THE BURNSI GENE AS A NUCLEAR MARKER FOR TRANSPLANTATION EXPERIMENTS IN FROGS

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INTRODUCTION

Genetic markers are becoming increasingly desirable in nuclear transplantation studies. Gurdon (1), in a recent review, has emphasized the value of mutant animals in the genetic analysis of somatic cells. Signoret, Briggs, and Humphrey (2) have discussed the need for genetically marked individuals in nuclear transplantation experiments to aid in the study of the problem of how genes control development. To this end, they have developed a procedure for the transplantation of nuclei in the axolotl (*Ambystoma mexicanum*), in which several mutant traits are known. Genetic variants suitable for nuclear transplantation studies have also been described in *Xenopus* by Fischberg (3).

It will be the purpose of this paper to report the results of utilizing nuclei from a *Rana pipiens* pigment pattern mutant (burnsi) in nuclear transplantation experiments. The burnsi frog is characterized by the diminution or lack of dorsal spots on the body. The frog was initially described as a new species by Weed (4), but subsequently was shown by breeding tests to differ from the wild-type principally by a single dominant gene (5). Recent studies by Volpe (6) reveal that the burnsi gene has variable expressivity. The magnitude of the expression of the burnsi gene is believed to be governed largely by a complex of modifying genes (7, 8). However, it is not known to what extent extragenic or environmental factors contribute to the phenotypic diversity.

The burnsi mutant would seem to be an ideal form for nuclear transplantation studies involving developmental genetics. Factors contributing to its usefulness include: (*a*) it is relatively common in Minnesota (9) and other parts of north-central United States; (*b*) it has been the subject of considerable genetic study (6–8, 10, 11); and (*c*) no variation in conventional nuclear transplantation procedure devised for *R. pipiens* (12) is necessary.

In the present study, diploid nuclei from burnsi embryos were transplanted into previously enucleated unfertilized eggs obtained from wild-type frogs. The objective was to ascertain whether the mutant burnsi gene would serve as a useful genetic marker for donor nuclei in transplantation experiments. Small isogenic groups, or clones, of burnsi individuals were established by transplanting nuclei from a single donor embryo. We wished to learn to what degree the genetically identical individuals would differ in the manifestation of the burnsi character.

MATERIALS AND METHODS

Burnsi frogs were obtained from the J. R. Schettle Frog Farm, Stillwater, Minnesota, and the Steinhilber Company, Oshkosh, Wisconsin. Wild-type *R. pipiens* were purchased from the J. M. Hazen Company, Alburg, Vermont.

Ovulation was induced by the method of Rugh (13). Blastulae for nuclear donors were produced by fertilizing freshly extruded eggs from a burnsi female with a sperm suspension of a burnsi male. At Shumway's Stage 9 (14), a single blastula was selected, freed from its jelly coat, and dissected in modified Niu and Twitty's (15) solution. The cell clusters rapidly dissociate in this calcium- and magnesium-free buffered salt solution, and single cells can be picked up with ease. Only dark animal hemisphere cells were chosen for nuclear donors. Recipient eggs

were prepared by activation and enucleation (16) followed by removal of the outer jelly coat. The transplantation was performed in Niu and Twitty's solution (17), following the procedure of Briggs and King (12). Operated eggs were placed in individual small Stender dishes containing diluted amphibian Ringer's solution.

and enucleated cytoplasm of unfertilized eggs of wild-type frogs is as follows:—

Total No. of eggs injected	303
No. of blastulae	160
No. of gastrulae	80
No. of embryos which hatched	47
No. of frogs carried to metamorphosis	21

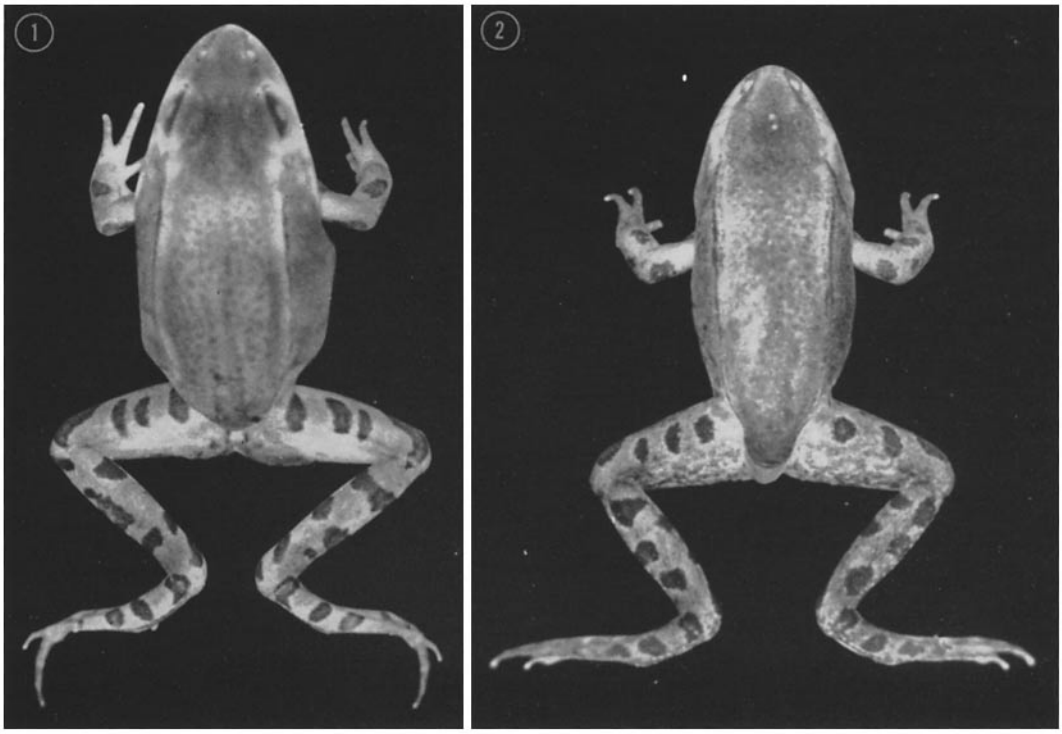

FIGURE 1 Nuclear transplant frog of mutant phenotype produced by transplanting a burnsi diploid blastula nucleus to an enucleated egg obtained from a wild-type *Rana pipiens*. × 2.

FIGURE 2 Control frog of mutant phenotype produced by fertilizing eggs obtained from burnsi frogs with sperm derived from burnsi males. × 2.

Hatched embryos were transferred to individual 1 quart squat bowls containing pond water. Those reaching the feeding stages were fed autoclaved lettuce *ad libitum* until metamorphosis.

Aceto-orcein chromosome squashes (18) were prepared from the tail-tips of swimming embryos (Shumway, Stages 22 to 25).

RESULTS

Embryonic nuclei derived from burnsi blastulae were injected into activated and enucleated unfertilized eggs of wild-type frogs.

A summary of the results of the transplantation of diploid burnsi blastula nuclei into the activated

All 21 of the juvenile frogs exhibited the burnsi phenotype. Figs. 1 and 2 show a diploid nuclear transplant burnsi frog and a control burnsi frog produced by normal fertilization for comparison. The answer to the first objective of the study is thus clear: the mutant burnsi gene does indeed serve as a convenient marker in nuclear transplantation studies.

In several experiments, more than one metamorphosed frog descended from the nuclei of a given blastula donor. Nuclear descendants of a single blastula comprise isogenic groups. Fig. 3 is a diagram of four such groups. The nuclear trans-

plant juvenile frogs (Fig. 3, line 5) are grouped according to their origin from a particular blastula (Fig. 3, line 2). Enucleated recipient eggs (line 4 of Fig. 3), for a given isogenic group, were derived from the same female. Blastulae for nuclear donors were obtained from three crosses of burnsi frogs (Fig. 3, line 1). The six juvenile control frogs were reared to metamorphosis. Of this number, eleven were wild-type, one was a burnsi type B, three were burnsi type D, two were burnsi type E, and three were burnsi type E_n. The uniformity of the pigment patterns within isogenic groups is in striking contrast with the diversity of phenotypic types obtained from a

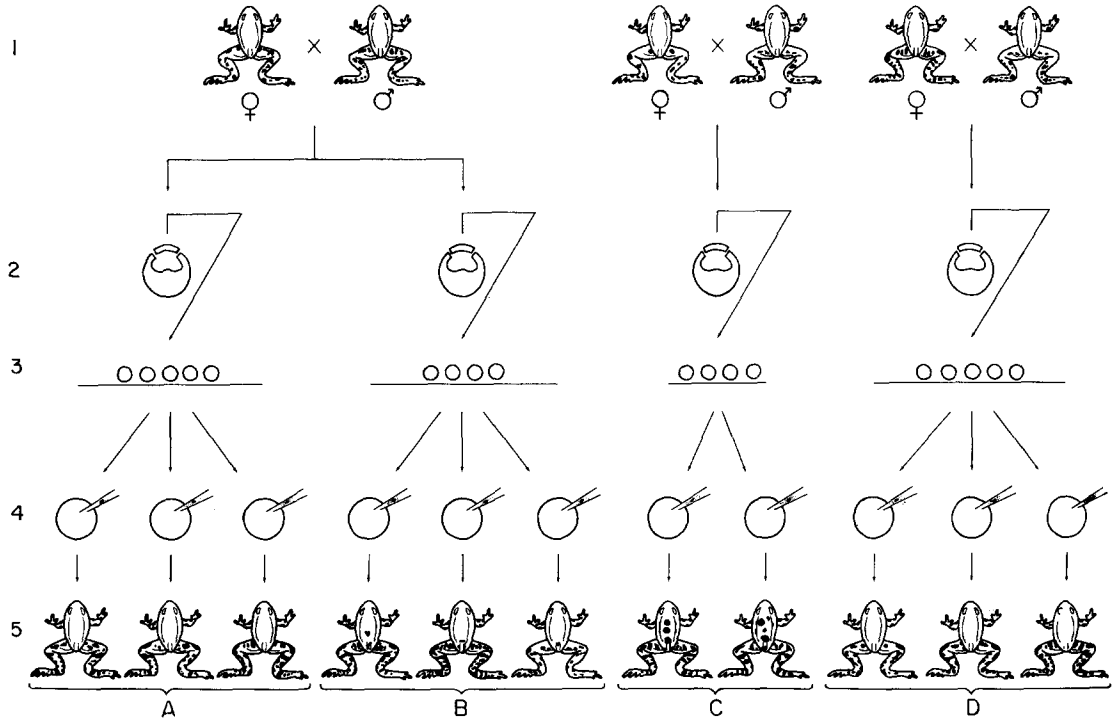

FIGURE 3 Diagram illustrating method for production of small isogenic groups of frogs. Adult frogs of burnsi phenotype (line *1*) yield eggs and sperm from which are produced donor blastulae (line *2*). Animal hemispheres of the donor blastulae are dissociated (line *3*) and transplanted (line *4*) to activated and enucleated recipient eggs. Adult nuclear transplant progeny (line *5*) are obtained. The frogs, forming small groups within brackets, are isogenic. The individuals of group *A* resemble each other in having immaculate dorsal body surfaces, the frogs of group *B* have small spots located posteriorly on their dorsal body surfaces, the pair of animals of group *C* have substantial dorsal pigmentation, and the animals of group *D*, like those of group *A*, resemble each other in having immaculate backs.

frogs of groups A and B were descended from two blastulae produced by crossing burnsi parents (Fig. 3, line 1) which possessed a large number of spots on their limbs. Frogs with spotting patterns similar to these parents have been classified as type E by Volpe (6). In ordinary diploid crosses, when two such burnsi frogs are crossed, some of the burnsi progeny resemble the parental type, others have less appendage spotting (types C and D), and some possess few to several spots on the back (type E_n). A control cross of burnsi frogs in the present study yielded similar results. Twenty normal diploid cross. The three frogs of group A had immaculate dorsal body surfaces and heavily spotted limbs (type E). There were a few spots on the bodies of each of the frogs of group B (type E_n). Large dorsal body spots occurred on both of the frogs of group C (type E_n). The frogs of group D had immaculate backs and heavily spotted appendages (type E). One other group of two frogs (not illustrated) were type E_n. There were eight other nuclear transplant burnsi frogs which were the sole individuals to reach transformation from a particular experiment.

The eight frogs varied with respect to the extent of spotting on their limbs and the presence or absence of a few dorsal body spots.

Although frogs within an isogenic group are basically alike, there is slight phenotypic intragroup variation. All frogs were grown under similar conditions, such as light, temperature, food, and water depth. Phenotypic diversity may be caused by slight variations in environmental factors not easily controlled, minor differences in the nuclear injection procedure, and diversity in egg cytoplasm (*e.g.*, eggs of the same female vary in size). Variations between the groups (*e.g.*, between group A and group B progeny) may be ascribed to genetic differences (presumably different recombinations of modifiers) among blastulae from the same parents.

The demonstration that isogenic groups can be produced by nuclear transplantation techniques fulfills an important condition for studying the interaction of heredity and environment. One can now measure the effects of environmental factors, *e.g.* light and temperature, by growing genotypically identical members under different conditions. Large numbers of progeny are needed for such a study. This aspect is within the bounds of future investigation.

Chromosome counts were made from aceto-orcein squashes of nuclear transplant embryo tail-tips. Eighteen of the 21 embryos were found to have the diploid chromosome number of 26. Three were ascertained to have the tetraploid number of 52. Examination of the cleavage records revealed that the diploid embryos reached the two-blastomere stage at a normal cleavage interval of about 3 hours after activation. The tetraploid embryos were found to have reached the two-blastomere stage after a delay of one cleavage interval. The tetraploid condition did not appear to alter the expression of the burnsi gene.

DISCUSSION

The twenty-one nuclear transplant frogs of this study were produced from the nuclei of nine blastulae. Since all the frogs were phenotypically burnsi, it may be concluded that the nine donor blastulae carried the burnsi gene. Adult burnsi collected from natural populations are reported to be heterozygous (5). Accordingly, a cross of eggs from a burnsi female with sperm of a burnsi male should result in a 3:1 ratio of burnsi frogs to the wild-type frogs. In this study, all of the donor blastulae carried the burnsi gene.

There was reason to believe that the burnsi gene would serve as a useful nuclear marker because of related experiments involving the kandiyohi (mottled) variant of *R. pipiens*. Frogs developing from a combination of enucleated eggs derived from wild-type females and nuclei containing the kandiyohi gene display the kandiyohi phenotype (19–21).

Gurdon (1) has stated that it is not yet known whether all somatic cell nuclei in an individual contain the same genes as were present in the zygote nucleus. Insight into this question is provided by an examination of nuclear descendants from individual blastulae. Gurdon (22) produced nuclear clones of *Xenopus laevis*. Unfortunately, striking pigment patterns are not obvious in this species. In contrast, the two well known mutant forms of *R. pipiens* differ sharply from the wild-type. McKinnell (20) has reported similarities in isogenic groups of metamorphosed wild-type and kandiyohi mutant frogs. The previous studies of the kandiyohi mutant, as well as the present studies of the burnsi mutant, provide evidence that, at least with respect to the mutant genes under consideration, all nuclei from a common blastula are genetically identical. That is, never have both wild-type and mutant phenotype individuals occurred among the nuclear-transplant progeny of a single blastula.

CONCLUSIONS

When nuclei containing the burnsi gene are transplanted to wild-type cytoplasm, the resulting progeny are burnsi progeny. The burnsi gene therefore serves well as a nuclear marker.

Offspring from a common blastula nuclear donor display striking similarities in pigment patterns. Environmental fluctuations, which may have included variations in recipient egg cytoplasmic quality and other subtle variations not easily controlled, were not sufficient to alter the intrinsic genetic control of the pigment patterns of these isogenic groups of frogs.

This investigation was supported in part by Public Health Service Research Grant CA-05935-02 from the National Cancer Institute and by a Grant from the Cancer Association of Greater New Orleans.

Received for publication, June 25, 1963.

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