Visualization of Intracellular Copper in Mottled Embryonic Mouse Fibroblasts by Micro-XRF and Optical Fluorescence Microscopy

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Copper is an essential element for the maintenance of human health. A number of diseases including Menkes' and Wilson's disease are caused by impaired copper transport and regulation. At present, the nature of cellular structures and organelles that may act as transient storage places and thus intimately participate in cellular copper homeostasis are elusive. To investigate how the intracellular copper distribution is altered in Menkes' disease, we utilized an embryonic mouse fibrolast cell line (802-1) that is defective in the Menkes' protein. We utilized microprobe synchrotron X-ray fluorescence (micro-XRF) to visualize the intracellular copper topography with submicron resolution, and thus to identify possible locations for copper storage. Our results suggest, Menkes deficient cells accumulate excessive copper in the nucleus under supplementation with 150 μM copper(II) chloride, but show a rather even distribution throughout the cytoplasm when grown in basal medium. In contrast, the corresponding wild-type cells (802-5) showed no sign of nuclear copper accumulation in high copper medium, but revealed patches of increased copper levels near the cell surface, presumably due to enhanced secretion. Furthermore, in an attempt to colocalize the elemental maps with subcellular structures and organelles, we used fluoro-nanogold labeled secondary anti-bodies as a tool for correlative optical fluorescence and micro-XRF microscopy.