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THE TRANSPLANTATION OF LIVING CELL NUCLEI

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I. Introduction

The problem of cell differentiation can at present be considered to have two main aspects: the first of these is the regulation of information transfer, a process determining what kind of molecules are synthesized in the cell; the second is the spatial arrangement of these molecules into cell structures, or the genesis of shape. The transplantation of living nuclei is a kind of experiment which has so far contributed mainly to the former aspect of differentiation, especially to the control of gene activity and to the stability of gene expression. It is therefore indirectly relevant to morphogenesis.

This review contains three main sections. The first describes the techniques that have been developed for transplanting nuclei in different organisms. The intention is to discuss the achievements and limitations of these techniques and hence the kind of problems to which each can most usefully be applied.

In the next section (III) the results of the different kinds of nuclear transfer experiments are summarized and are arranged according to the type of conclusion that can be drawn from them.

In Section IV the conclusions reached from nuclear transfer experiments are compared with relevant results from other kinds of experiments. Rather few other experimental approaches yield results which can be directly compared to those obtained from the manipulation of living nuclei, but it is at least possible to determine how far the results of nuclear transplantation are consistent with those of other experiments.

II. Techniques of Nuclear Transplantation

A. Transfer of Nuclei with Cytoplasm

The successful transplantation of living cell nuclei to a new cytoplasmic environment has now been achieved in several different organisms each of which offers certain advantages for this kind of operation. However each organism also presents some technical problems the solution of which always depends to a considerable extent on the dexterity of the operator, as well as on the development of special methods.

It should be pointed out at this stage that the same result as is achieved by nuclear transplantation can sometimes be realized by appropriate hybridization experiments. Astaurov and Ostriakova-Varshaver (1957), for example, heated eggs of *Bombyx mandarina* fertilized with sperm of *Bombyx mori* to 40° C for over 2 h. This prevented the egg nucleus in the second meiotic metaphase from leaving the egg periphery and allowed the fusion of two sperm nuclei to give diploid androgenetic hybrids. This method can only be used to combine egg cytoplasm with sperm nuclei. Nuclear transplantation must therefore be employed to introduce different kinds of somatic cell nuclei to egg cytoplasm.

1. *Amphibia*

Briggs and King (1952) were the first to obtain really satisfactory results from the transplantation of blastula cell nuclei to enucleated frog eggs. The principle of their method has been followed in all subsequently successful work with Amphibia (Fig. 1). Further details of the techniques outlined below may be found in the following references: Briggs and King (1953) and King and Briggs (1955) for *Rana*; Gurdon (1960b) and Elsdale *et al.* (1960) for *Xenopus*; Signoret *et al.* (1962) for *Ambystoma*; Signoret and Picheral (1962) for *Pleurodeles*.

Unfertilized eggs are laid by frogs several hours after they have received an injection of anterior pituitary glands or of gonadotrophic hormone. In some species, unfertilized eggs require activation before they will respond to an injected nucleus. In *Xenopus* no activation is

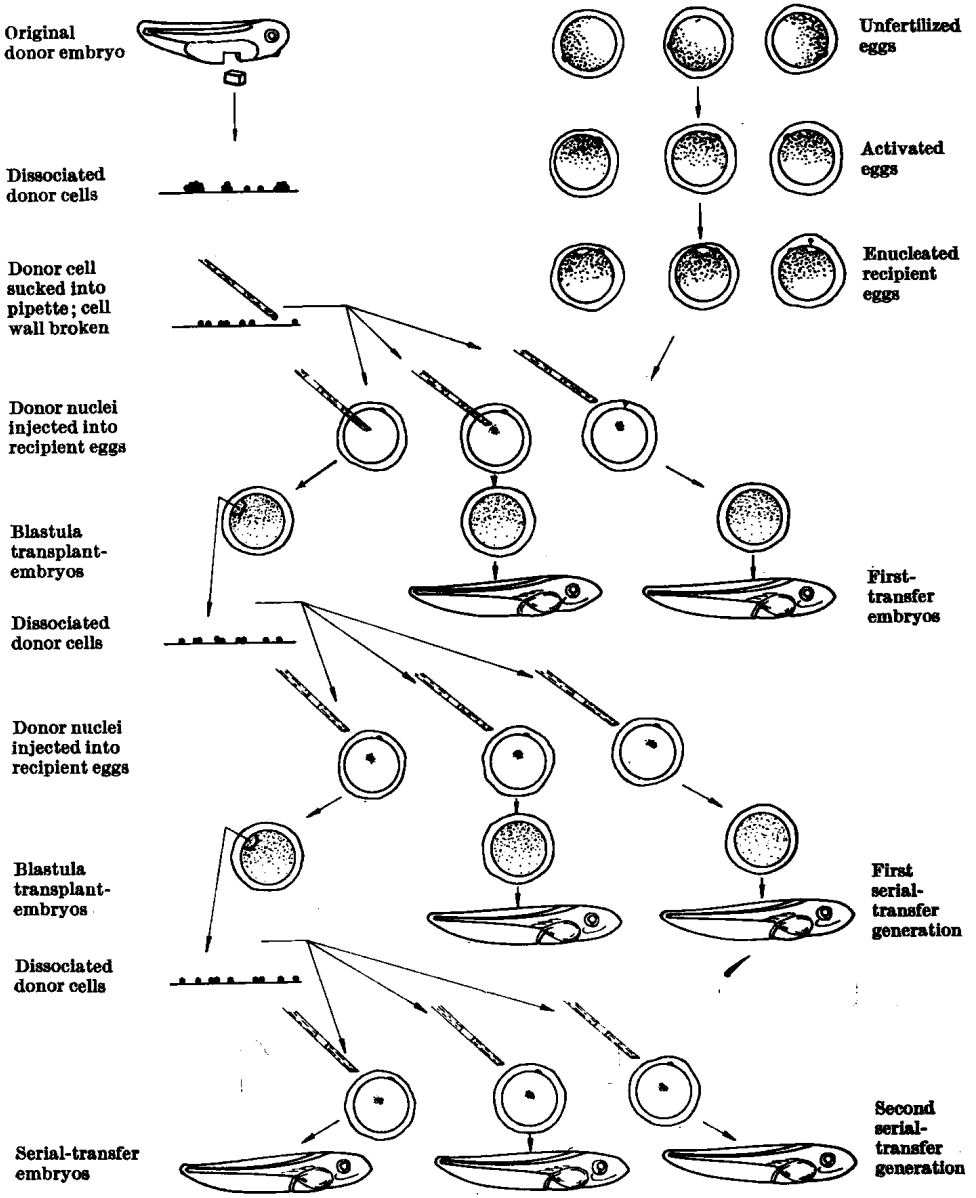

FIG. 1. Diagram of the nuclear transplantation procedure in Amphibia. (From Gurdon, 1963.)

needed, but in *Rana* it is done by pricking the egg with a clean glass needle, and in *Ambystoma* and *Pleurodeles* by an electric or hot shock. Various methods are employed to remove or kill the egg nucleus which lies just under the surface of the animal pole of the egg, where its position is marked by a microscopically dark spot. In *Rana*, a needle is used to make an exovate in the egg close to where the nucleus lies. In *Xenopus*, *Ambystoma* and *Pleurodeles* the egg nucleus is killed by a dose of ultraviolet irradiation which has no detectable effect on the cytoplasm of the egg. The donor tissue which will provide the nuclei to be transplanted is dissected out from the embryo and is then placed in a saline solution lacking calcium and magnesium, but usually containing between 10^{-3} and 10^{-4} M Versene, phosphate buffered at pH 8·1. This dissociates the cells which may be kept without deterioration for over 1 h in a saline solution containing calcium and magnesium. In order to transplant a nucleus, a donor cell is sucked up into a micropipette of such a size that the cell wall is broken but the nucleus is still surrounded

TABLE I

Species in which living cell nuclei have been successfully transplanted

Species	Total transfers	Late blastulae (% of total)	Tail-bud embryos (% of total)	Feeding tadpoles (% of total)	References
Rana pipiens	92	42	40 (usual range 40–60%)	36	Briggs and King (1957)
Rana sylvatica	24	13	?	8	Hennen (1963)
Rana nigromaculata	387	14	2	1	Sambuichi (1957)
Rana temporaria	416	7	0·5	—	Stroeva and
Rana arvalis	144	10	2	—	Nikitina (1960)
Xenopus laevis	279	62	38	36	Gurdon (1962c)
Ambystoma mexicanum	138	78	44	11	Signoret *et al.* (1962)
Pleurodeles waltlii	207	30	16	9	Picheral (1962)
Amoeba proteus					Danielli *et al.* (1955)
Amoeba discoides					
Apis mellifera	Many nuclei injected				DuPraw (1960)
Neurospora crassa					Wilson (1963)
Stentor coeruleus	Macronuclear grafts		High percentage of success		Tartar (1953)
Acetabularia mediterranea	Rhizoid grafts				Hämmerling (1953)
Acetabularia crenulata					
Acicularia schenckii					
Acetabularia species—transfer of isolated nuclei					Werz (1962)

by some of the cell cytoplasm. The whole broken cell is then injected into the centre of the enucleated recipient egg.

As a result of refinements in technique over several years, a high percentage of eggs receiving transplanted blastula nuclei will develop normally, especially in *Rana pipiens* and *Xenopus laevis* (Table I). As is also shown in Table I, a number of other amphibian species have been used for nuclear transplantation experiments. The main problems in using Amphibia for these experiments seem to be the tendency for the egg cytoplasm to leak out of the punctured membranes, and the difficulty of finding a means of artificial activation; however these cannot be all, as is shown by the unrewarded efforts of Lehman (1955) to transplant nuclei in newts.

Amphibia constitute very convenient material for nuclear transfer experiments partly because their eggs can withstand a remarkable amount of experimental punishment and partly because of the very large size of their cells compared to those of most other animals. They are particularly suitable for transplanting nuclei from different kinds of somatic cells, but cannot be used with the present methods for the transfer of nuclei to any other kind of cytoplasm than that of eggs.

2. *Insects*

A method of transplanting nuclei in the honeybee has been worked out by DuPraw (1960), who has very kindly allowed the author to quote much of his unpublished work in the following account. In the honeybee the sausage-shaped eggs are 1·5–2·0 mm long and, as is common in the insects, free nuclei are distributed throughout the cytoplasm during cleavage. The eggs remain syncytial until the blastoderm stage. By means of a fine glass pipette, some of the contents of a preblastoderm embryo are withdrawn, and the cytoplasm with several to many nuclei is injected into a fertilized (worker) or unfertilized (drone) egg through the animal pole (Fig. 2). Immediately after injection a cotton fibre is used to constrict off about 15–20% of the animal end of the egg which contains the egg or zygote nucleus. This treatment not only enucleates the egg but also prevents desiccation through the injection pore which otherwise occurs; it results in the development of dwarf but in other respects apparently normal larvae. It is interesting that the honeybee egg is extremely sensitive to temperature, and both the pipette and eggs must be kept at exactly 35·5° C.

DuPraw has obtained vigorous young larvae from the injection of several preblastoderm nuclei and cytoplasm into fertilized eggs. Indirect evidence from time-lapse photography indicates that the zygote nucleus is reliably excluded by the constriction. However, nuclear-transplant embryos do not survive until the adult stage, and therefore it has not

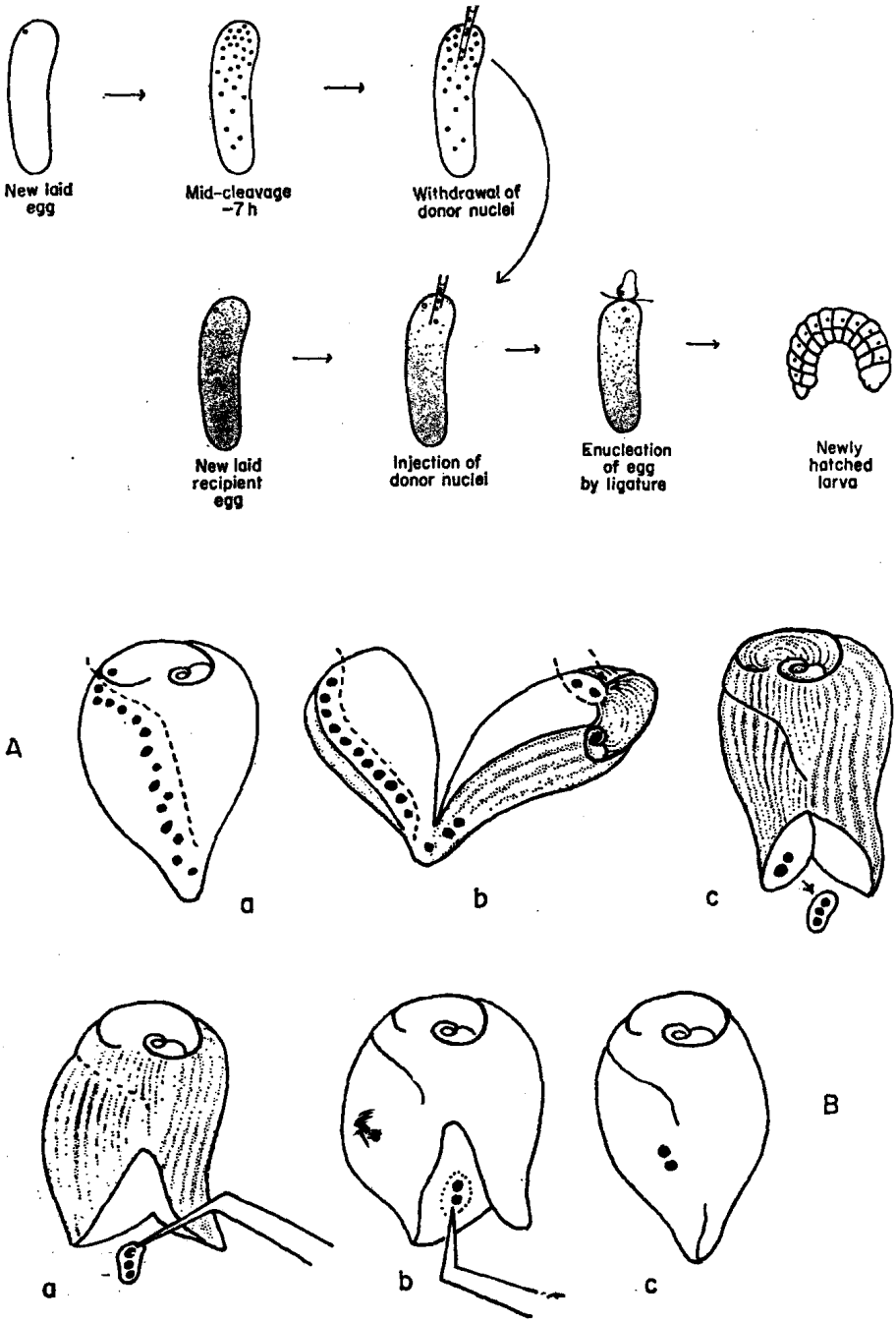

FIG. 2. Top: diagram of DuPraw's method of transplanting nuclei in the honeybee. See text for description. (From DuPraw, 1960.) Bottom: diagram of Tartar's method of grafting nuclei in *Stentor*. See text for description. (From Tartar, 1961.)

been possible to prove by use of genetic mutants that the zygote nucleus does not participate in development.

If it can be established that the injected nuclei are responsible for the development of the eggs, then DuPraw's experiments are the first to show that nuclear transplantation is feasible in insects. It should not be impossible to refine the technique so as to inject only one nucleus with correspondingly less cytoplasm. In the author's laboratory C. F. Graham (unpublished) has looked into the possibility of transplanting nuclei in *Drosophila* and *Calliphora*. So far no success has been obtained in *Drosophila*. In *Calliphora*, injected nuclei sometimes appear to divide a few times, but development does not proceed as far as the blastoderm stage (Fig. 4, F, G). The relatively 'mosaic' nature of many insect eggs makes it difficult to obtain normal larvae after manipulation of the egg, but there must be other reasons which are not yet apparent why nuclei fail to divide after transfer.

3. *Neurospora*

As a result of the very careful preparation of micropipettes and the use of certain structural and physiological peculiarities of *Neurospora*, Wilson (1963) has been able to transplant nuclei in this fungus. *Neurospora* has chains (hyphae) of elongated cells each of which contains twenty or more free nuclei (Fig. 3, D). The cells are separated by a septum; this is perforated by a pore which becomes sealed off if one of the cells on either side of it dies. Some of the contents of one cell, including several nuclei, are sucked up into a micropipette. The pipette is then inserted into a cell of the recipient hypha in such a way that the contents of the pipette are disgorged through the septal pore into the neighbouring cell. The volume of cytoplasm and number of nuclei introduced in this way generally amounts to about one-tenth of that already present in the recipient cell. After the pipette is withdrawn the punctured cell dies, and if the operation is successful the septum seals itself off. About one-third of the successful transfer-heterokaryons then commence growth.

The successful transplantation of nuclei has been proved by injecting nuclei from a strain unable to synthesize an amino acid (or vitamin) into cells which are unable to synthesize a different amino acid, and showing that the resulting heterokaryons will grow on minimal medium lacking both amino acids. Some strains always fail to grow after injection of nuclei from certain other strains; this can be attributed to incompatibility—a phenomenon which can be analysed further by this technique.

The main importance of these experiments is to demonstrate the possibility of manipulating and transplanting nuclei in *Neurospora*. This is the only organism in which nuclei can be transplanted and in

which a wide range of genetic mutants have been isolated. Limitations in the present form of the technique include the large amount of cytoplasm and the undefined number of nuclei that are injected. The inability to enucleate the recipient cells is not a serious limitation, since spores have been obtained from the injected nuclei as well as from the host nuclei (Wilson, 1963).

4. *Amoeba*

Comandon and de Fonbrune (1939) devised a method of enucleation and nuclear transplantation in *Amoeba*; this has been improved by Lorch and Danielli (1953).† The amoebae to be operated on are placed in a paraffin chamber in shallow hanging drops of Chalkley's saline medium (including a minute amount of protein from one cytolysed amoeba). An amoeba is enucleated by pushing its nucleus out with a blunt glass needle (Fig. 3, A–C). Such enucleated amoebae soon lose contact with the coverslip and show unco-ordinated streaming of the endoplasm, but survive for 10 days or more. Nuclear transplantation is carried out by holding two amoebae close together with a glass hook and then pushing a nucleus from one to the other with a blunt glass needle. After receiving a nucleus, an enucleated amoeba soon attaches to the glass and recommences co-ordinated streaming. Reactivation sometimes takes place within seconds but only does so after several hours if damage was done during the operation. Only a small amount of cytoplasm is usually carried over with the nucleus. Following nuclear transfer there is an average delay of 1–2 days before division in addition to the 3–4 days' delay in non-operated amoebae randomly selected from the culture. About 95% of the nuclear transfers between individuals of the same species are successful (Danielli *et al.*, 1955). Nuclear transplantation in *Amoeba* has been extensively used by Danielli and co-operators to determine the relative contribution of nucleus and cytoplasm in the control of differentiation (Section III, C) as well as by Goldstein to follow the movement of substances between nucleus and cytoplasm (Section III, D).

5. *Acetabularia*

Acetabularia is a green alga which consists during most of its life cycle of a branched rhizoid, containing a single large nucleus, a lengthy stalk, and a cap the shape of which helps to distinguish one species from another (Fig. 4, A, B). Two methods of transplanting nuclei have been used in *Acetabularia*. One involves the transfer of an almost isolated nucleus (Section II, B). The earlier method, which has been used more extensively, consists of making rhizoid grafts (Hämmerling, 1934). Two

† The technique of nuclear transplantation in amoeba has been reviewed recently by Goldstein (1963b).

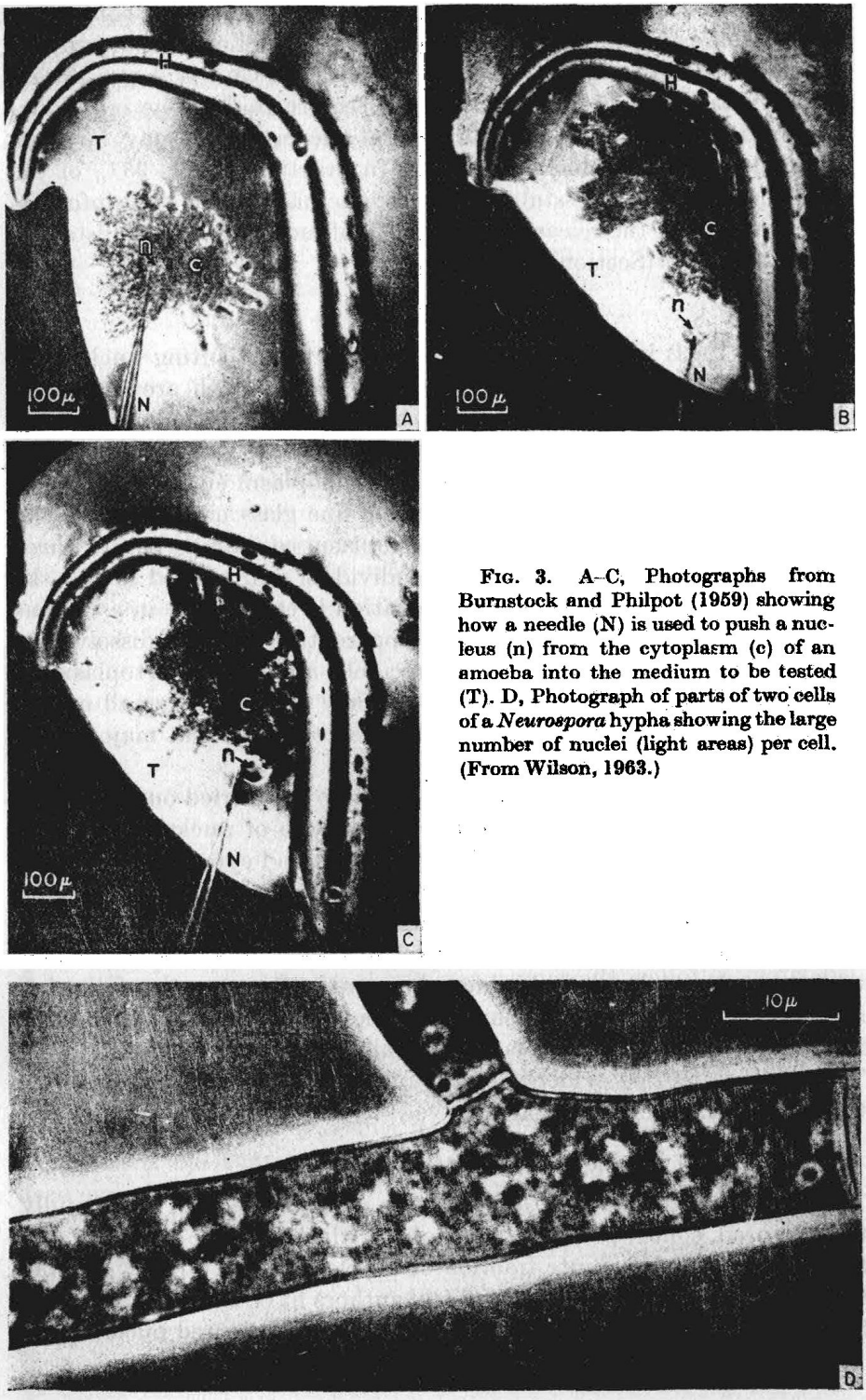

FIG. 3. A–C, Photographs from Burnstock and Philpot (1959) showing how a needle (N) is used to push a nucleus (n) from the cytoplasm (c) of an amoeba into the medium to be tested (T). D, Photograph of parts of two cells of a *Neurospora* hypha showing the large number of nuclei (light areas) per cell. (From Wilson, 1963.)