Video Article

Visualization of Mitochondrial DNA Replication In Individual Cells By EdU Signal Amplification

Kristine M. Haines[1], Eva L. Feldman[2], Stephen I. Lentz[3]

[1]Michigan Research Community, Undergraduate Research Opportunity Program, University of Michigan, Ann Arbor

[2]Neurology, University of Michigan, Ann Arbor

[3]Internal Medicine, Division of Metabolism, Endocrinology and Diabetes, University of Michigan, Ann Arbor

Correspondence to: Stephen I. Lentz at lentzs@umich.edu

URL: http://www.jove.com/video/2385
DOI: doi:10.3791/2385

Keywords: mitochondria, mitochondrial DNA (mtDNA), 5-ethynyl-2'-deoxyuridine (EdU), labeling, tyramide signal amplification, mtDNA biogenesis, dorsal root ganglion neurons

Date Published: 6/15/2015

Citation: Haines, K.M., Feldman, E.L., Lentz, S.I. Visualization of Mitochondrial DNA Replication In Individual Cells By EdU Signal Amplification. *J. Vis. Exp.* (), e2385, doi:10.3791/2385 (2015).

Abstract

Mitochondria are key regulators of cellular energy and mitochondrial biogenesis is an essential component of regulating mitochondria numbers in healthy cells1-3. One approach for monitoring mitochondrial biogenesis is to measure the rate of mitochondrial DNA (mtDNA) replication4. We developed a sensitive technique to label newly synthesized mtDNA in individual cells in order to study mtDNA biogenesis. The technique combines the incorporation of 5-ethynyl-2′-deoxyuridine (EdU)5-7 with a tyramide signal amplification (TSA)8 protocol to visualize mtDNA replication within subcellular compartments of neurons. EdU is superior to other thymidine analogs, such as 5-bromo-2-deoxyuridine (BrdU), because the initial click reaction to label EdU5-7 does not require the harsh acid treatments or enzyme digests that are required for exposing the BrdU epitope. The milder labeling of EdU allows for direct comparison of its incorporation with other cellular markers9-10. The ability to visualize and quantify mtDNA biogenesis provides an essential tool for investigating the mechanisms used to regulate mitochondrial biogenesis and would provide insight into the pathogenesis associated with drug toxicity, aging, cancer and neurodegenerative diseases. Our technique is applicable to sensory neurons as well as other cell types. The use of this technique to measure mtDNA biogenesis has significant implications in furthering the understanding of both normal cellular physiology as well as impaired disease states.

Disclosures

No conflicts of interest declared.