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The cloning of a frog

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Summary

It is relatively unusual for the Nobel Prize in Physiology or Medicine to be made, to a large extent, on the basis of a single author paper, published over 50 years ago, for work carried out by a graduate student. This was largely true of a paper published in 1962 in the journal *Development* (called at that time the *Journal of Embryology and Experimental Morphology*). The main subject of that paper was the production of normal tadpoles from the nuclei of intestinal epithelium cells of *Xenopus laevis*. In view of this unusual situation, I have been invited to comment on the 1962 paper.

Commentary

In 1962, a paper examining the developmental capacity of nuclei taken from intestinal epithelial cells of *Xenopus laevis* tadpoles was published (Gurdon, 1962) (Boxes 1, 2). This paper, which used the technique of nuclear transplantation originally described by Briggs and King (Briggs and King, 1952), showed that the nuclei of some intestinal epithelial cells from feeding tadpoles, after transplantation into enucleated eggs, could develop into normal feeding tadpoles. Several years later, the tadpoles produced in this work had matured into frogs and had been tested for sexual maturity and fertility. A subsequent paper (Gurdon and Uehlinger, 1966) was based entirely on the animals that had been produced and described in the 1962 paper. Below, I highlight some interesting features and outcomes of these early experiments.

In all experiments, the transplanted nuclei carried the nucleolar genetic marker (Elsdale et al., 1960), which was crucial for these experiments to be accepted by the scientific community. Intestinal epithelial cells from feeding tadpoles (Box 3) were used as donors of nuclei for transplantation into unmarked enucleated recipient eggs, and these were interspersed with experiments using nuclei taken from early embryo (blastula or gastrula) embryos. The proportion of total transplant embryos that reached a feeding tadpole stage was 1.5% when using intestinal nuclei as donors but 36% when using early embryo nuclei (Box 4). It was therefore already evident, at this time, that, as cells differentiate, their nuclei are a great deal less able to support development within enucleated eggs than are nuclei from embryonic cells. This reflects the phenomenon of 'resistance' that is so evident in current induced pluripotent stem cell (iPSC) experiments.

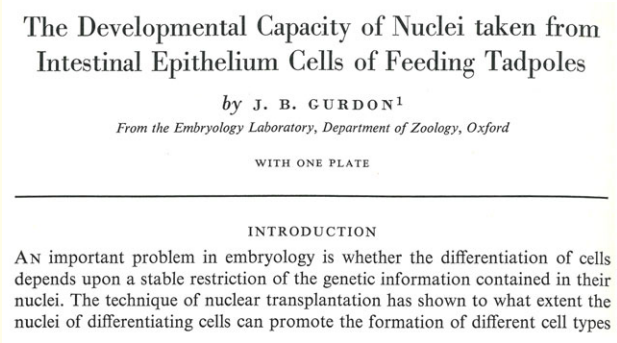
To investigate the reason for this decline in the success of nuclear transfers, the fate of transplanted nuclei in eggs was investigated by wax embedding and serial sectioning. In some cases no transplanted nucleus was found at all and this could have been because the nucleus was broken or pulled out of the egg as the injection pipette was withdrawn. Therefore, technical difficulties accounted for some of the failures. Furthermore, previous work had shown the desirability of breaking the donor cell to the least extent possible, thereby enabling it to be protected by donor cell cytoplasm. Therefore, in these experiments, I used the least amount of donor cell distortion that gave a reasonable proportion of broken donor cells. A number of the recipient eggs may therefore have received unbroken donor cells, which cannot participate in development.

It was also known from work in *Rana pipiens* (Di Berardino and King, 1967) that transplanted somatic nuclei often undergo severe chromosome breakage. This has been attributed to incomplete DNA replication after nuclear transfer. DNA synthesis normally requires ~6 hours in somatic cells but has to be completed within 1 hour of nuclear transfer to eggs. This is because eggs always divide into the two-cell stage 90 minutes after activation (which results from a micropipette injection). A failure to make the transition from a slow to a fast DNA replication cycle can therefore account for some of the nuclear damage sustained after transfer to eggs and, hence, for the abortive cleavages often observed with nuclei from differentiated cells.

Another common consequence of transplanting nuclei from differentiated cells is the formation of partially cleaved embryos, which either die prior to gastrulation or form abnormal gastrulae. Typically, these have normal-looking blastomeres in about half of the embryo while about half are undivided. A favoured interpretation of this is that somatic nuclei often fail to complete their DNA replication by the time the egg divides into two. It is thought that, in such cases, the whole replicating transplanted nucleus moves into one of the first two blastomeres where it can then further complete its DNA replication, while fertilised eggs continue carrying out their second replication, from two-cell to four-cell stages. The other blastomere hence receives no nucleus and so remains undivided.

To test this idea, serial nuclear transplantation was carried out in which normal cells from a partially cleaved embryo served as donors of nuclei for transplantation to another set of recipient eggs. The results published in the 1962 paper were quite striking.

Box 1.
The original title and opening lines of the 1962 paper. The issue of 'stable restriction of genetic information' is still important today.



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

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WITH ONE PLATE

INTRODUCTION

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

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Box 2.
Instructions for authors in 1962 stipulated the inclusion of a summary of the paper in both French and English. This was phased out during the following decade.

SUMMARY

1. Nuclei from differentiated intestinal epithelium cells of feeding tadpoles and from control blastulae of *Xenopus* have been transplanted into enucleated recipient eggs. The differentiated state of the intestinal epithelium cells was shown by their possession of a striated border.

2. The cleavage and embryonic development resulting from the intestinal epithelium cell nuclei was much more abnormal than that resulting from control blastula transfers.

3. 1½ per cent. (10 out of 726) of the first transfers of intestine nuclei resulted in normal feeding tadpoles.

RÉSUMÉ

Potentialités de développement de noyaux issus de cellules de l'épithélium intestinal de têtards se nourrissant

1. Des noyaux de cellules différenciées de l'épithélium intestinal et de blastulas témoins ont été transplantés dans des œufs énucléés chez le *Xénope*. On reconnaît l'état différencié des cellules de l'épithélium intestinal à la présence d'une bordure striée.

2. La segmentation et le développement embryonnaire obtenus à partir de noyaux de cellules de l'épithélium intestinal sont beaucoup plus anormaux que ceux obtenus à partir de noyaux de blastulas.

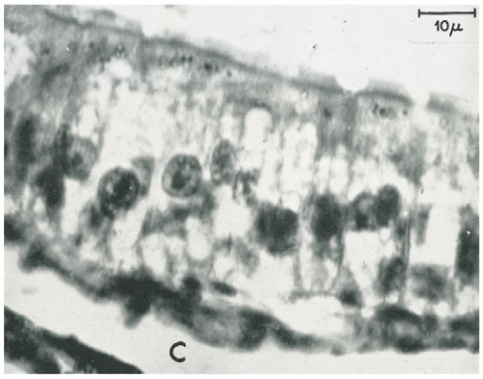
3. 1½ pour cent (10 sur 726) des transplantations simples ont donné des têtards normaux se nourrissant.

The first important observation is that the serial transfer of nuclei from partially cleaved embryos very often gave completely cleaved embryos and, hence, complete blastulae. In turn, many of these developed much further, even forming, in some cases, normal feeding tadpoles (Box 5). The conclusion that I believe to be correct is that the initially transplanted nuclei were spared from chromosome damage by having a second chance to complete their DNA replication as the recipient egg divided into two cells. Only when that had happened did the originally transplanted nucleus have to start division as one of the first two blastomeres divided into two cells at the time that control embryos from fertilised eggs were dividing from the two- to four-cell stage. In this way, I believe that the true genetic potential of an originally transplanted somatic nucleus can be revealed especially well when the initial first transfer embryo divides into a partial blastula. It was noticed that subsequent serial nuclear transfers up to a third or fourth generation did not yield any further improvement in the normality of development ultimately achieved. In some cases, as many as ten serial transfer experiments were carried out, a physically demanding kind of experiment requiring re-transfer every 12 hours for 5 days. There was therefore no case for believing that the developmental capacity of a transplanted nucleus can increase with serial transfer. Rather, the full genetic potential of such a somatic nucleus is often revealed most clearly by a serial nuclear transfer experiment (Gurdon, 1960).

The major conclusion from these nuclear transplant experiments was that two-thirds of the nuclei transplanted from intestinal epithelium cells could generate embryos that reach the muscular response stage (Box 6). This stage of development indicates normal muscle and nerve function; the muscle and nerve lineages are entirely unrelated to that of the intestine, which comes from the endoderm, highlighting that a nucleus is able to promote the formation of a differentiated cell type whilst still retaining the genetic information required for the formation of other differentiated somatic cell types in a normal tadpole.

The normal tadpoles derived from these early experiments were taken to Geneva where my supervisor Michail Fischberg moved to

Box 3.
Image of the intestinal epithelial cells used in the nuclear transfer experiments.



Donor cells

The differentiated cells used to provide donor nuclei were intestinal epithelium cells from the mid-intestine of feeding tadpoles (stages 46–48 of Nieuwkoop & Faber, 1956). These cells (plate) have the following features characteristic of their differentiated state: a tall columnar shape with basally situated nuclei; pigment granules inside the surface exposed to the gut lumen; and, most important, the striated border typical of cells having an absorptive function.

Box 4.
The Nobel Prize-winning results (highlighted in red). Altogether, ten normal feeding tadpoles were obtained from the transfer of intestinal epithelial cell 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%

Box 5.
A summary of the serial transplantation experiments, which highlights the full genetic potential of a somatic cell nucleus.

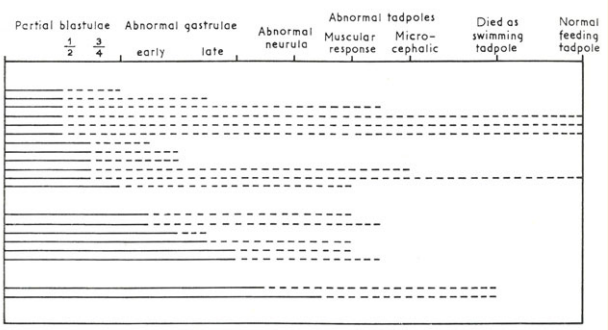


FIG. 2. Serial nuclear transfers from abnormal first-transfer embryos. Original tadpole intestine nuclei. Furthest differentiation attained by each first-transfer embryo (solid line) and by the most normal of the serial-transfer embryos derived from its nuclei (dotted line).

Box 6.
Combined results show that 70% of nuclei transplanted from intestinal epithelial cells could generate embryos that reached the muscular response stage (highlighted in red).

TABLE 5

Summary of conclusions reached regarding the developmental capacity of nuclei taken from differentiated and embryonic cells of Xenopus laevis

Donor nuclei	*Developmental capacity of nuclei. Stages of Nieuwkoop & Faber, 1956*	*Results of first-transfers only as percentage of total transfers*	*Combined results of first and serial transfers**		
			As percentage of total transfers	*As percentage of total transfers, less those resulting in no cleavage*	*As percentage of total transfers, less those resulting in no cleavage or abortive cleavage*
Intestinal epithelium cell nuclei	Capable of forming feeding tadpoles; stage 50	1·5% (10)	7% (49)	13% (49)	24% (49)
Blastula or gastrula cell nuclei		36% (100)	57% (158)	74% (158)	77% (158)
Intestinal epithelium cell nuclei	Capable of forming muscular response tadpoles; stage 26	2·3% (17)	20% (142)	37% (142)	70% (142)
Blastula or gastrula cell nuclei		48% (133)	65% (181)	85% (181)	88% (181)

The figures in brackets represent the number of individuals.
* The figures for serial transfers were calculated as described on p. 634.

take up the professorship of Zoology. He and his assistant Vreni Uehlinger cared for my tadpoles during my post-doctoral period in USA. They grew them up to adult frogs and returned them to me in Oxford for fertility testing. This led to the 1966 paper (Gurdon and Uehlinger, 1966) reporting 'fertility' of intestine nuclei and, of course, to many more studies that examine the concept of reprogramming.

Acknowledgements
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