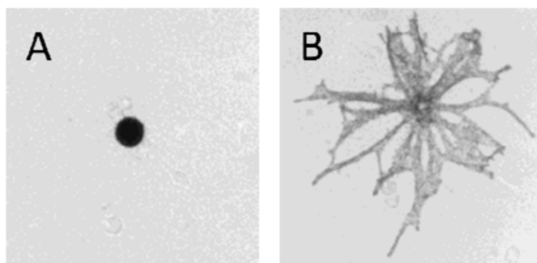
Primary cultures of functional Atlantic cod melanophores

Fish melanophores are terminally differentiated cells that derive from the neural crest. They are large and contain numerous amounts of dark pigment organelles – the melanosomes – that can be regulated to either all move towards the cell centre or disperse throughout the cell. This regulatory ability has made them a primary model system for understanding of intracellular transport. The following protocols were approved for primary cultures of Atlantic cod melanophores.

1. A 1-cm slice of dorsal fin is removed and placed in a sterile glass dish containing cell culture medium 199 with Hank's salts and supplements.
2. Medium is renewed and fin slice is cut into pieces of about 1 mm^2. Explants are placed in a sterile culture dish onto coverslips covered with a drop of medium then kept at 12°C for about 1 h (without drying) to promote cell adhesion. Medium is added to fill-up culture dishes.
3. Fin explants are further incubated at 12°C in darkness. Culture medium is changed twice a week. A monolayer of skin cells including melanophores will form around each explant.
4. To isolate melanophores from other skin cells, culture medium is changed to serum-free CO_2-independent medium used for culturing nerve cells. After a few days in this medium, only melanophores remain attached to the coverslips. Isolated melanophores can be kept for weeks under these conditions providing that medium is changed twice a week.
5. Pigment aggregation is induced with micromolar concentrations of noradrenaline or melatonin (A), and pigment dispersal is achieved by renewing culture medium and therefore removing noradrenaline / melatonine (B). For a faster dispersal, cAMP-elevating agent forskolin can be added to fresh culture medium. Cells are stable for immunocytochemistry, microinjections and other experimental manipulations.



Note: Although this protocol was developed and used for Atlantic cod melanophores, it is likely that the same protocol can be applied to other marine fish species.

Helen Nilsson Sköld
SLC-Kristineberg, Fiskebäckskil, Sweden

Fish: Captured Atlantic cods maintained in large basins supplied with running seawater at ambient temperature and salinity, and fed once a week ad libitum with crushed blue mussels (Mytilus edulis).

Apparatus: Inverted microscope with bright field illumination. Cell incubator set at 12°C.

Cell culture media: Medium 199 supplemented with Hank's salts, 10% fetal calf serum, 1% antibiotic / antimycotic solution and 1% L-glutamine (all from Life Technologies). Serum-free CO_2-independent medium supplemented with 1% antibiotic / antimycotic solution, 1% L-glutamine (Life Technologies), 5 µg/ml insulin, 5 µg/ml transferrin, 5 ng/ml sodium selenite (Boehringer Mannheim) and 1 mg/ml glucose.

Labware: Plastic cell culture dishes, glass coverslips, scalpels, forceps and serologic pipettes. All sterilized.

Additional information:

***Nilsson H & Wallin M** (1997) Evidence for several roles of dynein in pigment transport in melanophores. Cell Motil Cytoskel 38:397-409*

***Nilsson H, Rutberg M & Wallin M** (1996) Localization of kinesin and cytoplasmic dynein in cultured melanophores from Atlantic cod, Gadus morhua. Cell Motil Cytoskel 33:183-196*

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