

Review Article

Simple Laboratory methods to measure cell proliferation using DNA synthesis property

Madhavan H N^{1*}

* Dr. H.N. Madhavan, Director of Research & Professor of Microbiology, Sankara Nethralaya, L & T Microbiology Research Centre, 18, College Road, Chennai - 600006 India. drhnm@snmail.org

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Introduction

This is a mini-review on the techniques to measure proliferation of cells by estimation of DNA synthesis. This is not an exhaustive review of literature, but a bird's eye view of a few selected articles which may provide the technical details to the readers.

The nucleus of a cell occupies about 10-30% of the cells space, depends on the type of genetic material (DNA -DeoxyriboNucleic Acid). DNA is a long, double-stranded, helical molecule which carries the genetic information. Duplication of the DNA takes place by the phenomena of replication. One copy of double-stranded DNA molecule forms two double-stranded DNA molecules. DNA replication is the fundamental process used in all living organisms as it is the basis for biological inheritance. This process is known also as Mitosis in somatic cells. In Mitosis, the duplication process results in two genetically identical "daughter" cells from a single "parent" cell. The resulting double-stranded DNA molecules are identical; proof reading and error-checking mechanisms exist to ensure near perfect pair. Mitosis is divided into six

phases: prophase, prometaphase, metaphase, anaphase, telophase, and cytokinesis.

Methods of detecting and assay of DNA synthesis

In a cell, DNA replication must happen before cell division can occur and the biochemical pathway correlates well with DNA synthesis which is relatively specific for cell division. Measurement of new DNA synthesis is, therefore, essentially synonymous with measurement of cell proliferation. Direct measurement generally involves the incorporation of a labeled nucleoside into genomic DNA. Examples include the tritiated thymidine (³H]dT) and BrdU (bromodeoxyuridine) methods 1, 2. Radioactive tagging of newly synthesized DNA with ³H-labeled Thymidine (³H-T) is most frequently applied technique. In recent days, usage of BrdU non radioactive labeling has increased. Gratzner in 1982 2 developed a monoclonal antibody to BrdU and for identifying BrdU-labeled S-phase cells with immunoperoxidase, immunofluorescence, or avidin-biotin complexes 3, 4, 5, 6. Monoclonal antibody (MAb) techniques for detection of

BrdU have the advantages of simplicity and speed over standard autoradiography. Therefore, for a long period of time the cell biologists have been using the technique of thymidine labeling, whereby a group of chemicals such as BrdU are incorporated into the DNA of dividing cells. The advantages of non-radioactive immune techniques using BrdU or anti-BrdU are undisputed but the scope and limitations of the systems for detecting BrdU cannot always be clearly defined. Besides enabling microscopic detection at the cellular level, the enzyme immune assay technique represents a method for routine screening. Two different detection systems are available: 1) the conventional colorimetric test and 2) a chemiluminescence assay also based on the anti-BrdU technique. One of these test methods will be suitable for use depending on the problem and the available laboratory facilities.

Burns *et al*, 2005 7 showed evidence for BrdU labeled dead cells after introducing them into the brains of mice. BrdU is used in experiments to mark cell splitting at a particular time. This helps researchers to examine where those cells migrated and what new cell types they differentiated into. It has been shown that cells from one tissue marked with BrdU extracted and were transplanted into a second tissue with the assumption that the host cells had taken up the cells which could later be identified. Experimental evidences suggests that many thymidine-labeled stem cells die after being transplanted (as done for studies on stem cell transplantation research where many labeled stem cells die after being transplanted into the mice brain⁷). The released chemical were taken up by neighbouring cells, (dividing and non-dividing) which may be mistaken as transplanted cells. 7, 8

The tritiated thymidine ([³H]-T) labeling index has been used for estimation of the proportion of S-phase cells in asynchronous cell populations as described by Steel 9. Most investigators have found that BrdU has been equally good as [³H]-T in providing assays of S-phase fractions in labeling-index assays and

by bivariate flow cytometry 10, 11, 12. Incorporation of [³H]-T and BrdU were studied which showed, the same cells developed positive for both precursor analogues. 13, 14 Monoclonal antibody (MAb) techniques for detection of BrdU have the advantages of simplicity and speed over standard autoradiography required for radio-isotopes as used in ³H Thymidine technique. Lin & Allison (1993) stated that simultaneous labeling of cells with BrdU and [³H]-T was rather difficult because both these precursor analogues were incorporated by the salvage pathway which was the competitive binding for Thymidine kinase. 15 This study shows that Feulgen hydrolysis denatured the DNA of fixed cells sufficiently to allow detection of incorporated BrdU with monoclonal antibodies. Cells were then double-labeled with [³H]-T and BrdU, placed on slides, and Feulgen stained. The absorption cytometry of DNA content was measured on randomly selected cells. The removal of the BrdU or r31-4T grains after DNA measurements did not interfere with subsequent detection of the grains from the other label. BrdU and r3HJ-T can be used reliably in combination for identification of S-phase cells. This method eventually allows the microscope-based image analysis for selectively measuring DNA contents.

Macallan and colleagues developed a method for measuring DNA synthesis analyzing within the cells. 16 It consisted of administering [6, 6-²H₂] Glc or [U-¹³C] Glc, in this method genomic DNA was isolated, enzymatically hydrolyzed to free deoxyribonucleosides and it was prepared for GC-MS analysis of dA or dG isotopic enrichments, or both. Comparison of dA or dG to extracellular Glc enrichment (with a correction for intracellular dilution) revealed the fraction of newly synthesized DNA, by application of the precursor-product relationship. The technique was different from the widely used [³H]thymidine or BrdU techniques in the *de novo* nucleotide synthesis pathway, rather than the nucleoside salvage pathway was used to label DNA and deoxyribose rather than the base moiety was

labeled; purine rather than pyrimidine deoxyribonucleosides were analyzed; and stable isotopes rather than radioisotopes were used. The method can be applied for both *in-vitro* (cell culture) and *in-vivo* (animal model). This method has several advantages over previously available techniques for measuring the cell turnover which involves no radioactivity or potentially toxic metabolites, and is suitable for use in humans. The availability of a reliable and safe method for measuring cell proliferation in humans opens up a number of fundamental questions to direct experimental testing, including basic problems related to cancer, AIDS, and other pathologic states.

Conclusion

This is a small review which may be helpful for the beginners, to learn technical details.

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