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GFP as a Genetic Marker in Transgenic Fish

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Introduction

The use of transgenic fish has so far been chiefly limited by the lack of predictable, strong, tissue specific, and position-independent expression of transgenes. For genetic analysis, expression of a marker transgene, easily screenable in the living fish, could facilitate studies of gene targeting, insertional mutagenesis, lineage, and mutational analysis. For aquacultural applications, a transgene coding for disease resistance, food conversion, or environmental tolerance could be expressed. In most of these situations, expression should be tissue specific, position independent, and usually high level for maximum efficiency. This study will focus on the construction, assessment, and genetic uses of a vector that expresses the green fluorescent protein (GFP).

Materiasl and methods

DNAs for microinjection were prepared with a final concentration of approximately 2 μg/ml linear or circular DNA and 0.05% phenol red. Under a dissecting microscope, several hundred newly fertilized eggs in ERS were placed in a 35 mm petri dish of 1% agar containing a single depression of 1-2 mm.

Living embryos and whole mount tissues from adult fish were observed and photographed with either inverted fluorescence microscope or stereo fluorescence dissection microscope equipped with a camera. Embryos were individually transferred by pipette in an approximately 50 μl drop of ERS to an inverted 100 mm petri dish lid and observed in groups of about 50 embryos. To immobilize embryos for photography or detailed observation, 1 ml of a 20× stock anesthetic was added to the approximate 20 ml of ERS in a 100 mm petri dish. The 20× stock anesthetic was 3 g/L of tricaine methanesulfonate (MS-222), 20 mM Tris, pH 8, in ERS.

Results and Discussion

As a marker transgene, we have chosen the green fluorescent protein (GFP) (Chalfie et al., 1994) because it is easy to screen in living organisms, it is a

small transgene (717 bp), and it may be less sensitive to inactivation by methylation owing to its low CG dinucleotide content (1.4%).

As marker transgenes, growth hormone and tyrosinase have shown promise. Fish transgenic for growth hormone have been produced and have been phenotypically scored as larger fish (Chen et al., 1996) and, in one case, with a characteristic pigmentation and a heightened level of cranial-facial deformities (Devlin et al., 1995). The success of growth hormone may have been due to the minimal amount of expression needed for phenotypic effect. Medaka transgenic for tyrosinase have also been produced that were scorable for wild type pigmentation in a host pigment cell mutant background. (Matsumoto et al., 1992; Hyodo-Taguchi et al., 1997). However, the tissue specific expression of tyrosinase (pigment cells) as well as the requirement for mutant host lines may limit the usefulness of tyrosinase as a transgenic marker. For transgenic studies of tissue specificity, position independence, and strength of expression, novel reporter genes not normally found in the host provide the most useful prospects for evaluation.

A vector that consistently drives strong, ubiquitous, and position independent expression in fish throughout the life cycle has so far not been available. In this study we will show heritable, strong, nearly ubiquitous, and partially position independent expression of GFP using the fish expression vector and demonstrate the genetic screening of fish at stages of the life cycle from the egg to the adult.

References

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. (1994). Green fluorescent protein as a marker for gene expression. Science 263:802-805.

Chen, T.T., Vrolijk, N.H., Lu, J.K., Lin, C.M., Reimschuessel, R., and Dunham, R.A. (1996). Transgenic fish and its application in basic and applied research. Biotechnol Annu Rev 2:205-236.

Devlin, R., Yesaki, Y., Donaldson, E., and Hew, C. (1995). Transmission and phenotypic effects of an antifreeze/GH gene construct in coho salmon (Oncorhynchus kisutch). Aquaculture 137:161-169.

Hyodo-Taguchi, Y., Winkler, C., Kurihara, Y., Schartl, A., and Schartl, M. (1997). Phenotypic rescue of the albino mutation in the medakafish (Oryzias latipes) by a mouse tyrosinase transgene. Mech Dev 68:27-35.

Matsumoto, J., Akiyama, T., Hirose, E., Nakamura, M., Yamamoto, H., and Takeuchi, T. (1992). Expression and transmission of wild-type pigmentation in the skin of transgenic orange-colored variants of medaka (Oryzias latipes) bearing the gene for mouse tyrosinase. Pigment Cell Res 5:322-327.