The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles

by J. B. GURDON[1]

From the Embryology Laboratory, Department of Zoology, Oxford

AN important problem in embryology is whether the differentiation of cells depends upon a stable restriction of the genetic information contained in their nuclei. The technique of nuclear transplantation has shown to what extent the nuclei of differentiating cells can promote the formation of different cell types (e.g. King & Briggs, 1956; Gurdon, 1960*c*). Yet no experiments have so far been published on the transplantation of nuclei from fully differentiated normal

The donor cells used for these experiments were intestinal epithelium cells of feeding tadpoles. This is the final stage of differentiation of many of the endoderm cells whose nuclei have already been studied by means of nuclear transplantation experiments in *Xenopus*. The results to be described here may therefore be regarded as an extension of those previously obtained from differentiating endoderm cells (Gurdon, 1960*c*).

MATERIAL AND METHODS

The animals used for these experiments belong to the subspecies *Xenopus laevis laevis*. The transplantation technique has been carried out as described previously (Elsdale *et al.*, 1960), except that the donor tissue was exposed to the dissociating Versene solution (5×10^{-4} M) for 30–40 minutes. The *Xenopus* nuclear marker was used (Elsdale *et al.*, 1960), and marked donor nuclei were transplanted into unmarked recipient eggs. Among the transplant-embryos described below, all those which developed beyond the blastula stage contained marked nuclei, thus proving that they were derived from the transplanted nucleus and not from the egg nucleus. The nuclear marker can only be seen in embryos which have passed the blastula stage.

Donor cells

The differentiated cells used to provide donor nuclei were intestinal epithelium

Controls

Owing to the variable quality of the *Xenopus* recipient eggs laid in the laboratory (Gurdon, 1960*b*), the transplantation of intestinal epithelium cell nuclei has been accompanied by control transplantations of blastula or gastrula nuclei. Since no change in developmental capacity has been detected in *Xenopus* nuclei until after the late gastrula stage, either blastula or gastrula nuclei have been used as controls according to convenience.

RESULTS

Six experiments involving the transplantation of intestinal epithelium cell nuclei (referred to as intestine nuclei) gave similar results, and these have been combined in Table 1. In each experiment control transfers from blastulae or gastrulae (referred to as embryonic nuclei) were interspersed with transfers of intestine nuclei.

TABLE 1

The development resulting from the transplantation of nuclei from differentiated and embryonic cells of Xenopus laevis

Donor stage (Nieuwkoop & Faber, 1956)	*Total transfers*	*No cleavage*	*Total transfers resulting in cleavage*	*Development resulting from transplanted nuclei*								
				Abortive cleavage	*Partial cleavage*	*Complete blastulae*	*Arrested blastulae*	*Abnormal gastrulae*	*Abnormal post-neuralae*	*Stunted tadpoles*	*Died as swimming tadpoles*	*Normal feeding tadpoles*
Intestinal epithelium cell nuclei (stage 46–48)	726	347	379	175	156	48	18	8	5	6	1	10
	100%	48%	52%	24%	21·5%	6·5%	—	—	—	—	—	1·5%
Blastula or gastrula endoderm nuclei (stage 8–12)	279	66	213	8	32	173	4	17	19	27	6	100
	100%	24%	76%	3%	11%	62%	—	—	—	—	—	36%

The cytological analysis of eggs fixed soon after receiving transplanted nuclei

The procedure followed in this analysis was to transplant nuclei from one donor embryo into eggs laid by one frog. Soon after transplantation some of the eggs were taken at random and fixed while the remainder were allowed to develop as far as they were able. The fixed eggs were then serially sectioned and stained. Subsequent microscopic examination of the sections often revealed abnormalities of the transplanted nucleus and achromatic apparatus. The eggs which were not fixed served as exact controls since they were laid by the same frog as the fixed eggs and contained transplanted nuclei from the same donor

TABLE 2

The cytological analysis of eggs fixed 60–80 minutes after transplantation

	Number of eggs fixed	*Eggs with no developing nucleus*	*Chromosomes clumped at first mitosis*	*3–4 polar spindle at first mitosis*	*Normal at first mitosis*
(*a*) Tadpole nuclei from intestinal epithelium cells	70	22 out of 70 31·5%	3 out of 11* 27%	4 out of 11* 36%	—
(*b*) Nuclei from blastulae and gastrulae	59	8 out of 59 13·5%	0 out of 30* 0%	4 out of 30* 13%	20 out of 30* 67%

The transplantation of nuclei from abnormal nuclear transplant-embryos

Information on the cause and significance of partial blastulae and of abnormal post-blastulae has been obtained by means of serial nuclear transfers. The basic design of the experiments was as follows. Nuclear transfers were made using original intestine or embryonic donor cells. When the resulting 'first-transfer' embryos had differentiated as far as they were able, some of their endoderm nuclei were used for serial transfers, giving rise to the 'first serial-transfer' generation. As a result of experience, the best differentiation that will be achieved by an abnormal transplant-embryo can be judged to within narrow limits, before developmental arrest takes place and cell death sets in. For

stage of differentiation attained by each first-transfer embryo is shown by a solid line; the dotted continuation of this line represents the most normal differentiation achieved by any of the resulting serial-transfer embryos. It can be seen

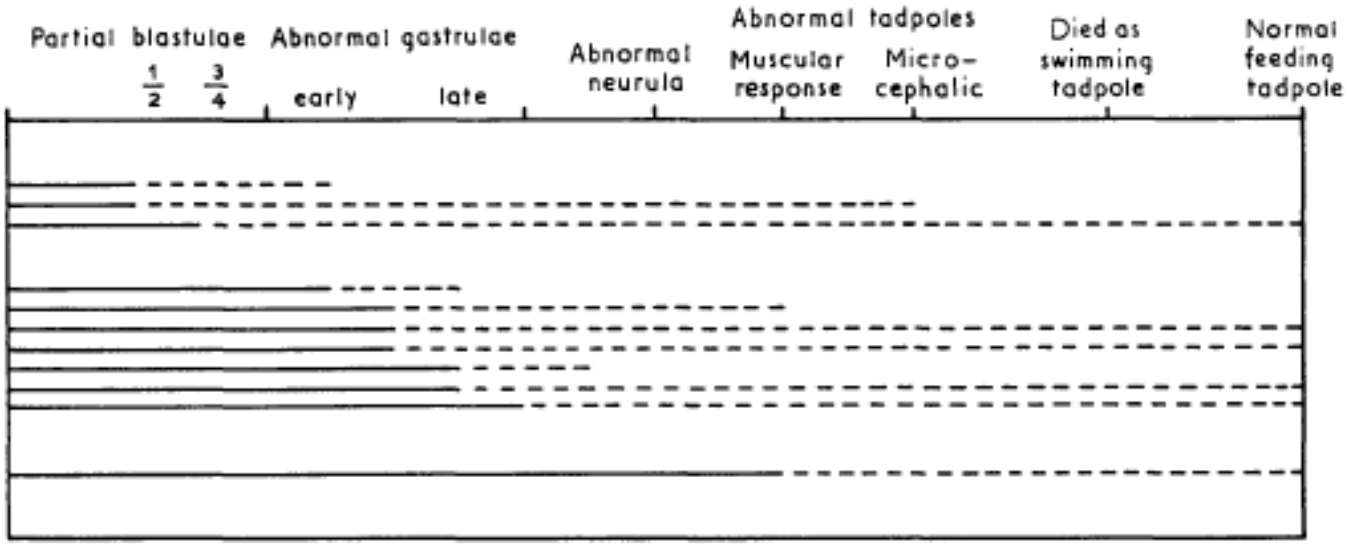

FIG. 1. Serial nuclear transfers from abnormal first-transfer embryos. Original gastrula donor nuclei (embryonic nuclei). Furthest differentiation attained by each first-transfer embryo (solid line) and by the most normal of the serial-transfer embryos derived from its nuclei (dotted line).

ju

Cellular differentiation is most probably initiated by the effect of the cytoplasmic environment on a nucleus, so that the nucleus provides specific genetic information which promotes the formation of a particular cell type (recent discussion by Fischberg & Blackler, 1961). Three possible ways in which this could happen are the following. First, nuclei might undergo a progressive loss of genetic material, so that cellular differentiation would result from the genetic material that is retained in different nuclei. Secondly, an inactivation of certain parts of the genetic material might take place, so that specific genetic information would be provided by the non-inactivated parts of a genome. This kind of inactivation would be stable under the normal conditions of cell mitosis. A theory of differentiation along these lines is suggested by various reports of stable nuclear changes in somatic cells (e.g. Brink, 1960). The third possibility is that the genetic information provided by a nucleus is entirely dependent on its cytoplasmic environment at any one time; in this case a nucleus would never undergo any stable changes having a qualitative effect on its function. This kind of system is suggested by the reversible appearance of puffs in the polytene chromosomes of insects (e.g. Breuer & Pavan, 1955) and by cases of metaplasia (e.g. Reyer, 1954). The first of these three possibilities is rendered very improbable by the results of the experiments reported in this article; these have shown that a nucleus may be responsible for the differentiation of one cell type while still possessing the capacity to form all other types of somatic cell in a feeding tadpole. It has previously been found that most of the normal feeding tadpoles resulting from transplanted nuclei of *Xenopus* will eventually form adult frogs (Gurdon, 1962*a*). However, the possibility still exists that intestine nuclei may have undergone stable changes restricting their capacity to form adult frogs and normal germ cells, since intestine nuclei have not yet been tested in these respects. These results are therefore consistent with any theory of cell differentiation which does not require that the nucleus of a differentiated cell has lost the genetic information required for the formation of other differentiated somatic cell types.