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A New Laser Pointer Driven Optical Microheater for Precise Local Heat Shock

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Abstract
The zebrafish has emerged as an important genetic model system for the study of vertebrate development. However, while genetics is a powerful tool for the study of early gene functions, the approach is more limited

when it comes to understanding later functions of genes that have essential roles in early embryogenesis. There is thus a need to manipulate gene expression at different times, and ideally only in some regions of the developing embryo. Methods for conditional gene regulation have been established in Drosophila, C.elegans and the mouse, utilizing conditional gene activation systems such as the Gal4-UAS system (fly) and the cre/lox recombination system (mouse). While these tools are also being developed in zebrafish, the accessibility of the zebrafish embryo makes other approaches both possible and desirable.

We have taken advantage of a heat-shock inducible system that uses the hsp70 promoter that is activated by cellular stress, such as heat. Having established that this global heat shock method allows temporal control of gene expression, we aimed to spatially control gene expression by applying controlled thermal heat to only a small region of the embryo. This would allow us to determine cell- and tissue-autonomous roles for developmentally important genes in an embryo with otherwise normal gene function. We have now developed a device that uses a laser to heat a defined region of the embryo, and thus activate the hsp70 promoter only in restricted regions of the embryo. The output of a 75 mW red laser pointer was focused into the 50 µm diameter core of an optical fiber, whose cleaved and coated end was used to heat, and thus induce, gene expression in a defined area. We have established conditions that allow controlled heating and trans-gene activation in small regions of the embryo without inducing cell death. This new tool will allow us to study the cell-autonomous roles of embryonic signaling molecules in cell differentiation, proliferation, and survival in a variety of tissues and at different times.

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