

SUB-NUCLEOLAR DOMAINS EXCLUDING NEWLY SYNTHESIZED RNA REVEALED BY  
MULTI-ISOTOPE IMAGING MASS SPECTROMETRY (MIMS)

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One of the determinants of gene expression is the spatial relation of DNA and RNA in the nucleus. MIMS allows one to study DNA-RNA localization simultaneously and at high resolution, providing information difficult if not impossible to obtain with other methods. While using MIMS to explore the colocalization of RNA and DNA in cultured cells, we found the presence of sub-nucleolar domains that exclude newly synthesized RNA.

Embryo fibroblasts were cultured with <sup>15</sup>N-uridine and bromodeoxyuridine as markers of newly synthesized RNA and DNA, respectively. They were fixed in the cultured dish, lifted and epon embedded. Sections 0.5 μm thick were deposited on Si and analyzed with MIMS (Cs<sup>+</sup> primary ion beam; 16kV acceleration voltage; approximately 1pA). As expected, the bromine signal (DNA) was restricted to the cell nuclei. There was strong <sup>81</sup>Br labelling along the nuclear envelope and around the nucleoli. In contrast, the <sup>15</sup>N signal (RNA) was strong within the nucleoli. An excess <sup>15</sup>N signal was also seen into the cytoplasm (endoplasmic reticulum). Strikingly, the <sup>15</sup>N signal was also strong along the nuclear envelope. Because of the parallel imaging of <sup>81</sup>Br and <sup>15</sup>N, we observed the colocalization of newly synthesized RNA and DNA. Colocalization is visible along the nuclear envelope and around the nucleoli, while there is clear separation of RNA from DNA within the nucleoli.

Localization of newly synthesized RNA requires distinguishing local excesses of <sup>15</sup>N over its natural occurrence.

Using the overlay of the <sup>15</sup>N image on the <sup>14</sup>N image with color coding, we revealed the localization of newly synthesized RNA. It was found in the nucleoli, along the nuclear envelope, and in the cytoplasm.

We used as a control the <sup>13</sup>C/<sup>12</sup>C ratio image obtained from the pixel by pixel ratio of the <sup>13</sup>C/<sup>14</sup>N image over the <sup>12</sup>C/<sup>14</sup>N image. The <sup>13</sup>C/<sup>12</sup>C ratio image had no intracellular contrast because in the absence of <sup>13</sup>C excess, <sup>13</sup>C remains a constant fraction of <sup>12</sup>C throughout the cells. This ensured that the isobares <sup>12</sup>C<sup>15</sup>N and <sup>13</sup>C<sup>14</sup>N were well separated.

By examining the nucleolus at the high spatial resolution provided by MIMS, we found sub-nucleolar domains that exclude newly synthesized RNA. The simultaneous parallel imaging provided by MIMS allowed us to determine that these domains were due to locally lower incorporation of <sup>15</sup>N-uridine rather than to instrument or sample artifacts. We suspect that this finding is related to the nucleolar organization of ribosomal proteins and to the putative role of the nucleolus in mRNA export or degradation (1,2,3).

### **References**

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