Gold-Tagged RNA Glitters in Electron Microscopy

The genetic messages of DNA molecules are transcribed into RNA molecules which, leaving the nucleus, play a key role in many fundamental life processes. Chemically, RNA molecules are long polymers consisting of adenine (A), cytosine (C), guanine (G) and uracil (U), linked together along a ribose sugar chain. In practice, RNA molecules often form large complexes with protein molecules to form biologically important particles several millions of Daltons in weight, such as ribosomes (which form proteins), spliceosomes (which process proto-RNA) and viruses. Electron microscopy techniques can help visualize these particles, but they can not readily distinguish the enclosed RNA molecule and determine its biologically relevant structure, since the weak X-ray scattering of the RNA is hard to distinguish from that of the protein in which it is embedded. Various attempts to label RNA molecules to enhance their electron microscopic visualization have met with only limited success.

Prof. Joseph Sperling and his colleagues have now discovered a way to specifically and covalently bind strongly scattering 2nm gold particles (nanogold) to randomly spaced locations along the RNA chain. Like highway beacons, these particles clearly delineate the RNA molecule's twisting path. To accomplish this feat, the investigators transcribe DNA into RNA in test tubes, using RNA-polymerase and special modified A, C, G or U-triphosphate building blocks. The modified molecules have a sulfur-containing terminal thiol (-SH) group added to their heterocyclic ring. Commercially available nanogold particles are then chemically bound to the thiol-containing locations via a maleimido group.

The investigators' transmission electron microscope images of these gold-tagged molecules (both ATP and UTP varieties) showed bright spots corresponding to gold clusters, arranged at nearly regular distances along an imaginary curve that presumably corresponds to the RNA chain. Confirmation was provided by atomic force microscope images of the same preparations, which showed knob-like structures whose height corresponded to the expected size of the nanogold particles. In the example shown (see figure), the RNA samples were deposited on freshly cleaved mica chips and dried in air.

The researchers' pioneering work, recently published in the *Journal of Structural Biology*, literally lights up the way for more detailed studies of the 3-dimensional structure of RNA molecules in biologically important protein-RNA complexes.