Cohen NS (1996): Intracellular Localization of the mRNAs of Argininosuccinate Synthetase and Argininosuccinate Lyase Around Liver Mitochondria, Visualized by High-Resolution In Situ Reverse Transcription-Polymerase Chain Reaction. J Cell Biochem 61:81–96.

Due to a typographical error, a number was incorrectly printed in the Abstract (page 81). The following is the revised Abstract with the corrected number printed in **boldface:**

Abstract Argininosuccinate synthetase and argininosuccinate lyase, two cytoplasmic enzymes of the urea cycle, are released into the soluble phase in the absence of detergent when cells are disrupted. Yet previous biochemical studies, as well as immunocytochemistry at the electron microscope level, have shown that these enzymes are localized around mitochondria in situ. Such intracellular localization of soluble enzymes requires mechanisms to deliver the proteins to the appropriate sites, where they may then be anchored by specific protein-protein interactions. A method was developed to examine the intracellular distribution of the mRNA of argininosuccinate synthetase and argininosuccinate lyase in intact rat liver at the ultrastructural level by in situ reverse transcription and the polymerase chain reaction, using primers targeting regions of the coding sequences of the rat enzymes, digoxigenin-dUTP as the label, and anti-digoxigenin/1nm gold plus silver enhancement as the detection method. The tissue was fixed in 4% paraformaldehyde/0.1% glutaraldehyde and embedded in Lowicryl. Examination of the numbers and the location of the silver grains, coupled with morphometric analysis of the electron micrographs, permitted the calculation of the silver "enrichment ratio" for each type of cell structure. These ratios showed that the mRNAs for argininosuccinate synthetase and argininosuccinate lyase were located next to the cytoplasmic side of the mitochondrial membrane and in the nearby endoplasmic reticulum. Most of the silver grains that were observed in the endoplasmic reticulum were within 200 nm of the mitochondria; it was not possible, however, to determine if those grains were actually associated with the reticular membranes. These studies demonstrate that the mRNAs of these two soluble cytoplasmic proteins are localized to the same limited regions where the proteins are situated. Translation of the proteins, therefore, must occur at these specific sites. The targeting of argininosuccinate synthetase and argininosuccinate lyase mRNAs to the immediate vicinity of the mitochondria may be the first step of the mechanisms by which the spatial organization of these soluble proteins in situ is accomplished. The targeting of mRNAs for soluble cytoplasmic proteins of organized metabolic pathways has not been demonstrated previously. These studies also show that in situ reverse transcription and the polymerase chain reaction at the ultrastructural level, which has not been previously reported, can be used to detect specific mRNAs; it should be extremely valuable for the intracellular detection of low-abundance mRNAs. © 1996 Wiley-Liss, Inc.

Key words: mRNA sorting, mRNA targeting, urea cycle, enzyme organization, cell organization, electron microscopy, digoxigenin