MethodsX 3 (2016) 378–385

Contents lists available at ScienceDirect

MethodsX

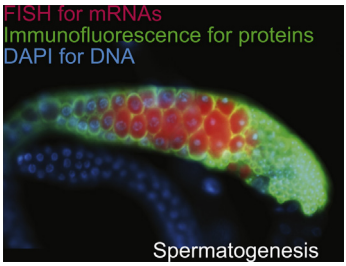
journal homepage: www.elsevier.com/locate/mex

A simple and rapid method for combining fluorescent in situ RNA hybridization (FISH) and immunofluorescence in the *C. elegans* germline

Dong Suk Yoon[a], DeQwon L. Pendergrass[a], Myon-Hee Lee[a,b,*]

[a] *Department of Medicine, Brody School of Medicine at East Carolina University, NC 27834, USA*
[b] *Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599, USA*

GRAPHICAL ABSTRACT



ABSTRACT

Imaging of RNAs and proteins in specific tissues has opened ample avenues to understand gene expression during development. Recently, a fluorescent *in situ* RNA hybridization (FISH) method has been developed to analyze the spatio-temporal expression patterns of endogenous mRNAs. However, combining FISH with immunofluorescence is challenging as the reaction conditions for the two procedures conflict in multiple ways. In this report, we developed a simple and rapid method to detect both RNAs and associated proteins with better preservation of the fine structure in the *C. elegans* germline. This method will provide new tools for *in vivo* imaging of RNAs and their associated proteins in the same germline, which also enables simultaneous visualization of RNA/protein complex at the cellular level *in vivo*.

- Developing a simple and rapid FISH method with better preservation of the fine structure.
- Combining FISH with immunofluorescence in *C. elegans* germline.
- Labeling extruded gonads, instead of the whole worms, to prevent non-specific somatic autofluorescence.

* Corresponding author at: Department of Medicine, Brody School of Medicine at East Carolina University, NC 27834, USA.
E-mail address: leemy@ecu.edu (M.-H. Lee).

http://dx.doi.org/10.1016/j.mex.2016.05.001

ARTICLE INFO
Keywords: Fluorescent *in situ* hybridization (FISH), Immunofluorescence, *C. elegans*, Germline
Article history: Received 26 February 2016; Accepted 3 May 2016; Available online 6 May 2016

Background

Tracking expression and subcellular localization of RNAs and proteins during development provides important clues to understand their biological and physiological function. In conventional *in situ* RNA hybridization methods, sliced tissues are incubated with biotin or digoxygenin-labeled RNA probes that are synthesized by PCR, and then endogenous RNAs:RNA probe hybrids are visualized by antibodies against biotin or digoxygenin (Fig. 1A and B). This two-step procedure exhibited low sensitivity and non-specific probe binding. Therefore, a highly sensitive RNA detection method was required to analyze the *in vivo* subcellular localization of endogenous RNAs with better preservation of the fine structure.

Recently, an advanced fluorescent *in situ* RNA hybridization (henceforth called "FISH") method using fluorescent (e.g., Alexa 594, Alexa 488, Cy3, or Cy5)-labelled small RNA molecule (~20 nucleotides) probes has been developed (Fig. 1C and D) in multiple organisms, including *C. elegans* whole worms and embryos [1], yeast [2], zebrafish embryos [3], and Drosophila egg chamber [4]. Particularly, this FISH method enables the analysis of several different RNAs simultaneously using multi-color fluorescence and the analysis of transcriptional activity at the cellular level. Although this method has made a significant progression in analyzing the expression pattern and subcellular localization of the specific RNAs, combining FISH with immunofluorescence (henceforth called "IF") method to detect RNAs and their associated proteins simultaneously is a practically challenge due to different hybridization conditions (Saline-sodium citrate (SSC) for FISH; Phosphate-buffered saline (PBS) for IF), different reaction temperatures ($\geq$30 °C, usually with formamide for FISH; 4–24 °C, no harsh chemicals for IF), and RNase sensitivity [5] (Table 1). To date, this combining method has been developed only in Drosophila egg chamber [4] and embryos [5]. Therefore, developing the FISH/IF combining protocol will lead to advanced biochemical, cellular, and functional analysis of RNA:protein complex in the specific cells or tissues.

The nematode *Caenorhabditis elegans* (*C. elegans*) is an attractive model organism for study of basic biological and biomedical sciences. Particularly, RNA regulations by RNA-binding factors (e.g., RNA-binding proteins and microRNAs) have been studied intensively in the *C. elegans* germline [6,7]. These RNA regulations control germline stem cell (GSC) maintenance, mitosis/meiosis decision, and sex determination [8]. Aberrant regulations result in sterility and germline tumors [7]. Therefore, the simultaneous analysis of RNAs and RNA-binding proteins in the specific cell types and tissues provides a powerful tool for investigating its cellular and physiological function *in vivo*. To date, in *C. elegans*, FISH method has been developed in only whole worms and embryos [1], but not in germlines. Moreover, FISH/IF combining protocol has not yet been developed because the reaction conditions for the two procedures conflict in multiple ways (Table 1). We here present a simple protocol to visualize RNAs and proteins simultaneously using FISH and IF in the same *C. elegans* germline. This method enables detection of RNA/protein complex with better preservation of the fine structure at the cellular level.

Method details

Probe design and synthesis

Stellaris FISH probes were designed as described by Ji and van Oudenaarden [1]. Briefly, FISH probe length is 17–22 base pairs, while probe spacing is no less than 2 base pairs. Probe GC contents are about 45%, and the number of probes for each target RNA are between 30 and 96 depending on target transcript length. Typically, 48 probes are used to ensure good signal quality. A web-based probe design software is available at: http://www.biosearchtech.com/stellarisdesigner/