single cells of the 4- and 8-cell embryos can develop into adult animals, and twins, triplets and quadruplets have been produced. Thousands of chimaeric animals have been created using different developmental stages and different techniques.

In recent years it has been pointed out that this great developmental flexibility of early embryonic cells is manifested only under experimental conditions *in vitro* and that when embryos are left undisturbed they develop according to a predetermined pattern which stem from the organization of the egg. There are thus two sources of information which apparently are in conflict, and have to be somehow reconciled. My proposition is that the mammalian egg and the early embryo have indeed a system of spatial organization and signalling that guarantees a repeatable course of developmental events. Lack of a morphogenetic pattern would result in the developmental chaos. However, each developmental system characterizes itself also by some (greater or smaller) regulative capabilities. In mammals these capabilities are very large but perhaps *in vivo*, i.e. in the maternal womb, embryos rarely make use of them for developmental repairs. But due to these abilities identical twins can spontaneously develop, and in this way Nature confirms the view of many experimental embryologists, including myself, that developmental patterning in early mammalian development is very labile. This, in turn, permits us to manipulate the embryonic development, hopefully to the benefit of biomedical sciences.