Bivariate flow-cytometric analysis of regulation of 3T3 cell proliferation

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Using acridine orange (AO) as a dye for simultaneous measurement of DNA- and RNA-content of lymphocytes, Darzynkiewicz (1) was able to distinguish between quiescent (G_0-state) and cycling cells. Working with 3T3 cells, Smets (2) reported an increased stainability of DNA by AO after stimulation with high serum from quiescence.
We used similar staining methods to study population dynamics of 3T3 cells, especially the kinetics of density-dependent growth inhibition as well as the cellular response to stimulation by high serum reseeding.
Measurements were carried out on a flow-cytometer ICP 22 (Biophysics) equipped for two-parameter analysis, interfaced to a microcomputer CBM 3032 (Commodore) via an analog-to-digital converter, allowing online single and dual parameter analysis of the data.
Our results confirm that RNA content and DNA stainability are reduced in quiescent cells as compared to cells from a growing population.
The shift during normal growth from the high to the low fluorescence state starts when the population reaches saturation density and is largely finished after several days.
Stimulation of a quiescent culture by raising the serum content of the medium or by replating to lower densities first leads to an increase of DNA stainability, followed by an increase of RNA content.
Cells stimulated by reseeding begin to synthesize RNA during their lag phase of growth and reach high RNA contents when exponentially growing.
In contrast, cells stimulated at high cell densities by rising external serum concentration in our experiments were able to resume proliferation without regaining the high RNA content of exponentially growing cells.

1) Darzynkiewicz, Z., in: Melamed, M.R., Mullaney, P.F., Mendelsohn, M.K., (1979), Flow Cytometry and Sorting, Wiley, New York, p. 285.

2) Smets, L.A., (1973), Exptl. Cell Res. 79, 239

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