of paths. What we prove is that the tangent bundle (thus represented) is a fiber deformation retract of the path space.

The retraction is easy to construct. The essential point is to find a parameter value t^* for each path which varies continuously with the path and has the property that for $0 \leq t \leq t^*$ the points $x(t)$ and $x(0)$ are less than one unit of distance apart. Then a unique shortest geodesic runs from $x(0)$ to $x(t)$.

To define t^*, let $d(t)$ be the distance from $x(t)$ to $x(0)$. Then we define a continuous monotone decreasing function $\delta(t)$ by

$$\delta(t) = \min_{\tau \leq t} |1 - d(\tau)|.$$

This function $\delta(t)$ varies continuously with the path; hence we can define t^* by the equation

$$t^* = \delta(t^*).$$

Using t^*, the deformation of the paths into geodesics of unit length can be defined as a three-stage process. First, contract the path along itself until it becomes the subpath ending at the parameter value t^*. We may now deform it into the geodesic segment from $x(0)$ to $x(t^*)$, with the use of the geodesic segments from $x(0)$ to $x(t)$, $0 \leq t \leq t^*$. Finally, the geodesic segment from $x(0)$ to $x(t^*)$ is gradually extended until it has unit length.

[1] Réné Thom, *Ann. sci. École norm. supér.* (ser. 3), **69**, 109–182, 1952.

[2] The path space is a true fiber space in the sense that the covering homotopy theorem holds. This fact is actually not needed to justify any of the results we obtain by use of the path space, but it deserves a remark.

One can show by construction that covering homotopy holds locally, even if there is no global differentiability structure on M, so we at least have a fiber space in Hu's sense. But then a recent (unpublished) theorem of Hurewicz shows that this local property implies the global covering homotopy property under very general conditions. Hence the name "fiber space" is justified.

[3] This assumption can be avoided, if desired, by changing the later construction. It is obviously realizable for compact manifolds by scale change and is fairly easily justified in general, so we include no proof of its realizability.

CHANGES IN THE NUCLEI OF DIFFERENTIATING GASTRULA CELLS, AS DEMONSTRATED BY NUCLEAR TRANSPLANTATION

BY T. J. KING AND R. BRIGGS

INSTITUTE FOR CANCER RESEARCH AND LANKENAU HOSPITAL RESEARCH INSTITUTE, PHILADELPHIA, PENNSYLVANIA

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The hypothesis that the nucleus controls the differentiation of embryonic cells dates back to the time of Roux and Weismann. Originally it involved the assumption of a segregation of nuclear determiners of differentiation during cleavage. When put to the test, the hypothesis was found wanting.[1] Embryological experimentation showed that the distribution of nuclei during the early cleavages could be altered without producing a corresponding alteration of the developmental pattern. Furthermore, cytological evidence of the equational nature of mitosis was

against any theory of differentiation involving somatic segregation. Finally, modifications of the hypothesis which required permanent changes in gene activity were difficult to accept. So far as was known, such changes (i.e., mutations) occur infrequently and unpredictably, while what was required was a co-ordinated series of directed gene changes. Taken together, this evidence indicated that somatic cells contain identical nuclei. However, definite proof of this fact was obtained only for the early cleavage stages, and the possibility remained that nuclear changes, perhaps of a type not heretofore recognized, might occur later in development.[2]

In an effort to detect changes in the properties of nuclei during differentiation, we have developed a method for transferring nuclei of embryonic cells into enucleated eggs of the frog *Rana pipiens*.[3, 4]. The earlier work with this procedure showed that living nuclei of undifferentiated blastula cells could be successfully transplanted. The recipient eggs developed into normal embryos, demonstrating that there had been no irreversible changes in the nuclei during pregastrula development.

More recently, nuclei of chorda-mesoderm and presumptive medullary plate of the late gastrula were similarly tested.[5] The donor cells were small and difficult to handle, and fewer of the attempted transfers were successful than had been the case in the previous experiments. About 8 per cent of the test eggs developed into normal blastulae which, on the basis of their appearance, would have been expected to differentiate normally. Instead of so developing, about half the blastulae were arrested in blastula and gastrula stages. Of the remainder, a few developed into larvae, while the majority were arrested in various embryonic stages. At the time there were two possible interpretations of this result. One was that the nuclei of determined areas of the late gastrula had undergone a change restricting their capacity to promote certain of the varied types of differentiation required for the co-ordinated development of the egg. Such a nuclear change could easily account for the cessation of the development of the test eggs. The second possibility was that the nuclei might have been damaged in the course of the transfer in such a way as to lead to the same result. Since this possibility could not be excluded, we adopted it as the more conservative interpretation and emphasized the fact that some of the test eggs did develop normally. This indicated that at least some, and possibly all, of the late gastrula nuclei were unchanged, despite the fact that the tissues were determined.

Recently the experiments mentioned above have been repeated and extended, using an improved procedure for nuclear transplantation. The improvement consists mainly of the use of trypsin and versene (ethylenediamine tetra acetic acid) to aid in dissecting the embryo and isolating individual cells of known type in undamaged condition.[6] The embryo is first placed in 0.5 per cent commercial (Difco) trypsin made up in a Niu-Twitty solution[7] lacking Ca^{++} and Mg^{++} and buffered at pH 7.1 with phosphate. The trypsin has a preferential action on the material cementing the embryonic layers together. As it acts, the layers can be neatly separated. A portion of the desired layer is now placed in $5 \times 10^{-4}M$ versene, also made up in the modified Niu-Twitty solution. The versene quickly affects the intercellular material—within 5–10 minutes the cells round up and are transferred to the regular Niu-Twitty solution. They can now be picked up singly in the transfer pipette, and the nuclei can be transplanted in the manner previously described.[4]

The improved transplantation procedure was first tested on nuclei of undifferentiated early gastrula cells, with gratifying results (Table 1). The proportion of attempted transfers resulting in normal cleavage of the recipient eggs was increased from 21 to 41 per cent. There was also a pronounced improvement in the later development of the test eggs, as indicated in the table.

TABLE 1

TRANSPLANTABILITY OF NUCLEI AS INFLUENCED BY METHOD OF DONOR CELL ISOLATION
(Donor Cells from Animal Hemisphere of Early Gastrulae)

METHOD OF DONOR CELL ISOLATION	NO. EGGS INJECTED WITH NUCLEI	COMPLETE BLASTULAE	DEVELOPMENT OF BLASTULAE		
			Arrested Blastulae and Gastrulae	Arrested Neurulae and Postneurulae	Larvae (10–12 Mm.)
Dissection with glass needles*	135 (100%)	29 (21%)	10	12	7
Trypsin plus versene	71 (100%)	29 (41%)	1	3	25

NOTE: There is no difference in the type of cleavage that can be correlated with the sources of nuclei. In all cases some nuclear transfers fail completely; others give rise to abortive or "nucleusless" blastulae which never develop further. The remainder, which comprise the genuinely cleaved eggs, give rise to partial or complete blastulae. In this and the following tables we will consider only the formation and development of the complete blastulae.

* T. J. King and R. Briggs, *J. Embryol. Exptl. Morphol.*, **2**, 78, 1954.

The experiments with late gastrula chorda-mesoderm nuclei were now repeated. With the new procedure the proportion of recipient eggs displaying normal cleavage and blastula formation was more than doubled—from 8 to 22 per cent—indicating that more of the nuclei were being transplanted in undamaged condition (Table 2). On this basis one would expect a definite improvement in the later development of the blastulae. However, no such improvement was observed. The majority of the blastulae were still arrested in blastula, gastrula, or abnormal postneurula stages. Sections of the latter embryos showed that the inductor system (notochord and somites) was differentiated, while the central nervous system was very deficient and occasionally absent. These experiments indicated that the deficiencies in the development of the "chorda-mesoderm embryos" were not, as we had previously thought, entirely the result of nuclear damage but rather reflected an intrinsic change in the "differentiative" properties of the chorda-mesoderm nuclei.

TABLE 2

TRANSPLANTABILITY OF NUCLEI AS INFLUENCED BY METHOD OF DONOR CELL ISOLATION
(Donor Cells from Chorda-Mesoderm of Late Gastrulae)

METHOD OF DONOR CELL ISOLATION	NO. EGGS INJECTED WITH NUCLEI	COMPLETE BLASTULAE	DEVELOPMENT OF BLASTULAE		
			Arrested Blastulae and Gastrulae	Arrested Neuralae and Postneurulae	Larvae (10–12 Mm.)
Dissection with glass needles*	242 (100%)	20 (8%)	10	6	4
Trypsin plus versene	83 (100%)	18 (22%)	8	8	2

* See Table 1.

In order to obtain more decisive evidence of nuclear changes during cell differentiation, a series of experiments was done on nuclei of the presumptive mid-gut region of late gastrulae. The cells in this region (floor of archenteron) are large and are easily handled during the transplantation operation, which can therefore be carried out with minimal risk of damage to the nuclei. The results of these experiments showed, first, that the endoderm nuclei elicited normal cleavage and blastula formation in 40 per cent of the test eggs—this being almost exactly the same result as was obtained in control experiments with nuclei of undifferentiated early gas-

trula cells (Table 3). However, the later development of the two groups of blastulae was quite different. Most of the control blastulae developed into larvae, while the "endoderm blastulae" were usually arrested in blastula, gastrula, or abnormal embryonic stages.

TABLE 3

DEVELOPMENT OF EGGS INJECTED WITH ENDODERM NUCLEI OF LATE GASTRULAE

			Development of Blastulae		
Source of Nuclei	No. Eggs Injected with Nuclei	Complete Blastulae	Arrested Blastulae and Gastrulae	Arrested Neurulae and Postneurulae	Larvae (10–12 Mm.)
Early gastrula (St. 10), animal hemisphere	71 (100%)	29 (41%)	1	3	25
Late gastrula (St. 12), endoderm	67 (100%)	26* (40%)	8	13	5

* There is some variation in the results in the separate experiments. In one experiment 3 out of 11 complete blastulae developed into larvae. In the other two experiments 1 out of 10, and 1 out of 5 blastulae developed into larvae, respectively.

The abnormal embryos were very interesting. They grew to a length of about 6 mm. and displayed a combination of deficiencies which we have not observed in other embryos. This syndrome is characterized by a loss of the integrity of the epidermis, which becomes thickened in some places and thin or absent in others. Internally, the notochord is well developed, somites are large but abnormal in forms and the gut is developed as well as the general condition of the embryo allows. All ectodermal derivatives, however, are very poorly developed and display degenerative nuclear changes. It should be added that these effects on development are not observed when endoderm cytoplasm alone is injected into either enucleated or normally nucleated eggs.

From the results described above it now appears definite that nuclei undergo certain changes during differentiation. These have been best worked out for the endoderm nuclei of late gastrulae. The transplantation tests show that these nuclei have retained the ability to participate normally in cleavage but are usually restricted in their potentiality for differentiation. In some cases chorda-mesoderm is formed, and some attempt at the differentiation of central nervous system and other ectodermal derivatives ensues. In others, development stops sooner, before any neural structures appear. Finally, experiments not reported here reveal that at later developmental stages there is a loss of the capacity of these nuclei to enter into cleavage of egg cytoplasm. All together, this suggests a progressive specialization of nuclear function during cell differentiation. Concerning its nature there is as yet very little known. How it may differ in different tissues, whether it depends upon alterations in chromosomal or other aspects of nuclear function, and the question of correlated changes in cytoplasmic elements are among the problems remaining to be worked out.

A more detailed account of these experiments, and of those involving nuclear transfers from later developmental stages, is in preparation.

Summary.—Nuclei of chorda-mesoderm and endoderm of late gastrulae (*R. pipiens*) were transferred to enucleated eggs. In the successful cases the recipient eggs cleaved normally and developed into complete blastulae, the majority of which were arrested in blastula, gastrula, or abnormal embryonic stages. Evidence is presented to indicate that the failure of the test eggs to differentiate normally is

due to intrinsic restrictions in potentiality for differentiation on the part of the nuclei of the late gastrula.

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[1] For a review of early literature on this subject see E. B. Wilson, *The Cell in Development and Heredity* (3d ed.; New York: Macmillan Co., 1925).

[2] J. Schultz, *Cancer Research*, **7**, 41, 1947.

[3] R. Briggs and T. J. King, these PROCEEDINGS, **38**, 455, 1952.

[4] R. Briggs and T. J. King, *J. Exptl. Zool.*, **122**, 485, 1953.

[5] T. J. King and R. Briggs, *J. Embryol. Exptl. Morphol.*, **2**, 78, 1954.

[6] P. Rous and F. S. Jones, *J. Exptl. Med.*, **23**, 549, 1916; P. B. Medawar, *Nature*, **148**, 783, 1941; A. Moscona, *Exptl. Cell Research*, **3**, 535, 1952; C. Grobstein, *J. Morphol.*, **93**, 19, 1953; E. C. Slater and K. W. Cleland, *Nature*, **170**, 118, 1952; N. G. Anderson, *Science*, **117**, 627, 1953.

[7] M. C. Niu and V. C. Twitty, these PROCEEDINGS, **39**, 985, 1953.