SEQUENCE-SPECIFIC VISUALIZATION OF MITOCHONDRIAL DNA OR RNA IN NUCLEOIDS BY DIRECT STORM

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Mitochondria are bioenergetics power plants of eukaryotic organisms and important contributors to metabolism and biosyntheses and basic physiological processes, such as cell proliferation, apoptosis, stress signaling and insulin secretion. Their dysfunction is implicated in aging, diabetes and cancerogenesis. Mitochondrial network in each cell contains 10 to 1000 copies of autonomous mitochondrial DNA (mtDNA) clustered in nucleoprotein structures called nucleoids, which encodes for 13 proteins, 22 tRNAs and 2 rRNAs. Mitochondrial replication, nucleoid segregation, transcription and further RNA processing are key steps of mitochondrial biogenesis and function. Surprisingly, although studies of nuclear genetic apparatus represent one of the most exposed fields of biology, little is known about mitochondrial nucleoids and RNA. We applied the dSTORM nanoscopy in combination with the molecular beacon hybridization technique for sequence-specific super-resolution visualization of mitochondrial DNA and RNA. Molecular beacons are short DNA probes with self-hybridization sequences at 5’ and 3’ ends with attached fluorescent molecule and quencher, respectively [1]. Therefore, beacons emit fluorescence only upon hybridization to its specific target thus increasing the signal/noise ratio. We constructed molecular beacons with attached Alexa Fluor and Cy3B fluorophores which are able to photobleach in reducing media. Photobleaching allows us to localize hybridized beacon with dSTORM nanoscopy (with 25 nm resolution). We combined this technique with dSTORM immunocytochemistry for nucleoid components and with FPALM localization of Eos-tagged nucleoid proteins. Using this technique, we characterized distribution of mt-RNR2 ribosomal RNA and noncoding RNA from the D-loop region in the vicinity of mitochondrial nucleoids.

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