Abstract

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Subcellular imaging of RNA distribution and DNA replication in single mammalian cells with SIMS: the localization of heat shock induced RNA in relation to the distribution of intranuclear bound calcium.

Chandra S.

Cornell SIMS Laboratory, Department of Earth and Atmospheric Sciences, Snee Hall, Cornell University, Ithaca, NY 14853, USA.

BACKGROUND: The subcellular localization of RNA for understanding transcriptional activity by using RNA precursors, like 5-bromouridine (BrU), generally requires chemical fixation and staining of cells with monoclonal antibody for imaging BrU-containing RNA in individual cells. Although effective for RNA localization, the native chemical composition of diffusible ions and molecules is destroyed in this approach and one cannot study their spatial relationship with RNA localization sites in this sample type.

OBJECTIVE: This work presents a novel secondary ion mass spectrometry approach in cryogenically prepared cells, which allows the same cell imaging of RNA (and/or replicating DNA) distribution in relation to intracellular chemical composition.

METHODS: The heat shock treatment of HeLa cells was used as a model system because the transcription of heat shock genes is activated during heat shock while other transcriptional activities of the cell are suppressed. The HeLa cells were heat-shocked for 1 h at 42 degrees C in presence of 100 muM BrU and/or 100 microM IdU (5-iododeoxyuridine). Following the heat shock treatments, the cells were cryogenically prepared with our sandwich freeze-fracture method and freeze-dried prior to secondary ion mass spectrometry analysis. A CAMECA IMS 3f secondary ion mass spectrometry ion microscope (CAMECA, Paris, France) capable of producing elemental (isotopic) distributions with a spatial resolution of 500 nm was used in the study. Secondary ion mass spectrometry analysis of fractured freeze-dried HeLa cells revealed well-preserved intracellular (39)K and (23)Na concentrations in heat-shocked cells. Both DNA replication and RNA distribution (total RNA) were imaged directly in the same cell by secondary ion mass spectrometry imaging of masses (127)I (from IdU) and (81)Br (from BrU), respectively.

RESULTS: Surprisingly, the nucleus of heat-shocked cells contained spatially resolved regions with elevated levels of bound calcium (approximately 0.75 mM total calcium instead of 0.50 mM total calcium in the nucleoplasm). These regions spatially correlated with depleted levels of BrU-RNA in (81)Br secondary ion mass spectrometry images. The remainder of intranuclear regions displayed the presence of BrU-RNA with heterogeneous distribution.

CONCLUSIONS: These observations indicate that calcium in its bound form may play a fundamental role in processes such as transcription and/or processing and storage of RNA. The shape of intranuclear regions with elevated levels of bound calcium resembled the heat shock induced nuclear bodies in HeLa cells. The analysis of cryogenically prepared frozen freeze-dried cells provides an ideal sample type for further understanding of the role of bound calcium in transcription of genes under physiological and pathological conditions.

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