Cell Proliferation Method: Click Chemistry Based on BrdU Coupling for Multiplex Antibody Staining

Paolo Cappella,[1] Fabio Gasparri,[1] Maurizio Pulici,[2] and Jürgen Moll[1]

[1]Department of Biology, Drug Discovery Oncology, Nerviano Medical Sciences Srl, Milan, Italy
[2]Department of Chemistry, Drug Discovery Oncology, Nerviano Medical Sciences Srl, Milan, Italy

ABSTRACT

Determination of incorporation of the thymidine analog 5-bromo-2′-deoxyuridine (BrdU) into DNA is a widely used method to analyze the cell cycle (see *UNIT 7.7*). However, DNA denaturation is required for BrdU detection with the consequence that most protein epitopes are destroyed and their immunocytochemical detection for multiplex analysis is not possible. A novel assay is presented for identifying cells in active S-phase that does not require the DNA denaturation step but nevertheless detects BrdU. For this purpose, cells were pulsed for a short time by an alkenyl deoxyuridine (5-ethynyl-2′-deoxyuridine, EdU), which is incorporated into DNA. The nucleotide exposed ethynyl residue was then derivatized by a copper-catalyzed cycloaddition reaction ("click chemistry" coupling) using a BrdU azide probe. The resulting DNA-bound bromouracil moieties were then detected by commercial anti-BrdU monoclonal antibodies without the need for a denaturation step. This method has been tested using several cell lines and is preferred over traditional BrdU detection since it is more sensitive and allows multicolor and multiplex analysis in FCM and imaging. *Curr. Protoc. Cytom.* 45:7.34.1-7.34.13. © 2008 by John Wiley & Sons, Inc.

Keywords: BrdU azide • BMA • ethynyl deoxyuridine • click chemistry • cell cycle • multiplex analysis • chemical reporter

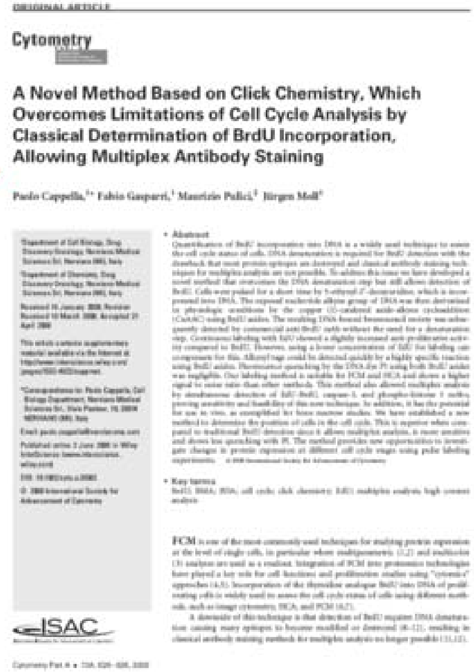
INTRODUCTION

For the history of determination of BrdU incorporation and its applications in cell biology, refer to *UNITS 7.7 & 7.14*. The major drawback of the current methods is that a DNA denaturation step is required to make BrdU accessible to an antibody. This harsh treatment destroys many protein epitopes (Tang et al., 2007). A highly sensitive method that overcomes this limitation and broadens the application of the technique by applying an alternative chemistry is described here. In recent years, a bio-orthogonal approach (Breinbauer and Kohn, 2003) has been introduced as a strategy to insert chemical tags into biological molecules such as proteins, glycans, lipids, and nucleic acids (Kolb and Sharpless, 2003; Link and Tirell, 2003; Hsu et al., 2007). These chemical tags can be detected after a two-step labeling reaction, which typically involves carbonyl tags detected by Shiff bases, azide tags detected by Staudinger ligation, and terminal alkyne detected by copper-catalyzed cycloaddition reactions ("click chemistry").

The "click chemistry" bio-conjugation reaction using the cycloaddition of azides and acetylenes exhibits favorable thermodynamic properties accompanied by high specificity and a high quantitative yield of the end product in aqueous solvents at physiological temperatures, providing 1,4-disubstituted triazoles with nearly complete regioselectivity (Rostovtsev et al., 2002; Bock et al., 2006).

Current Protocols in Cytometry 7.34.1-7.34.13, July 2008
Published online July 2008 in Wiley Interscience (www.interscience.wiley.com).
DOI: 10.1002/0471142956.cy0734s45

Cell proliferation method: click chemistry based on BrdU coupling for multiplex antibody staining. Cappella P, Gasparri F, Pulici M, Moll J. Curr Protoc Cytom. 2008 Jul ;Chapter 7: Unit 7.34.


ORIGINAL ARTICLE

Cytometry

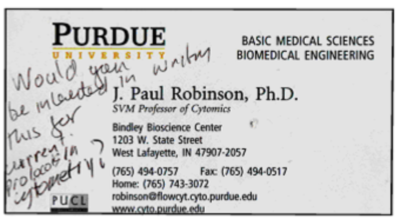
A Novel Method Based on Click Chemistry, Which Overcomes Limitations of Cell Cycle Analysis by Classical Determination of BrdU Incorporation, Allowing Multiplex Antibody Staining

Paolo Cappella, Fabio Gasparri, Maurizio Pulici, Jürgen Moll

• Abstract

• Key terms

ISAC


PURDUE UNIVERSITY
BASIC MEDICAL SCIENCES
BIOMEDICAL ENGINEERING
J. Paul Robinson, Ph.D.
SVM Professor of Cytomics
Bindley Bioscience Center
1203 W. State Street
West Lafayette, IN 47907-2057
(765) 494-0757 Fax: (765) 494-0517
Home: (765) 743-3072
robinson@flowcyt.cyto.purdue.edu
www.cyto.purdue.edu
PUCL

Would you be interested in writing this for current protocols in cytometry?

Current Protocols of Cytometry:
Chapter 7 introduction to Nucleic Acid Analysis

An enterely new approch to detect DNA replication, based on so called "click chemistry", is presented in UNIT 7.34. [...] . It is likely that different variant of "click chemistry" will provide new methods to detect DNA replication that will be prefered in future applications in cytometry.

Prof. Z. Darzynkiewicz

Cell Proliferation Method: Click Chemistry Based on BrdU Coupling for Multiplex Antibody Staining

UNIT 7.34

Paolo Cappella,[1] Fabio Gasparri,[1] Maurizio Pulici,[2] and Jürgen Moll[1]

[1]Department of Biology, Drug Discovery Oncology, Nerviano Medical Sciences Srl, Milan, Italy
[2]Department of Chemistry, Drug Discovery Oncology, Nerviano Medical Sciences Srl, Milan, Italy

ABSTRACT

Determination of incorporation of the thymidine analog 5-bromo-2′-deoxyuridine (BrdU) into DNA is a widely used method to analyze the cell cycle (see UNIT 7.7). However, DNA denaturation is required for BrdU detection with the consequence that most protein epitopes are destroyed and their immunocytochemical detection for multiplex analysis is not possible. A novel assay is presented for identifying cells in active S-phase that does not require the DNA denaturation step but nevertheless detects BrdU. For this purpose, cells were pulsed for a short time by an alkenyl deoxyuridine (5-ethynyl-2′-deoxyuridine, EdU), which is incorporated into DNA. The nucleotide exposed ethynyl residue was then derivatized by a copper-catalyzed cycloaddition reaction ("click chemistry" coupling) using a BrdU azide probe. The resulting DNA-bound bromouracil moieties were then detected by commercial anti-BrdU monoclonal antibodies without the need for a denaturation step. This method has been tested using several cell lines and is preferred over traditional BrdU detection since it is more sensitive and allows multicolor and multiplex analysis in FCM and imaging. *Curr. Protoc. Cytom.* 45:7.34.1-7.34.13. © 2008 by John Wiley & Sons, Inc.

Keywords: BrdU azide • BMA • ethynyl deoxyuridine • click chemistry • cell cycle • multiplex analysis • chemical reporter

INTRODUCTION

For the history of determination of BrdU incorporation and its applications in cell biology, refer to UNITS 7.7 & 7.14. The major drawback of the current methods is that a DNA denaturation step is required to make BrdU accessible to an antibody. This harsh treatment destroys many protein epitopes (Tang et al., 2007). A highly sensitive method that overcomes this limitation and broadens the application of the technique by applying an alternative chemistry is described here. In recent years, a bio-orthogonal approach (Breinbauer and Kohn, 2003) has been introduced as a strategy to insert chemical tags into biological molecules such as proteins, glycans, lipids, and nucleic acids (Kolb and Sharpless, 2003; Link and Tirell, 2003; Hsu et al., 2007). These chemical tags can be detected after a two-step labeling reaction, which typically involves carbonyl tags detected by Shiff bases, azide tags detected by Staudinger ligation, and terminal alkyne detected by copper-catalyzed cycloaddition reactions ("click chemistry").

The "click chemistry" bio-conjugation reaction using the cycloaddition of azides and acetylenes exhibits favorable thermodynamic properties accompanied by high specificity and a high quantitative yield of the end product in aqueous solvents at physiological temperatures, providing 1,4-disubstituted triazoles with nearly complete regioselectivity (Rostovtsev et al., 2002; Bock et al., 2006).

Current Protocols in Cytometry 7.34.1-7.34.13, July 2008
Published online July 2008 in Wiley Interscience (www.interscience.wiley.com).
DOI: 10.1002/0471142956.cy0734s45

ORIGINAL ARTICLE

Cytometry PART A

A Novel Method Based on Click Chemistry, Which Overcomes Limitations of Cell Cycle Analysis by Classical Determination of BrdU Incorporation, Allowing Multiplex Antibody Staining

Paolo Cappella,[1]* Fabio Gasparri,[1] Maurizio Pulici,[2] Jürgen Moll[1]

[1]Department of Cell Biology, Drug Discovery Oncology, Nerviano Medical Sciences Srl, Nerviano (Mi), Italy

[2]Department of Chemistry, Drug Discovery Oncology, Nerviano Medical Sciences Srl, Nerviano (Mi), Italy

Received 16 January 2008; Revision Received 19 March 2008; Accepted 21 April 2008

This article contains supplementary material available via the Internet at http://www.interscience.wiley.com/jpages/1552-4922/suppmat

*Correspondence to: Paolo Cappella, Cell Biology Department, Nerviano Medical Sciences Srl, Viale Pasteur, 10, 20014 NERVIANO (MI), Italy

Email: paolo.cappella@nervianoms.com

Published online 2 June 2008 in Wiley InterScience (www.interscience.wiley.com)

DOI: 10.1002/cyto.a.20582

Abstract

Quantification of BrdU incorporation into DNA is a widely used technique to assess the cell cycle status of cells. DNA denaturation is required for BrdU detection with the drawback that most protein epitopes are destroyed and classical antibody staining techniques for multiplex analysis are not possible. To address this issue we have developed a novel method that overcomes the DNA denaturation step but still allows detection of BrdU. Cells were pulsed for a short time by 5-ethynyl-2′-deoxyuridine, which is incorporated into DNA. The exposed nucleotide alkyne group of DNA was then derivatized in physiologic conditions by the copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) using BrdU azides. The resulting DNA-bound bromouracil moiety was subsequently detected by commercial anti-BrdU mAb without the need for a denaturation step. Continuous labeling with EdU showed a slightly increased anti-proliferative activity compared to BrdU. However, using a lower concentration of EdU for labeling can compensate for this. Alkynyl tags could be detected quickly by a highly specific reaction using BrdU azides. Fluorescence quenching by the DNA dye PI using both BrdU azides was negligible. Our labeling method is suitable for FCM and HCA and shows a higher signal to noise ratio than other methods. This method also allowed multiplex analysis by simultaneous detection of EdU-BrdU, caspase-3, and phospho-histone 3 mAbs, proving sensitivity and feasibility of this new technique. In addition, it has the potential for use in vivo, as exemplified for bone marrow studies. We have established a new method to determine the position of cells in the cell cycle. This is superior when compared to traditional BrdU detection since it allows multiplex analysis, is more sensitive and shows less quenching with PI. The method provides new opportunities to investigate changes in protein expression at different cell cycle stages using pulse labeling experiments. © 2008 International Society for Advancement of Cytometry

Key terms

BrdU; BMA; BDA; cell cycle; click chemistry; EdU; multiplex analysis; high content analysis

FCM is one of the most commonly used techniques for studying protein expression at the level of single cells, in particular where multiparametric (1,2) and multicolor (3) analyses are used as a readout. Integration of FCM into proteomics technologies have played a key role for cell functions and proliferation studies using "cytomics" approches (4,5). Incorporation of the thymidine analogue BrdU into DNA of proliferating cells is widely used to assess the cell cycle status of cells using different methods, such as image cytometry, HCA, and FCM (6,7).

A downside of this technique is that detection of BrdU requires DNA denaturation causing many epitopes to become modified or destroyed (8–12), resulting in classical antibody staining methods for multiplex analysis no longer possible (11,12).

A novel method based on click chemistry, which overcomes limitations of cell cycle analysis by classical determination of BrdU incorporation, allowing multiplex antibody staining. Cappella P, Gasparri F, Pulici M, Moll J. Cytometry A. 2008 Jul;73(7):626-36.