METABOLIC LABELING/AUTORADIOGRAPHY OF CELLS

Information on the cellular sites of synthetic processes in individual cells can be readily obtained using the techniques of autoradiography. Cells are exposed to radioactively labeled precursors which become metabolically incorporated. These isotopes are then detected by exposing the cells to a photographic emulsion. Emitted rays activate silver grains in the emulsion. Upon development, location and number of grains corresponds to the distribution and amount of radioactivity.

Procedure:

1. Preparation of Cells

Culture cells on dishes or, preferably, coverslips (CS).
Expose to radioisotopes, as required.
Wash coverslips twice with PBS. Fix in methanol (or 95% EtOH) on ice, for 15 minutes.
Extract with 5% TCA, on ice, for 30 minutes.
Rinse twice with dH_2O. Air dry.
Mount coverslip (cell side up) on slide with drop of Permount. Allow to harden overnight.

2. Emulsion

All procedures are done in a dark room with red light (use No. 2 red filter).
Melt emulsion (KODAK NTB-2 or NTB-3) at 60°C prior to use. Dilute 1:1 with dH_2O.
Add emulsion to dish and decant, leaving it on long enough to coat cells or dip slide into stock dilution. Do not mix used and unused emulsion.
Drain for 1 minute to remove excess emulsion. Place in a dark box. Include desiccant and allow to sit for several days (as required) at room temperature or 4°C.

3. Development

Cover sample with D-19 developer and incubate for 3 minutes.
Stop development by washing with 3% acetic acid.
Incubate in Kodak fixer for 3 minutes.
Wash extensively with H_2O.
Drain dry and observe. Under low power, look for clusters of silver grains over cells. Calculate the percentage of cells that are labeled.
Under high power, what can be said about the localization of grains over the cells?

References:

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