Welcome to MSU!

MSU Office of Environmental Health and Safety

Don’t start a fire

Credit: New Jersey Herald
- Ignition sources
- Papers and combustibles
- Electrical overheating
- Flammable liquids
- Traveling vapors
- Reactive chemistry
Go with the Cheap and Easy

Credit: UVA EHS
Say no to Cookbook Chemistry

A protocol for studying the kinetics of RNA within cultured cells: application to ribosomal RNA

Marc Thire1, Françoise Lemay2, Nicolas Thelen3, Auric Chaumet-Colliez2, Nathalie Luluy4, Hélène Beblivon2 & Dominique Pithon2

This protocol describes a microfocusing method for high-resolution investigation of the kinetics of RNA within the cell. This involves the incorporation of biotinylated-5'-tri-phosphate in RNA of living cells by liquidation followed by immunocytochemical detection of RNAs. The use of the same antibody/labelled probe with fluorescein or with gold particle revealed the three-dimensional organization of sites containing labelled RNAs on their precise localization by using confocal and infrastructural microscopy, respectively. Comparison of the three-dimensional reconstruction obtained from the series of optical sections and ultrathin sections was extremely helpful to assess the topological and spatial dynamics of RNAs from their synthesis to its subcellular location to the cytoplasm. Combined with immunocytochemical labeling of proteins involved in different nuclear activities and with highly resolved three-dimensional reconstructions of the labelling, this method should also provide a significant contribution for understanding of the functional, subcellular organization of the cell nucleus. The entire protocol can be completed in ~10 d.

INTRODUCTION
For a long time, the only data concerning the dynamics of RNA molecule within the nucleolus were obtained from autoradiographical studies after a cell uptake of labelled uridine. However, the physiological and rapid kinetics of the incorporation of biotinylated-5'-tri-phosphate in RNA of living cells that we have developed allows an intracellular localization of biotinylated RNAs by using confocal and infrastructural microscopy, respectively. Comparison of the three-dimensional reconstruction obtained from the series of optical sections and ultrathin sections was extremely helpful to assess the topological and spatial dynamics of RNAs from their synthesis to its subcellular location to the cytoplasm. Combined with immunocytochemical labeling of proteins involved in different nuclear activities and with highly resolved three-dimensional reconstructions of the labelling, this method should also provide a significant contribution for understanding of the functional, subcellular organization of the cell nucleus. The entire protocol can be completed in ~10 d.

MATERIALS

• HeLa cells (EGC Promochem, ATCC, cat. no. CCL-2)
• Dulbecco's modified Eagle medium (Gibco-Invitrogen, cat. no. 41966)
• Fetal bovine serum (Gibco-Invitrogen, cat. no. 2012-06)
• t-Aminocaproic acid (Sigma-Aldrich, cat. no. 06422)
• Actinomycin D (Sigma-Aldrich, cat. no. 75265-8)
• Penicillin-streptomycin (Gibco-Invitrogen, cat. no. 15140)
• Furomere 6 transduction reagent (Rösch Diagnostics, cat. no. 1814 443)
• BrUTP (Sigma-Aldrich, cat. no. B-7866)
• N6A (Sigma-Aldrich, cat. no. S-7655)
• NaH2PO4 (Merck, cat. no. 6579)
• K2HPO4 (Merck, cat. no. 6873)
• Na2HPO4 (Merck, cat. no. 1698)
• Formaldehyde (20% wt/vol), 2 ml amoule, Ladd Research, cat. no. 20.295

CAUTION

• Glutaraldehyde (70% wt/vol), 2 ml amoule, Ladd Research, cat. no. 20.100

CAUTION

• Torant, allergen, carcinogen.
• 5% (v/v) ethanol (Merck, cat. no. 1.0899.1, 2500)
• 2% (w/v) EDTA (Merck, cat. no. 1.0899.1, 1000)
• Dodecyl succinic anhydride (Ladd Research, cat. no. 21340)
• Methyl succinic anhydride (MNA, Ladd Research, cat. no. 21350)
• 2,3,5-Dimethyl-2-hydroxynaphthalene phenol (DMP-30, Ladd Research, cat. no. 21370)
• L-X 112 (Ladd Research, cat. no. 21330)
• Bovine serum albumin (BSA, fraction V, Boehringer Mannheim, cat. no. 720108)
• Normal goat serum (Sigma-Aldrich, cat. no. G 003)
• Normal sheep serum (Sigma-Aldrich, cat. no. S 7772)
• Methanol (Merck, cat. no. 1.08600.2500)
• Triton X-100 (Sigma-Aldrich, cat. no. 234729)
• Mouse monoclonal anti-bromodesoxyuridine antibody (100 ng ml-1).
Be a good neighbor

No chemicals down the sink

No needles in the garbage
Accidents happen, don’t hide it!

- Here for your questions or concerns
- Assist you in finding the answer you need
- Get you the right protective equipment
- Advocate on your behalf
- Don’t have to give your name!
- 517-355-0153 business hours
- 517-355-2222 after hours
- 24/7/365