# Geene Structures and Molecular Interactions of the Human 52-kD and 60- kD SS-A/Ro Auroantigeens

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Autoantibodies to SS-A/Ro are among the most common autoimmune antibodies in systemic rheumatic diseases such as Sjogren's Syndrome and systemic lupus erythematosus. Two distinct cellular proteins of 52 and 60 kD are recognized by the human SS-A/Ro autoantibodies. The 52-kD protein belongs to a small protein family with an N-terminal zinc-finger domain (RING finger), two center coiled-coil domains, and a C-terminal domain that is highly similar to that of the proto-oncoprotein rfp. In contrast, thee 60-kD protein belongs to a family of RNA-binding proteins and can bind directly to small cytoplasmic RNAs known as hY-RNAs. From our cDNA analysis and genomic DNA cloning, alternatively spliced mRNAs for both 52 and 60-kD proteins have been detected. The complete gene for the full -length 52-kD protein spans 10kb with total of seven exons. A previously identified cDNA 52 $\beta$ with deletion of one of the two coiled-coils can now be accounted for as a product of alternative mRNA splicing omitting exon 4 (leucine zipper). For the 60-kD autoantigen, the gene spans>30kb with a total of 10 exons. Alternative mRNA splicing is also responsible for the expression of two 60-kD proteins ( $\alpha$ and $\beta$ -type) differing only at the C-terminal residues. Studies in the gene expression of SS - A/Ro proteins may provide insights in the regulation and expression of different spliced products that may participate in the autoimmune responses.

Since anti-SS-A/Ro sera often recognize both 52 and 60-kD proteins, it is postulated that the two proteins are associated and the protein complex is the in vivo immunogen. To study of the interaction of these proteins in vivo, cDNAs for both 52 and 60-kD SS-A/Ro and another related autoantigen SS-B/La were subcloned into a eukaryotic expression vector driven by a CMV promoter. HEp-2 cells were transfected with a DNA construct and then examined the next day by indirect immunofluorescence using specific antibodies. Transient transfection with the SS-B/La construct yielded moderate overexpression of SS-B/La detected in the nucleus and cytoplasm as would be expected from its normal distribution. In con-

trast, overexpression of the predominant form of 52-kD SS-A/Ro (52 $\alpha$ ) or 60-kD SS-A/Ro (60 $\alpha$ ) showed aberrant localization. Normal subcellular location for the 52 $\alpha$ is almost exclusively in the nucleus while the transfection of 'native' 52 $\alpha$ construct alone (non-fusion protein) showed an intense accumulation in the cytoplasm as if overexpressed proteins lacked sufficient 'carriers' for transport into the nucleus and were trapped in the cytoplasm. Although the normal subcellular location for the 60-kD SS-A/Ro is also predominantly in the nucleus (like 52-kD SS -A/Ro), transfection of 'native' 60 $\alpha$ construct yielded intense staining of the nucleolus suggesting that 60 $\alpha$ can readily cross the nuclear and nucleolar boundary. The aberrant localization of overexpressed 60 $\alpha$ in the nucleolus was 'rescued' back to the 'normal' nuclear/cytoplasmic compartment in co-transfection experiments with both 52 $\alpha$ and 60 $\alpha$ plasmids. Cotransfection of constructs for 60 $\alpha$ and 52 $\beta$ did not rescue 60 $\alpha$ from the nucleolus suggesting that the leucine zipper of the 52-kD protein is important for interaction with 60 $\alpha$ . Cotransfection of 60 $\alpha$ and SS-B/La also did not affect the nucleolar accumulation of 60 $\alpha$ . These data strongly suggest that there are functional associations of the two abundant forms of SS-A/Ro proteins possibly via the leucine zipper region of the 52-kD protein. The in vivo association of 60 $\alpha$ and 52 $\alpha$ supports the Particle hypothesis that autoantibody response may be directed to a complex of the two proteins and may explain the co-expression of human autoantibodies to both antigens in most autoimune sera with anti-SS-A/Ro specificity.