Hydra: Research Methods

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Chapter 40

Eliminating Interstitial Cells with Nitrogen Mustard

Charles N. David

PURPOSE

To remove interstitial cells and differentiating nematoblasts from hydra leaving a shell of epithelial and gland cells.

INTRODUCTION

Treatment of hydra with nitrogen mustard (NM) causes the rapid elimination of interstitial cells and differentiating nemotoblasts from the tissue (Diehl and Burnett, 1964). Such hydra have been used to investigate the role of interstitial cells in budding and regeneration (Diehl and Burnett, 1965*a,b*). In addition, NM-treated hydra have been used as feeder layers for the culture of interstitial cells added to them by grafting (Diehl and Burnett, 1966) or reaggregation techniques (David and Murphy, 1977). The method, described here for *Hydra attenuata*, is essentially that of Diehl and Burnett (1964), who used *Hydra pseudoligactis* and *Hydra pirardi*.

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COMMENTS

NM is a potent alkylating agent capable of adding ethyl residues to nucleic acids including DNA (see Goodman and Gilman, 1974, for review). It is strongly cytotoxic to proliferating cells; nonproliferating or slowly proliferating cells appear to be less affected.

NM acts on cells at any stage of the cell cycle. Progression through the cycle, however, is usually blocked in the G_2 (premitotic) phase. Cells blocked in G_2 continue synthesizing RNA and protein and often become enlarged due to unbalanced growth.

Treatment of hydra with 0.01% NM for 10 min leads to the disappearance from hydra tissue of interstitial cells and differentiating nematoblasts over the next 4–8 days. This process can be easily monitored by observing interstitial cells in whole mounts of hydra stained with Toluidine Blue (Diehl and Burnett, 1964) or by counting interstitial cells in macerations of treated animals (David, 1973). Subsequently the number of differentiated nerves and nematocytes also declines. By comparison, the epithelial and gland cell populations appear to be less affected. Five days after NM treatment, hydra consist of a shell of epithelial and gland cells, and in this condition they survive for 4 weeks or longer.

The effects of NM on hydra cells are best explained with reference to the proliferation kinetics of hydra cell types. Interstitial cells are rapidly proliferating cells with generation times of 18–27 hr (Campbell and David, 1974). They are rapidly killed by NM and eliminated from hydra tissue, probably by phagocytosis by epithelial cells. Epithelial cells have cell generation times of 3 days in well-fed hydra and more than 6 days in starving hydra (David and Campbell, 1972). They are also killed by NM, but because of their longer cell cycle they survive longer than interstitial cells and thus give rise to animals consisting only of epithelial cells. Gland cells have a similar cell cycle to that of epithelial cells and also survive for long periods in NM-treated hydra.

MATERIALS

Two percent (w/v) sodium thiosulfate and 0.01% NM (Sigma Chemical Co.,). *Special precaution*: NM is toxic and should be handled with care. Carry out as much of the procedure as possible in a fume hood. In addition, have 1–2 liters of 2% sodium thiosulfate solution available to detoxify any spilled NM solution as well as any unused reagent.

PROCEDURES

Because NM is unstable in water, prepare the reagent from the dry powder immediately before use. Add NM to hydra at a final concentration of 0.01%. Mix the solution thoroughly by stirring with a pipet, taking care that all hydra are in suspension and not stuck to the sides of the dish. After 10 min allow the hydra to settle, decant the solution and fill the dish with fresh medium. Repeat the washing procedure four times to stop the action of NM on the hydra. Thereafter wash the hydra at least once each day in order to remove dead hydra and debris from the dish.

After NM treatment hydra can be fed for about a week until their nematocytes are depleted (Diehl and Burnett, 1964). However, feeding stimulates cell cycling and accelerates the death of treated animals. If the purpose of NM treatment is to prepare hydra free of interstitial cells, then one feeding after NM treatment appears to optimally stimulate the disappearance of interstitial cells without adversely affecting the survival of the host animal.

A final concentration of 0.01% NM is usually effective in completely eliminating interstitial cells from tissue without immediately destroying the animals. However, the precise concentration of NM required to eliminate interstitial cells varies slightly between batches of NM. Thus, it is useful to test several concentrations when using a freshly opened bottle of NM. If, after prolonged use, the NM in a bottle loses some of its potency, increase the concentration of NM used in experiments. However, do not use more NM than necessary because it does destroy epithelial cells and thus may noticeably shorten the survival of treated animals.

SPECIAL APPLICATIONS

NM-treated hydra free of interstitial cells are useful hosts in which to study the fate of interstitial cells added to them by grafting or reaggregation techniques. When normal hydra tissue is grafted into NM-treated tissue, interstitial cells migrate from the normal tissue into the NM tissue, where they proliferate and differentiate normally (Diehl and Burnett, 1966). Interstitial cells can also be introduced into NM tissue by preparing aggregates from dissociated NM-treated hydra (David and Murphy, 1977). Such aggregates regenerate normal hydra structures. When small amounts of normal tissue are dissociated and added to such NM aggregates, the added interstitial cells proliferate and differentiate normally. The number of cells

seeded in such aggregates can be easily controlled, and the aggregates function effectively as tiny tissue culture dishes (see Chapter 33).

REFERENCES

Campbell, R. D., and David, C. N. 1974. Cell cycle kinetics and development of *Hydra attenuata*. II. Interstitial cells. *J. Cell Sci.* **16**:349–358.

David, C. N. 1973. A quantitative method for maceration of hydra tissue. *Wilhelm Roux Arch. Entwicklungsmech. Org.* **171**:259–268.

David, C. N., and Campbell, R. D. 1972. Cell cycle kinetics and development of *Hydra attenuata*. I Epithelial cells. *J. Cell Sci.* **11**:577–568.

David, C. N., and Murphy, S. 1977. Characterization of interstitial stem cells in hydra by cloning. *Dev. Biol.* **58**:372–383.

Diehl, F., and Burnett, A. L. 1964. The role of interstitial cells in the maintenance of hydra. I. Specific destruction of interstitial cells in normal, sexual and non-budding animals. *J. Exp. Zool.* **155**:253–259.

Diehl, F., and Burnett, A. L. 1965*a*. The role of interstitial cells in the maintenance of hydra. II. Budding. *J. Exp. Zool.* **158**:283–298.

Diehl, F., and Burnett, A. L. 1965*b*. The role of interstitial cells in the maintenance of hydra. III. Regeneration of hypostome and tentacles. *J. Exp. Zool.* **158**:299–318.

Diehl, F., and Burnett, A. L. 1966. The role of interstitial cells in the maintenance of hydra. IV. Migration of interstitial cells in homografts and heterografts. *J. Exp. Zool.* **163**:125–140.

Goodman, L. S., and Gilman, A. 1974. *The Pharmacological Basis of Therapeutics*. Macmillan, New York.

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