C15

Survey of Target Proteins of Nucleoredoxin

Yeong-Man Yi* and Sa-Ouk Kang

Laboratory of Biophysics, School of Biological Sciences, Seoul National University

Nucleoredoxin (NRX) is a 435-amino-acid redox protein with similarity to TRX but with a -Trp-Cys-Pro-Pro-Cys- catalytic site (instead of -Trp-Cys-Gly-Pro-Cys-). It has been cloned from a mouse YAC library and localized to the nucleus

In this study, amino acid sequences of rat and human NRX were determined by RT-PCR and genomic PCR. The expression level of *NRX* transcript was shown in proliferating CG-4 (central glial) cell and differentiated cell. It appeared at all the stage. Recombinant NRX protein had insulin disulfide reducing activity. Sulfhydryl group modifications with iodoacetic acid showed that the N-terminal cysteine in the active site is exposed and seems to be reactive while C-terminal cysteine is buried as the same case with TRX or DsbA. To capture the target proteins of NRX, wild type NRX and a mutant in which cysteine substituted to serine was immobilized on the resin. After the resins were incubated with cell extract, eluted proteins with excess DTT were separated on 2-D gel. Comparing the spots of eluted proteins from the resin of wild type NRX with those from C208S mutant, two more spots from mutant protein were apparent. MALDI mass spectra of each protein digested with trypsin were acquired and the peaks were submitted for data base search. A candidate of one spot was TRX-like U5 SnRNP protein and another was serine threonine protein phosphatase V (rat PPV).