STRIDE (SensiTive Recognition of Individual DNA Ends): DIRECT IN SITU DETECTION OF INDIVIDUAL SINGLE- AND DOUBLE-STRAND BREAKS INDUCED BY CRISPR/Cas9

Magdalena Kordon¹, Mirosław Zarębski¹, Kamil Solarczyk¹, Hanhui Ma², Thoru Pederson², Jurek W. Dobrucki¹
¹Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Cell Biophysics, 30-387 Kraków, Poland
²University of Massachusetts Medical School, Department of Biochemistry and Molecular Pharmacology, Worcester MA 01605, USA
E-mail: jerzy.dobrucki@uj.edu.pl

KEY WORDS: DNA damage, DNA breaks, TUNEL assay, Nick Translation assay, STRIDE assay, CRISPR/Cas9.

The goal of this work was to directly detect in situ DNA cuts and nicks induced by the CRISPR/Cas9 system, using fluorescence microscopy methods. Some of the techniques that are routinely used for detection of DNA breaks in situ, such as terminal deoxynucleotidyltransferase dUTP nick end labelling (TUNEL) or the nick translation (NT) assay, allow for direct labelling of DNA breaks. However, since the methods are based on standard immunofluorescent staining, they are limited by typical problems associated with these techniques - low signal-to-noise ratio and unspecific staining. On the other hand, detection of DNA breaks by staining histone γH2AX or other DNA repair factors, although more sensitive, requires active signalling and triggered DNA repair mechanisms, and may yield false positive signals. Finally, methods such as SCSA (sperm chromatin structure assay), SCD (sperm chromatin dispersion test) or the Comet assay, albeit frequently used for informing about the overall degree of chromatin fragmentation, do not detect individual DNA breaks. Here, we present a method called SensiTive Recognition of Individual DNA Ends (STRIDE), which allows for specific, very sensitive in situ detection of DNA breaks in the nuclei of fixed cells. The method, in its two independent variants, allows the distinction between double strand- and single strand-breaks (dSTRIDE and sSTRIDE, respectively).

To detect in situ individual DNA breaks made by nuclease-active, GFP-tagged SpCas9 or the nicking activity of nickase mCherry-tagged SpCas9n, were localized in fixed human U2OS cells. In some experiments we deployed nuclease-inactive GFP-tagged dCas9 as a non-cutting negative control. We targeted the subtelomeric region of the long arm of chromosome 3 that contains a unique repetitive sequence. The single lesions were induced 349 bp or 394 bp downstream from the chromosome specific region of repetitive sequence. Multiple as well as individual cleavages made by SpCas9 within the target site were efficiently detected using dSTRIDE, and manifested by colocalization of the dSTRIDE and Cas9 signals. Similarly, DNA nicks produced by nickase SpCas9n were detected by sSTRIDE. In summary, dSTRIDE and sSTRIDE are capable of detecting clusters as well as individual DNA breaks induced by the action of the CRISPR/Cas9 system.