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To bind or not to bind: Characterization of binding interactions between X29 and U8snoRNA

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U8-snoRNP is involved in the processing of the 5.8S and the 28S rRNA, both of which are needed for the formation of the large ribosomal subunit (Peculis and Steitz, 1991). A nucleolar protein, dubbed X29, has the ability to bind and decap U8snoRNA, giving it the capacity to degrade U8RNA (Tomasevic and Peculis, 1999; Ghosh et al, 2004). Initially found in *Xenopus*, X29 is evolutionarily conserved in vertebrates from humans to sea squirts (MT, unpublished). The x-ray crystallography structure of X29 shows the protein can exist in the form of a homodimer (Scarsdale et al, 2006). The goal of this project was to determine whether the homodimer form or the monomeric version of X29 binds U8RNA and is catalytically active, as well as identify the protein:protein and protein:RNA contacts. I used chemical crosslinkers and identified a 60kD putative crosslink in X29, and formation of a 60kD band was also identified with the human homologue, H29K, but this forms with a lower efficiency. 4-thio-U mediated RNA (UV) crosslinking was used to identify the binding sites for X29 on U8RNA. All crosslinking assays were performed with mutant or truncated RNAs and proteins to more precisely map sites of interaction. The data from X29 were compared to that of H29K to determine whether the protein's activities were conserved among or differed between species. The results of these experiments showed that X29 and H29K both show abilities to form dimers and crosslink to U8 though to differing efficiencies. The U8 mutants have identified putative interaction sites between U8RNA and the proteins, which we are in the process of mapping more precisely.