Nuclear transplantation from intestinal epithelial cells of early and late Xenopus laevis tadpoles

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SUMMARY

The aim of these experiments was to test whether the developmental potential of nuclei from intestinal epithelial cells of Xenopus laevis tadpoles declined during the life of the functional larval gut. The results of transplantation of nuclei from three different stages of development were compared: stages 46–48, when feeding begins and while yolk is still present but before the formation of the typhlosole; stage 57, just prior to the onset of metamorphic reorganization; and stage 54, an intermediate stage. The results showed that there was no change in developmental potential of these nuclei during the life of the larval gut, thereby disproving the hypothesis that nuclear transplants from intestinal epithelial cells of early tadpoles of X. laevis will support extensive development because the cells are not fully functional. However, nuclei from the intestinal epithelial cells were less able to support development than blastula nuclei. It was concluded therefore, that the developmental potential of the gut nuclei is restricted relative to that of the blastula nuclei, but that these restrictions are reversed in a small proportion of cases.

INTRODUCTION

The results of nuclear transplantation studies have generally been interpreted as showing that as cells differentiate, their developmental potential progressively decreases, a process which involves the gradual acquisition of restrictions by their nuclei (see Briggs & King, 1957; Gurdon, 1963, 1974; Simnett, 1974). The stability of these restrictions is uncertain, since there is controversy as to whether nuclei of differentiated cells can support extensive development on transplantation to enucleated eggs (Simnett, 1974; Gurdon, Laskey & Reeves, 1975; McKinnell, Steven & Labat, 1976). Numerous experiments have shown that only a small proportion of transplanted nuclei from populations of differentiated cells can promote development of recipient eggs. Emphasis on the successful results has led to the suggestion that development is limited because of the adverse effects on nuclear performance of technical and physiological factors such as incompatibility in mitotic activity and stage of cell cycle between the donor nucleus and the recipient egg. McAvoy,

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Dixon & Marshall (1975) have shown that these latter factors are not important, at least in the system they studied. Emphasis on the unsuccessful results, on the other hand, has led to the hypothesis that development is due either to the presence of a small proportion of 'undifferentiated cells' or to the fact that none of the donor cells are 'fully differentiated'. For example, successful transplants with nuclei of intestinal epithelial cells from larval *Xenopus laevis* have been attributed to basal cells (Hamburger, 1971), primordial germ cells (Di Berardino & King, 1967) or cells with a ciliary brush border (Ebert & Kaighn, 1966). Market & Ursprung (1971, p. 133) have commented that 'in... *Xenopus laevis*, it has proved possible to obtain normal development using nuclei derived from the intestinal epithelium of larvae. However, even these cells, although apparently mature and functional are still not from an adult organism...' thereby implying that the gut epithelium at stages 46–48 (the stages used (Gurdon, 1962)) may not be fully differentiated.

Recent studies of the morphology and function of the cells of the larval gut epithelium in *X. laevis* have found no evidence to support the suggestion that undifferentiated cells are present (Marshall & Dixon, in preparation). The question remains, however, whether the gut at stages 46–48 is fully differentiated. Feeding begins about stage 45, yolk is still present until about stage 48, and the major mucosal fold, the typhlosole, does not form until about stages 49–51. The larval gut epithelium ceases to exist after metamorphosis, the first manifestations of which are seen at stages 58–59. Therefore the question arises whether, as the gut changes, there is a corresponding decline in the ability of the epithelial nuclei to support development.

The aim of this study was to compare the results of transplantation of nuclei of intestinal epithelial cells from three different stages in the life of the functional larval gut: stages 46–48, the beginning; stage 54, an intermediate stage and stage 57, just prior to the onset of metamorphosis. We conclude that since there are no differences in the ability of any of these types of nuclei to support development, intestinal epithelial cells from early stages are no less differentiated than cells from later stages which unquestionably are structurally and functionally specialized.

**Materials and Methods**

Stages of development of *X. laevis* embryos and tadpoles were identified according to the Normal Table of Nieuwkoop & Faber, (1967).

*Dissociation of donor cells.* Pieces of gut were dissected from the mid-intestine of early feeding tadpoles (stages 46–48) or from just below the bile duct of mature tadpoles (stages 54 and 57), minced into small pieces, and incubated in 1 % trypsin in modified Ca-, Mg-free Barth's saline (after Gurdon & Laskey, 1970) with gentle agitation for 30 min. The dissociated cells were then washed with three changes of Ca-, Mg-free saline.

The jelly was removed from stage-9–11 embryos with cysteine-papain (2:1)
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solution in Holtfreter's saline at pH 8. The embryos were then washed in Ca-, Mg-free saline and gently torn open with fine needles, releasing the cells.

Recipient eggs. About 50–100 eggs at a time were stripped from a frog previously injected with gonadotrophic hormones, covered with Holtfreter's saline and left for 7 min to allow them to become more resistant to handling (after Gurdon, 1960a). They were then transferred to Petri dishes containing a thin film of 1% agarose and orientated with the animal pole uppermost. Between 7–22 min after stripping, the eggs were irradiated with ultraviolet light (253.7 nm, 8000–12000 ergs/mm², Sylvania G8T5 source) to inactivate the egg nucleus and soften the jelly membrane so it could be pierced easily by a micropipette (after Gurdon, 1960b).

Nuclear transplantation. Suspensions of dissociated cells contained a number of cell types: epithelial cells, red blood cells, connective tissue cells, muscle cells and lymphocytes. Brush borders could usually be seen for only a short time after dissociation, but epithelial cells could be recognized without difficulty because of their size, checked by comparison with clumps of undissociated epithelium, and general morphology.

Transplantation of nuclei was carried out according to the technique described by Elsdale, Gurdon & Fischberg (1960). Each batch of eggs was used for 45 min and during that time approximately 30 transplants could be carried out, roughly 15 of each of the two gut cell stages being compared. A total of 60 transplants from eggs of the same animal was considered a complete experiment. Comparative development to blastula was analysed statistically using the Mantel–Haenszel test (see Snedecor & Cochran, 1967).

Controls. Sixty eggs from two batches were irradiated in the usual way, fertilized with sperm and their development then compared with that of untreated eggs from the same batch. The aim of this experiment was to demonstrate that irradiation inactivated the egg nucleus, as shown by the development of haploid tadpoles (see Hamilton, 1963; King, 1966). Development of these embryos was normal to neurula, but in tail-bud stages, abnormalities such as oedema and cell leakage were observed. By hatching stages, the embryos were stunted and only one normal tadpole developed. In addition in each experiment at least 20 eggs (a total of 380) were irradiated and pricked to simulate the transplant procedure (i.e. no nucleus was passed into the egg). The aim was to determine the developmental capacity of activated eggs lacking a functional nucleus. Normal first and second cleavage planes were sometimes seen, but no egg reached the blastula stage.

These results show that (i) the nucleus of the recipient egg was inactivated with a high rate of success and (ii) development did not take place in the absence of an introduced nucleus. We conclude therefore, that development of recipient eggs was due to the transplanted nucleus. Furthermore, we compared the overall patterns of development of eggs receiving nuclei from different developmental stages and thus the effect of other influences was cancelled out.
Table 1. Comparison between the developmental potential of stage-46–48, stage-57 intestinal epithelial and blastula nuclei as tested by nuclear transplantation

Based on six experiments.

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<tbody>
<tr>
<td>Blastula</td>
<td>153 (100 %)</td>
<td>60</td>
<td>26</td>
<td>34</td>
<td>27</td>
<td>19</td>
<td>12</td>
<td>9</td>
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<tr>
<td>St. 46–48</td>
<td>210 (100 %)</td>
<td>43</td>
<td>15</td>
<td>18</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>4</td>
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<tr>
<td>St. 57</td>
<td>202 (100 %)</td>
<td>25</td>
<td>7</td>
<td>13</td>
<td>7</td>
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* Stages according to Nieuwkoop & Faber (1967).
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Transplants of nuclei from blastula cells provided a test of egg quality and the effectiveness of the transplant procedure since the success rate using these nuclei should be high. The results (Table 1) show that both these parameters were satisfactory.

**RESULTS**

*Comparison of nuclear transplants from stage-46-48, stage-54 and stage-57 intestinal epithelial cells (Tables 1, 2)*

There was no significant difference in development to blastula ($P < 0.05$) between eggs which received nuclei from stage-46-48 tadpoles and those which received nuclei from stage-54 tadpoles.

In both cases, approximately 20% of the transplant embryos which commenced cleavage began to gastrulate and later development was also similar. We conclude therefore that stage-46-48 epithelial cell nuclei have the same developmental potential as stage-54 epithelial cell nuclei.

When the development of stage-46-48 and stage-57 transplants was compared, it was again found that there was no significant difference in development to blastula ($P < 0.05$). In both cases, approximately one quarter of the embryos which commenced cleavage began to gastrulate, and later development was similar. We conclude therefore that there are no differences in capacity to support development between nuclei from stages 46-48, 54 and 57.

A total of four transplant embryos developed into normal feeding tadpoles. Of these, three died at stages 49/50; the remaining one, which had received a nucleus from a stage-54 tadpole, metamorphosed successfully.

Histological examination of three of the six stage-57 epithelia used to provide donor cells revealed that the cells appeared normal. There were no signs of premature metamorphosis such as cell sloughing, increased numbers of macrophages or nests of undifferentiated cells which would have presumably affected the results of nuclear transplantation from these tissues.

*Comparison of nuclear transplants from intestinal epithelial cells and blastula cells (Table 1)*

Blastula nuclei initiated cleavage roughly two to three times as frequently as gut nuclei. These differences were greatly accentuated as development proceeded, largely during gastrulation, which blastula transplants accomplished on the average about four times as frequently as gut nuclei. We conclude therefore that there are differences in potential between blastula nuclei and gut nuclei which are manifested in the initiation of cleavage and during gastrulation.

**DISCUSSION**

Experiments which demonstrated that an egg cannot develop without a functional nucleus and that u.v. irradiation inactivates the egg nucleus prove beyond reasonable doubt that when a recipient egg developed, it was due to an
Table 2. *Comparison between the developmental potential of stage-48 and stage-54 intestinal epithelial nuclei as tested by nuclear transplantation*

Based on three experiments.

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<tr>
<td>St. 46–48</td>
<td>110</td>
<td>30</td>
<td>16 (14.5%)</td>
<td>8 (7.3%)</td>
<td>6 (5.5%)</td>
<td>1 (0.9%)</td>
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<td></td>
<td>(100 %)</td>
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<tr>
<td>St. 54</td>
<td>110</td>
<td>37</td>
<td>25 (22.7%)</td>
<td>10 (9.1%)</td>
<td>7 (6.4%)</td>
<td>3 (2.7%)</td>
<td>3 (27%)</td>
<td>2 (1.8%)</td>
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<td>(100 %)</td>
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* Stages according to Nieuwkoop & Faber (1967).
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introduced nucleus. This conclusion is reinforced by the results of all other nuclear transplantation experiments (Briggs & King, 1953; Signoret, Briggs & Humphrey, 1962; Burgess, 1967; Gurdon, 1974).

The results of nuclear transplantation experiments in which the donor nuclei were taken from intestinal epithelial cells of either stage-46-48, stage-54 or stage-57 tadpoles show that there is no change in developmental potential of the nuclei of these cells during the life of the larval gut. Therefore, any suggestion that the stage-46-48 gut epithelium is not fully differentiated because of the presence of yolk in the cytoplasm or because normal feeding has not yet begun is disproved.

Studies of the morphology and function of the epithelial cells of the larval intestine (Marshall & Dixon, in preparation) have led to the conclusion that successful development of eggs receiving transplants of nuclei from this tissue cannot be accounted for by invoking the presence of undifferentiated cells such as ‘basal’ cells. The present study has shown that successful development cannot be accounted for on the basis that the cells used were not differentiated. Only one conclusion remains: that successful development of recipient eggs is due to the transfer of nuclei from differentiated cells.

The capacity of nuclei from tadpole and adult gut epithelial cells (McAvoy et al. 1975) can be compared, with caution, if the results are normalized on the basis of blastula nuclear transplants. When this is done, it appears that adult gut epithelial nuclei are much less capable of supporting development of recipient eggs but this should be tested directly.

Comparison of the development of eggs receiving nuclear transplants from blastula cells with those receiving transplants from tadpole gut epithelial cells showed that nuclei from the more differentiated gut cells are less able to support development than the nuclei from the embryonic cells. This result leads to the conclusion that the potential of the gut nuclei becomes restricted as the cells differentiate although the restrictions are reversible in a small proportion of cases. This view is supported by a number of recent findings. For example, advanced larval stages and feeding tadpoles have developed from transplants of adult skin cell nuclei (Gurdon, 1974; Gurdon et al. 1975), and of adult intestinal epithelial nuclei (McAvoy et al. 1975). Furthermore, Hennen (1970) increased the proportion of successful transplants of gastrula and tail-bud nuclei by the addition of spermine to the transplantation medium and by altering the temperature at which the nuclear transfers were carried out, thereby showing that reversibility depends on the conditions of the experiment. Thus, reversibility and therefore stability of the differentiated state are relative rather than absolute cellular parameters.

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REFERENCES


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