DYNAMIC FUNCTIONAL AND STRUCTURAL ANALYSIS OF LIVING CELLS: NEW TOOLS FOR VITAL STAINING OF NUCLEAR DNA AND FOR CHARACTERISATION OF CELL MOTION

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ABSTRACT

Increasing interest has been paid to applications of fluorescence measurements to analyze physiological mechanisms in living cells. However, few studies have taken advantage of DNA quantification by fluorometry for dynamic assessment of chromatin organization as well as cell motion during the cell cycle. This approach involves both optimal conditions for DNA staining and cell tracking methods. In this context, this report describes a stoichiometric method for nuclear DNA specific staining, using the bisbenzimidazole dye Hoechst 33342 associated with verapamil, a calcium membrane channel blocker. This method makes it possible to correlate variations of nuclear DNA content with cell motion in cells that are maintained alive. Motion measurement is the second goal of this paper and it explains the snake-spline method, and the associated cell following method.

KEY WORDS: Living cell, Cell boundaries, DNA staining, Hoechst 33342, Chromatin, Segmentation, Motion analysis, B-splines, Active contour.

1. INTRODUCTION

Chromatin is the main component of the nucleus and has been widely studied by molecular biology methods providing information at the primary levels of chromatin packing. However the structural organisation of chromatin in the cell nucleus remains largely unknown, especially in living cells.

Fluorescence microscopy is well suited to study components of living cells as well as fixed cells (Taylor & Wang, 1980). But until now, most of the studies dealing with nuclear DNA have been realized on fixed cells. This is essentially due to the lack of staining techniques usable on living cells. Among the many DNA specific dyes that are commercially available (Latt & Langlois, 1990), only a handful can be used in the special context of living cells, taking into account their dynamics of incorporation and release, their toxicity and the effects induced on the progression of cells through the cell cycle (Erba et al., 1988).

Studying living cells by means of fluorescence microscopy requires non-perturbant optimal conditions for staining. The staining has to be non toxic, specific, and stoichiometric (Paillasson et al., 1994, 1995).

The first step of the present work was to optimize conditions of Hoechst 33342 (Hoe342) staining in order to study the time course evolution of living cell nuclei. Optimal conditions for staining nuclear DNA were determined on L929 murine fibroblasts, in order to use the lowest possible concentration of dye and thus to limit cell cycle perturbations. This part of the work consisted in evaluating the staining stoichiometry and measuring the induced effects on the cell viability and cell cycle progress.

This defined technique of DNA staining was then used to follow up cell motion. The study of cell motion involves the knowledge of the velocity vector field corresponding to the deformations of the cellular and nuclear boundaries. This vector field can be obtained through optical flow techniques (Horn, 1981; Duncan, 1992), if one has at each time the contours of the cell boundaries: the corresponding step is called segmentation and it follows in general a preliminary step of contrast enhancement of the grey level gradient on these boundaries. Contrast enhancement algorithms consist in applying either continuous differential operators (Cottet, 1991; Cottet & Germain, 1993) (reaction diffusion partial differential equations) or their discrete versions, i.e. formal neural networks, for real time treatments. The study of chromatin organization and cell motion is of basic importance in cell biology to provide new insights in the cell behaviour under normal and pathological conditions. The chromatin structure is not static and there is strong evidence that chromatin shows different levels of organization according to the state of proliferation and/or differentiation (Santisteban et al., 1992). Cell motion is a mechanism involved in metastasis and invasion processes and is basic to the spread of cancer. This paper addresses two different methods: a non perturbant DNA staining and a live cell image segmentation procedure for studying the relationship between chromatin organization and cell motion.

2. MATERIALS AND METHODS

2.1 DNA analysis

2.1.1 Cells and reagents

Anchorage dependent L929 murine fibroblasts (ATCC, CCL1) were grown to log phase using antibiotic free Minimum Essential Medium (MEM, Techgen), supplemented with 5% fetal bovine serum (FBS).

Several dishes were used according to the chosen method of analysis: 25 cm² flasks (Costar), and culture chambers (Lab Tech) for flow cytometry and image analysis,