Spatiotemporal control of embryonic gene expression using caged morpholinos.

Journal: Methods Cell Biol
Publication Year: 2011
Authors: Ilya A Shestopalov, James K Chen
PubMed link: 21924162
Funding Grants: Stanford CIRM Training Program, Stanford CIRM Training Program

Public Summary:
Insights obtained by studying embryonic development are both intellectually satisfying and informative to the clinical efforts in stem cell-based tissue regeneration via stem cells. Since the time of Aristotle, scientists have strived to explain this fascinating phenomenon using a ‘perturb and observe’ paradigm to study model organisms. These pioneering developmental biologists meticulously analyzed embryos from sea urchins, chickens, frogs, and other animals by injecting them with dyes to follow cell lineages and conducting surgical procedures to study interactions between tissues. Their investigations revealed conserved embryological processes among evolutionary diverse organisms and introduced concepts such as tissue-organizing centers and morphogen gradients. Over the past three decades, the zebrafish model system has emerged as one of the mainstream models of development with unique advantages over mammalian systems. Zebrafish share many common developmental mechanisms with other vertebrates, such as mammals. However, unlike mice, they develop rapidly, externally, and in large clutch sizes. These properties facilitate microinjection, imaging, and enable large scale screens for chemicals that affect development. The ease of microinjecting zebrafish eggs has allowed the use of synthetic and nonnatural oligonucleotide molecules to interrogate functions of genes involved in zebrafish development. In this review, we survey the capabilities and limitations of various oligonucleotide-based technologies for perturbing RNA function and tracking RNA expression. We also examine recent strategies for achieving control of oligonucleotide function within different tissues of the embryo and at different time points in development. In particular, we describe the use of light-gated technologies that exploit the optical transparency of zebrafish embryos.

Scientific Abstract:
Embryonic development depends on spatial and temporal control of gene function, and deciphering the molecular mechanisms that underlie pattern formation requires methods for perturbing gene expression with similar precision. Emerging chemical technologies can enable such perturbations, as exemplified by the use of caged morpholino (cMO) oligonucleotides to photo-inactivate genes in zebrafish embryos with spatiotemporal control. This chapter describes general principles for cMO design and methods for cMO assembly in three steps from commercially available reagents. Experimental techniques for the microinjection and photoactivation of these reagents are described in detail, as well as the preparation and application of caged fluorescein dextran (cFD) for labeling irradiated cells. Using these protocols, cMOs can be effective tools for functional genomic studies in zebrafish and other model organisms.