The Use of LacZ Fusion Constructs as a Cell Lineage Marker in the Axolotl

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Introduction

The *lacZ* gene of *Escherichia coli*, which codes for β-galactosidase, has been used extensively as a reporter gene in animal systems (Hall et al., 1983; Hiromi et al., 1985; Sanes et al., 1986; Turner and Cepko, 1987). This is largely due to the lack of interfering neutral β-galactosidases in animal cells (Neilsen et al., 1983) and the ease with which enzyme activity can be detected by histochemical staining.

In this study, we examined the expression of *lacZ* fusion constructs, to monitor gene expression in the axolotl. Several eukaryotic promoters were tested. The best results were obtained with the mouse *hsp68* promoter. The expression from this promoter was constitutive, and was observed in almost all cells. When this plasmid was injected into individual blastomeres a patch of expression was observed indicating that such a construct may be useful as a lineage marker.

Materials and Methods

Plasmids used for microinjection. The plasmids used in this study are listed in Table 1. Plasmid DNA used for microinjection was prepared by the boiling method (Holmes and Quigley, 1981). The DNA was further purified by precipitation with 20% polyethylene glycol, 0.5 M NaCl. Prior to microinjection, the DNA was linearized, extracted with phenol, phenol-chloroform, and ethanol precipitated.

Microinjection of axolotl eggs. Axolotl eggs were obtained from spawnings performed at the University of Ottawa axolotl colony. The eggs were manually dejelled using watchmaker's forceps, and were microinjected within 30 minutes of egg deposition. The microinjection was performed according to the method of Stephens et al. (1981). The eggs were injected in 25% Steinberg's solution (Asashima et al., 1989) containing 5% ficoll. Approximately one nanogram of DNA (in 75 nL of water), was injected into each egg. If the amount of DNA was increased to greater than 2 ng, the percentage of surviving embryos was reduced to about 10-20%. Following injection, the eggs were allowed to develop overnight in the Steinberg's-ficoll solution, and were subsequently transferred to 25% Holtfreter's media (Asashima et al., 1989) containing 0.01% penicillin and streptomycin. The embryos were staged according to Bordzilovskaya et al. (1989).

Histochemical staining of embryos for β-galactosidase. Embryos were fixed overnight at 4°C in PBS (phosphate buffered saline, pH 7.5) containing 2% formaldehyde and 0.2% glutaraldehyde. Following fixation, the embryos were rinsed three times over 24 hours in PBS, and were stained for 1-18 hours at room temperature in the solution described by Sanes et al. (1986) containing 400 μg/mL X-Gal (4-Cl-5-Br-3-indolyl-β galactoside).

Results and Discussion

Expression of exogenous sequences in fertilized axolotl eggs. In this study, we examined the possibility of using fertilized axolotl eggs to express exogenous DNA sequences. The use of the *lacZ* gene of *E. coli* as a reporter allowed for direct visualization of individual cells expressing the injected DNA. Several *lacZ* fusion constructs were tested with different promoters (Table 1). Embryos were injected at the one cell stage and were allowed to develop to late neurula or tail bud stages. They were then stained for β-galactosidase activity.

All but the *copia* promoter gave β-galactosidase activity. Of the three remaining, the strongest expression was observed with the plasmid pKP110 containing the mouse *hsp68* promoter. (Strong expression refers to the intensity of staining for β-galactosidase). Surprisingly, embryos which were heat shocked did not show any difference in expression from embryos which had not been heat shocked. No β-galactosidase activity was detected in uninjected embryos.

Embryos injected with pKP110 at the one cell stage were, in most cases, labelled in all

Table 1. Plasmids used in this study

Plasmid	promoter	reference
pCH110	SV40	Pharmacia corp.
pMWLTR	Drosophila copia	M. Whiteley
pCAB17	mouse phosphoglycerate kinase	Dr. M. McBurney, U. of Ottawa
pKP110	mouse hsp68	Kothary et al.,1989
phspPTlacZpA	mouse hsp68	Kothary et al., 1989

All plasmids consisted of a eukaryotic promoter fused to the *E. coli lacZ* coding sequence and SV40 polyadenylation site.

quadrants (Table 2, Fig. 1A). Less frequently, a single patch of β-galactosidase activity was observed, suggesting that integration was delayed past first cleavage. Alternately, the DNA may have been partitioned into individual blastomeres during early cleavage, but if this occurred unevenly, it would inevitably result in labelling of non-contiguous sectors. Labelling of non-contiguous sectors was not observed.

We have also observed β-galactosidase activity in the tail tips from 5-month-old animals, although the proportion of cells expressing β-galactosidase in these tail tips is difficult to assess due to the presence of interfering pigment.

Embryos injected into a single blastomere at the two cell stage gave a pattern of expression which ranged from half of the embryo being labelled to a discrete sector or patch (Table 3, Fig. 1B). Injection of one cell at the four cell stage gave rise to an embryo with a labelled sector. These patterns of expression suggested that those cells expressing β-galactosidase activity arose from a single cell. However, β-galactosidase expression was not observed in all cells either throughout the embryo (one cell stage injection) or within labelled sectors (2 and 4 cell injection). Similarly, heterogeneous expression was observed with the other constructs, most notably with pCAB17 (Fig. 2).

Heterogeneous expression of the *E. coli lacZ* gene in a clonal population of cells has previously been reported, both in tissue culture (MacGregor et al., 1987) and in transgenic mice (Kothary et al., 1989). Kothary et al. (1989) have suggested that the presence of a bacterial translational start site might be responsible for inefficient translation of the *lacZ* transcripts. When this site was replaced with a eukaryotic translation initiation site, they observed a three fold enhancement in expression in transfected cells. When we tested expression of this construct (phspPTlacZpA) in the axolotl, we also found more homogeneous expression

Table 2. Microinjection of pKP110 at the one cell stage

Exp#	1	2	3	control
Total	50	50	50	50
Infertile	8	9	4	13
Abortive	12	18	9	7
Exogastrula	7	5	10	6
Normal	23	18	27	24
Blue	7	10	11	-
BlueP*	2	1	3	-
%Blue	30	55	41	-

Embryos were injected with 1 ng of XbaI linearized pKP110, and were allowed to develop to late neurula or early tail bud stages of development. They were then stained for β-galactosidase activity. Each experiment represents a different spawning. Blue refers to the number of embryos which were blue in all quadrants; blueP* is the number of embryos with a patch of β-galactosidase activity. In the control experiment, embryos were injected with 75 nL of water.

Table 3. Microinjection of pKP110 in two and four cell stage embryos

Exp#	1	2	3
Total	45	20	15
Exogastrula	12	7	3
Normal	33	13	12
BlueP*	17	7	10
%Blue	51	53	83

Embryos were injected with pKP110 into a cell as described above, and were allowed to develop to late neurula, or early tail bud stages of development, and were then stained for β-galactosidase activity. Each experiment represents a different spawning. BlueP* is the number of embryos with a blue patch of β-galactosidase activity, and %blue is the percentage of embryos showing β-galactosidase activity.

(Fig. 1C).

Another possibility suggested by MacGregor et al. (1987) is that differing degrees of methylation in individual cells could be affecting the degree of transcription of the bacterial gene. This may explain the mosaic expression from the mouse phosphoglycerate kinase promoter, which is known to be affected by methylation in the mouse.

The use of lacZ constructs as a lineage marker. We believe that a plasmid which expresses β-galactosidase constitutively in the axolotl has potential as a lineage label. β-galactosidase has already been used successfully as a lineage marker in the rat and mouse (Sanes et al., 1986; Turner and Cepko, 1987) and offers advantages in ease of detection over markers, such as triploidy, currently in use (Armstrong and Muneoka, 1989). Labelled cells can be identified visually with histochemical staining of whole embryos, or more precisely in sectioned material. Though such a label is most useful if a high proportion of the descendents of the injected cell are labelled, for most experiments valid inferences can still be drawn even if not all are labelled, as a labelled descendant can only have arisen from the injected founder cell.

Experiments with injections into a single blastomere at the two and four cell stage demonstrate the potential usefulness of this system. We have also successfully injected individual blastomeres up the 64 cell stage, and have been able to identify labelled cells in tail bud embryos. Ideally, germ-line transmission of sequences would provide a valuable source of marked cells for the construction of chimeras to study cell lineage, at later stages, although this could still be accomplished with donor embryos injected at the one cell stage.

M.W. was funded by a post graduate fellowship from the Natural Sciences and Engineering Research Council of Canada (NSERC). This research was also funded by a NSERC grant to J.B.A.

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Figure 1. Examples of β-galactosidase activity in embryos injected with the plasmid pKP110 (mouse *hsp68* promoter), or phspPTlacZpA (mouse *hsp68* promoter). (A) Wild type embryo (late neurula) injected with the plasmid pKP110 at the one cell stage showing intense staining in a large proportion of cells. (B) Wild type embryo (tail bud) injected with the plasmid pKP110 into one cell at the two cell stage. Note the intense staining of most of the cells in the head. (C) Wild type embryo (tail bud) injected with the plasmid phspPTlacZpA at the one cell stage.

Figure 2. Examples of β-galactosidase activity in embryos injected with the plasmid pCAB17 (mouse PGK promoter). (A) Albino embryo (tail bud) injected at the one cell stage showing cells labelled both internally and externally. (B) Wild type embryo (tail bud) injected into one cell at the two cell stage showing a sector of β-galactosidase activity in the head.

Fig 1

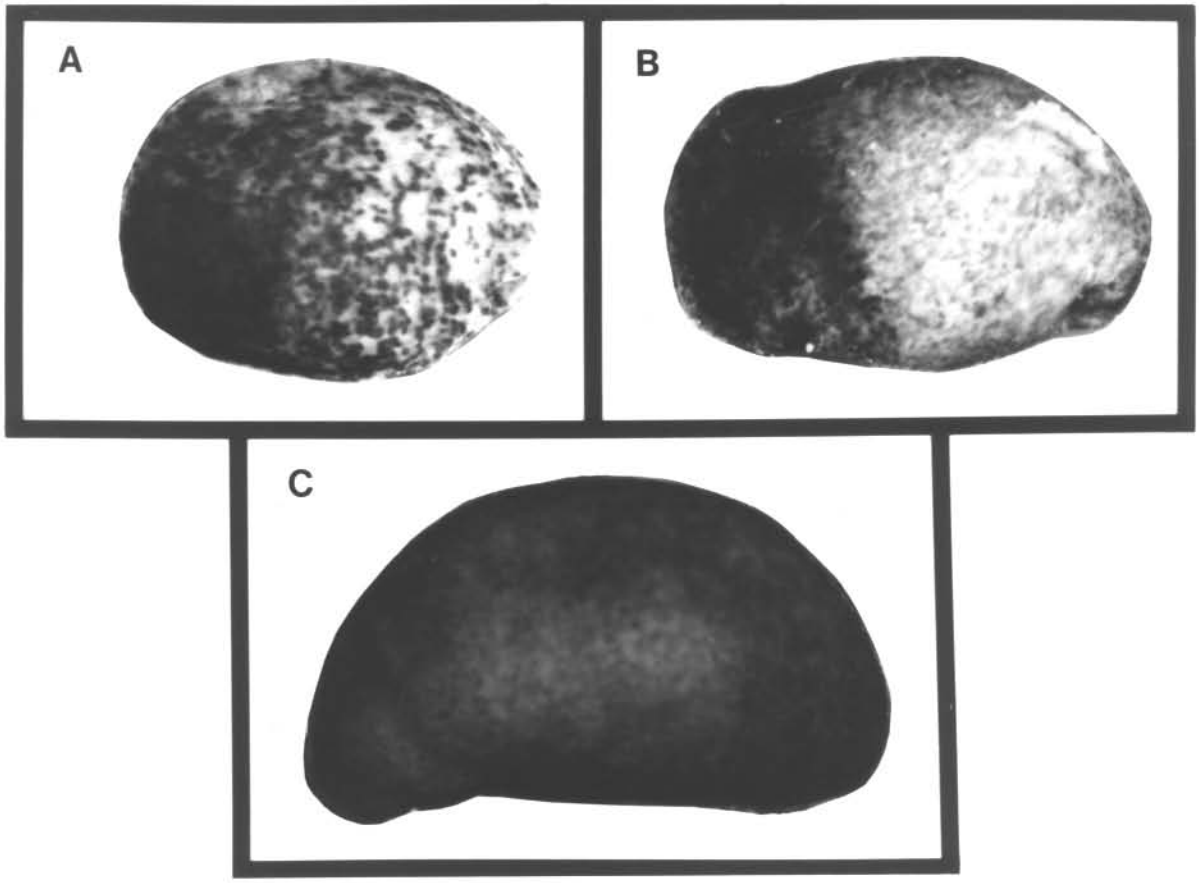

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Fig 2

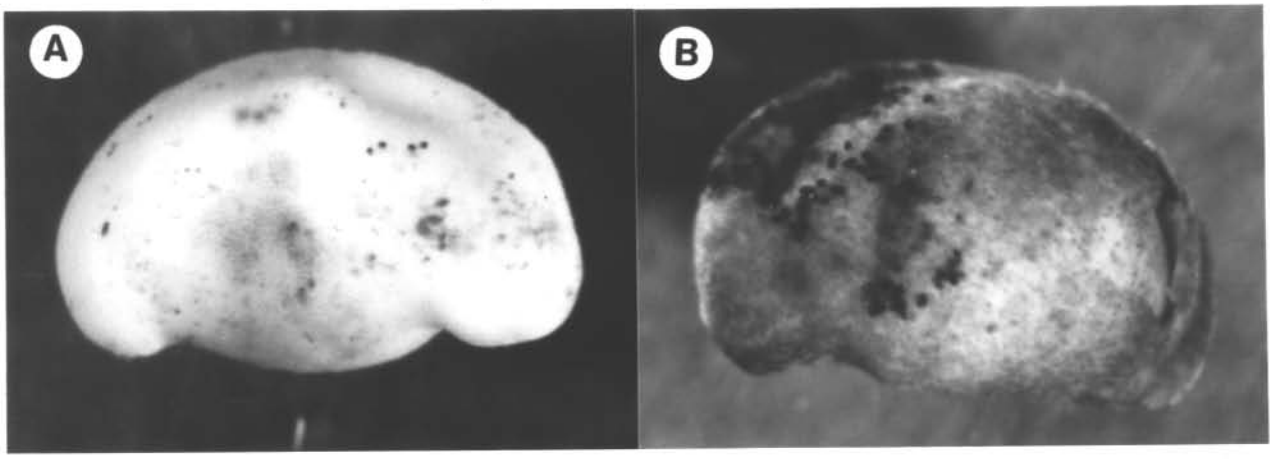

Fig 2