DEVELOPMENTAL DYNAMICS 242:503–517, 2013

Fixation/Permeabilization: New Alternative Procedure for Immunofluorescence and mRNA *In Situ* Hybridization of Vertebrate and Invertebrate Embryos

Juan Fernández* and Ricardo Fuentes

A new procedure is described to visualize the spatial pattern of expression of proteins and mRNAs in cryosections or whole-mounted leech, *Drosophila*, zebrafish, and chick embryos. Our principal contribution is in the use of a nonconventional fixation/permeabilization procedure based on the use of formaldehyde or paraformaldehyde combined with a short C-chain carboxylic acid. Detergents, methanol, and proteinases were omitted. Hybridization procedures were modified from those of routinely used protocols developed for the same embryos. Results showed that cytoskeletal and other cytoplasmic proteins, as well as different mRNAs, were clearly visualized in the expected regions of the embryos. Our procedure has several advantages over currently used protocols: is simpler, produces better general preservation of cells, yields reliable results, and can be used for embryos of different taxa at different developmental stages. It is hypothesized that short C-chain aliphatic carboxylic acids modulate the cross-linking effect of aldehyde fixatives on cell proteins. *Developmental Dynamics 242:503–517, 2013*. © 2013 Wiley Periodicals, Inc.

Key words: fixation/permeabilization; immunofluorescence; mRNA *in situ* hybridization; invertebrate embryos

Key findings:

- Our fixation/permeabilization procedure for immunofluorescence and mRNA *in situ* hybridization is a highly versatile procedure that works efficiently in diverse cell types of embryos of different taxa at different developmental stages.
- The same fixation/permeabilization procedure allows independent or simultaneous immunofluorescence staining and mRNA *in situ* hybridization.
- The fixation/permeabilization procedure combines good structural preservation of the cytoplasm with high reactivity of immunogenic sites and mRNA.
- Fixation/permeabilization allows good immunofluorescence staining and mRNA *in situ* hybridization of both whole-mounted and cryosectioned embryos.
- Short C-chain carboxylic acids appear to modulate the rate at which formaldehyde cross-links cell proteins.

Accepted 26 January 2013

INTRODUCTION

Immunofluorescence and RNA *in situ* hybridization are microscopy techniques that are extensively used for the localization of antigens and mRNAs with single cell resolution. Both techniques include a preparatory fixation and permeabilization steps. Fixation is a procedure intended to stabilize the cell structure, preserving it as closely as possible to that of the living cell. It may be achieved by precipitating or additive fixatives. Precipitating fixatives, such as cold organic solvents (methanol, acetone, ethanol, or mixtures of them), denature proteins and hence disrupt their three-dimensional

Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile
Grant sponsor: The University of Chile.
***Correspondence to: Juan Fernández, Laboratory of Developmental Cell Biology, Department of Biology, Faculty of Sciences, University of Chile, Casilla 653, Santiago, Chile. E-mail: jfernand@abello.dic.uchile.cl**

DOI: 10.1002/dvdy.23943
Published online 6 February 2013 in Wiley Online Library (wileyonlinelibrary.com).