

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <http://orca.cf.ac.uk/114141/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Smith, Paul J. 2016. EdU and BrdU incorporation resolve their differences. *Cell Cycle* 15 (12) , pp. 1527-1528. 10.1080/15384101.2016.1171654 file

Publishers page: <http://dx.doi.org/10.1080/15384101.2016.1171654>
<<http://dx.doi.org/10.1080/15384101.2016.1171654>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



EdU and BrdU incorporation resolve their differences

Paul J Smith*; Cancer & Genetics Institute, School of Medicine, Cardiff University, UK, CF14 4XN, *smithpj2@cf.ac.uk DOI: XXXX

The paper by Pierzyńska-Mach et al. ¹ provides a salutary comparison of two existing methods for detecting DNA synthesis events at the single cell level. The focus is on the patterns of unscheduled DNA synthesis (UDS) linked to ongoing UVC-induced DNA repair. The research underlines the insights gained through super-resolution microscopy. Detection methods have typically exploited the ability of various analogues of the pyrimidine deoxynucleoside thymidine to become readily incorporated into replicating DNA. Subsequent analysis can identify tagged cells and report the rate, extent and genomic location of discrete DNA synthesis events. Earlier applications using tritiated thymidine autoradiography were demanding and were replaced by 5-bromo-2-deoxyuridine (BrdU) with detection using antibodies ². However, a significant drawback of the latter approach is the need to denature nuclear DNA to allow epitope access for the detecting antibody – raising the worry that all is not what it seems.

To address this doubt the authors have used a method to detect UDS based on the incorporation of 5-ethynyl-2'-deoxyuridine (EdU). This employs detection by "click" chemistry whereby incorporated EdU is tagged by a fluorescent azide through a copper-catalyzed cycloaddition reaction ³. Apart from the removal of the need for a denaturation step of uncertain efficiency, the small molecular size of the fluorescent azide allows for less compromised access to the sites of precursor incorporation. The current Pierzyńska-Mach et al. study ¹ focuses on an exploration of this particular EdU advantage to resolve intranuclear UDS reflecting the latter stage of UVC-induced nucleotide excision repair (NER). The paper points out that the typical finding using BrdU that incorporation apparently occurs at discrete "foci" in UV-irradiated nuclei, is more likely to be a shortcoming of the BrdU method rather than representing a structural reality. Indeed, using sensitive detectors in fluorescence microscopy and the improved clarity of super-resolution microscopy the authors have demonstrated the dispersed nature of EdU-monitored NER throughout UV-irradiated nuclei of human cells. This offers exciting opportunities to ask entirely new questions as to the nuclear location and dynamics of repair in individual cells - free of a level of artefact.

This has implications in situations where UDS, for example, is used to identify repair deficiency. Higher resolution analysis of EdU incorporation could clearly inform the nature of intranuclear heterogeneity for UDS events induced by a variety of genotoxic insults. Further, the increased resolution potential of EdU will help to resolve more subtle differences between the patterns and extent of repair events in cells of different origins or according to their location within 3D tissue architectures. It is important to remember that the BrdU and EdU techniques are not mutually exclusive, allowing for informative pulsing regimens and co-analysis ⁴. Here caution is needed since EdU is an antimetabolite. In fission yeast, for example, EdU activates the rad3-dependent checkpoint potentially limiting its application to short term studies.

Resolution is critical since it is appreciated that the induction of DNA photo-damage is not random with sequence selectivity, nucleotide context and cellular shielding all affecting intranuclear CPD distribution. The assembly of multiple protein complexes and chromatin remodeling also limit the recognition and subsequent repair of damage. For example, telomeric sequences present favorable targets for CPD induction while apparently shunning the opportunity for NER-linked repair. Furthermore, UVC induction of CPDs is photoreversible⁵, with higher fluencies approaching an equilibrium. This is a complication when attempting to assess the saturation of repair capacity versus CPD load.

Although UVC is not represented in solar terrestrial exposure wavelengths (<295 nm), there is clear evidence of the continuing importance of CPDs in environmental carcinogenesis. Indeed, the photosensitivity disorder xeroderma pigmentosum provides a paradigm for the carcinogenic consequences of defects in NER, with the EdU-based UDS assay potentially representing a convenient method for XP diagnosis⁶. There is a significant induction of CPDs in whole human skin by genotoxic UVB radiation (290-320 nm) and the compromised repair capacity of cells exposed to UVA radiation (320-400 nm)⁷. Accordingly, high resolution imaging of NER induced by environmental wavelength combinations in relevant tissue architectures will contribute to our understanding of skin carcinogenesis. However, the next challenge will be to explore alternative approaches for live-cell monitoring of the dynamics of NER and linkage to downstream cellular events.

1. Pierzyńska-Mach A. Subnuclear localization, rates and effectiveness of UVC-induced unscheduled DNA synthesis visualized by fluorescence widefield, confocal and super-resolution microscopy. *Cell Cycle* 2016; X:X.
2. Gratzner HG. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: A new reagent for detection of DNA replication. *Science (New York, NY)* 1982; 218:474-5.
3. Salic A, Mitchison TJ. A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 2008; 105:2415-20.
4. Bradford JA, Clarke ST. Dual-pulse labeling using 5-ethynyl-2'-deoxyuridine (EdU) and 5-bromo-2'-deoxyuridine (BrdU) in flow cytometry. *Current protocols in cytometry / editorial board, J Paul Robinson, managing editor [et al]* 2011; Chapter 7:Unit 7.38.
5. Law YK, Forties RA, Liu X, Poirier MG, Kohler B. Sequence-dependent thymine dimer formation and photoreversal rates in double-stranded DNA. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology* 2013; 12:1431-9.
6. Limsirichaikul S, Niimi A, Fawcett H, Lehmann A, Yamashita S, Ogi T. A rapid non-radioactive technique for measurement of repair synthesis in primary human fibroblasts by incorporation of ethynyl deoxyuridine (EdU). *Nucleic acids research* 2009; 37:e31.

7. Mouret S, Baudouin C, Charveron M, Favier A, Cadet J, Douki T. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proceedings of the National Academy of Sciences of the United States of America* 2006; 103:13765-70.