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Evidence that endogenous G-quadruplex DNA mediates stress granule assembly in response to oxidative stress

Kevin D Raney
University of Arkansas for Medical Sciences, USA

DNA sequences that consist of appropriately spaced guanine repeats can readily fold into stable structures termed G-quadruplex DNA (G4DNA). These sequences occur in non-random sites throughout genomic DNA, such as the promoters of proto-oncogenes, but are especially concentrated in telomeres and human mitochondrial DNA. Human cancer and neurodegenerative diseases have been linked to misregulation of G4DNA or G4RNA. In order to understand the mechanisms and functions of G4DNA, the proteins that bind and/or unfold these structures need to be identified. We performed a quantitative proteomics analysis using G4DNA as bait and the top hit was the DHX36 RNA helicase, a protein known to tightly bind and unfold G4DNA. Surprisingly, the other major proteins discovered, such as TIA1 and YB1 can associate with mRNA within cytoplasmic stress granules. Stress granules are dynamic assemblies of protein and mRNA that regulate translation in response to the cellular environment. To support the proteomics data, fluorescence co-localization experiments were performed by introducing fluorescently labeled G4DNA into cells. Multiple stress granule proteins were found to co-localize in the cytoplasm with exogenous G4DNA introduced into cells by transfection. A quadruplex specific antibody, BG4, was used to examine the localization of endogenous G4DNA in cells after exposure to oxidative stress. G4DNA appears to increase in the cytoplasm after treatment of cells with hydrogen peroxide. The fluorescent foci were found to co-localize with proteins known to appear in stress granules such as TIA1 and G3BP. G4DNA is normally observed in the nucleus, but this evidence supports its appearance in the cytoplasm after oxidative stress. G4DNA is resistant to nuclease activity, allowing it to bind to proteins that are found in stress granules. Since one role of stress granules is to modulate translation, we propose that excised G4DNA can serve as a signaling molecule that modulates gene expression in response to oxidative stress.

Biography

Kevin Raney is interested in the enzymology and chemistry of nucleic acid enzymes. Helicases are enzymes that manipulate DNA and RNA in all aspects of nucleic acid metabolism. We are studying an enzyme called Pif1, which is involved in many aspects of DNA metabolism ranging from telomere maintenance to transcription. Pif1 binds tightly to unusual DNA structures called quadruplexes, for which the biological functions are being intensively explored. We have recently discovered a possible signaling mechanism by which cells respond to DNA damage. During oxidative stress, guanine residues are oxidized, leading to excision of the damaged DNA. When the excised DNA consists of specific sequences containing runs of guanine, the resulting DNA fragment can fold into a stable structure called quadruplex DNA. Telomeric DNA is particularly susceptible to oxidative stress and contains sequences that readily fold into quadruplex structures. The excised DNA quadruplexes can bind to proteins such as DHX36 (a helicase), leading to the formation of sub-organelles called stress granules. The functional role of stress granules is to modulate translation. Hence, this mechanism provides a stepwise chemical mechanism for the cell to respond to DNA damage leading to changes in translation.

raneykevind@uams.edu

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