Initiation of the transgenic *lacZ* gene expression in medaka (*Oryzias latipes*) embryos

Huai-Jen Tsai* and Shu-Huei Wang  
Institute of Fisheries Sciences, National Taiwan University, Taipei, Taiwan 106

Koji Inoue  
Central Research Laboratory, Nippon Suisan Kaisha, 559-6 Kitamoto, Hachioji, Tokyo 192, Japan

Shigeru Takagi  
NTT Basic Research Laboratories, 3-1, Morinosato Wakamya, Atsugi-shi, Kanagawa 243-01, Japan

Minoru Kimura  
School of Medicine, Tokai University, Bohseidai, Isehara, Kanagawa 259-11, Japan

Yoko Wakamatsu and Kenjiro Ozato  
Bioscience Center, Nagoya University, Furocho, Chikusa-ku, Nagoya 464-01, Japan

Abstract

In total, 4165 medaka (*Oryzias latipes*) oocytes were injected with three DNA constructs separately, and results showed that exogenous *lacZ* expression was transient and stage-dependent. The initiation of the transgene expression was at the mid-blastula stage for embryos derived from oocytes injected with pmwZ, containing the long terminal repeat (LTR) of the Rous sarcoma virus, and with pCAGGS-*lacZ*, containing the enhancer and promoter of the immediate early gene of the human cytomegalovirus, respectively, whereas embryos derived from oocytes injected with pMoZtk, containing the LTR of the Moloney murine leukemia virus, started expression at the late-blastula stage. These reveal that the earliest onset of the exogenous *lacZ* gene should be by the mid-blastula stage. Therefore the mid-blastula transition phenomenon in embryogenesis known in other animal species exists in medaka embryos.

Introduction

Transfer of foreign DNA into developing embryos has been used widely to analyze gene expression during development. Medaka (*Oryzias latipes*) provides a useful and convenient experimental system for studying transgene expression (Ozato et al., 1989). Eggs are produced daily on a year round basis and can be fertilized in vitro. Spawning can be controlled by light conditions, and the transparency of embryos makes embryological manipulation possible. Moreover, the manipulation of microinjection into the medaka oocyte nucleus is well established (Ozato et al., 1989).

Transient expression systems of foreign genes during embryogenesis have been used for functional analysis of the regulatory regions of developmental genes in *Drosophila* (Steller and Pirrotta, 1984; Martin et al., 1986), sea urchin (McMahon et al., 1985), frog (Etkin and Balcells, 1985; Krone and Heikilla, 1989), and fish (Chong and Vielkind, 1989; Winkler et al., 1991).

At the two-cell stage, there is a sharp fall in the level of total and poly(A)+ RNA (Clegg and Piko, 1983) in the translation of globin messenger RNA injected into the mouse zygote (Brinster et al., 1980). Ueno et al. (1987) have also demonstrated that the most zygotic gene expression in mouse embryos begins after the two-cell stage. On the other hand, when chloramphenicol acetyltransferase (CAT) controlled by the CyIIIa cytoskeletal actin gene was injected into unfertilized eggs of the sea urchin, the CAT enzyme appeared in transgenic sea urchin after the early blastula stage, equivalent to the same developmental schedule of turning on the endogenous CyIIIa actin gene (Franks et al., 1988; Hough-Evans et al., 1988). They reported that the exogenous gene constructs have been found to be regulated faithfully in sea urchin embryos, demonstrating that the activation of exogenous genes was parallel to that of...