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The Role of Zinc in Transcriptional Control of Xenopus 5S RNA Gene

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Zinc is essential to nucleic acid synthesis and function. While the universal presence of Zn in enzymes which bind DNA or RNA as template or substrate has been recognized, its participation in the function of a regulatory protein involved in nucleic acid synthesis is not known.

Transcription of Xenopus 5S RNA genes is dependent upon an intragenic DNA sequence of approximately 50 base pairs. A Xenopus transcription regulatory protein, factor A, has been found to interact specifically with this intragenic control region and direct accurate initiation of 5S RNA transcription by RNA polymerase III. Factor A also binds 5S RNA in Xenopus immature oocyte in the form of a 1:1 stable 7S particle, suggesting an autoregulatory mechanism for the transcription.

Analysis of highly purified preparations of the 7S particle by atomic absorption spectrometry led us to the discovery that it contains two moles of tightly bound Zn ions per mole of particle. The refractory nature of the Zn ions upon extensive dialysis against EDTA or 1,10-phenanthroline suggests that they are an integral part of the particle. However, Zn ions can be readily removed by the metal chelators after the particle has been treated with RNase to liberate factor A. Thus, the Zn ions in the 7S particle are most likely located at the contact domain between 5S RNA and factor A. Alternatively, they may be buried in factor A and become exposed to the chelator due to a conformational change of the protein following removal of 5S RNA from the particle.

Evidence that Zn is involved in the specific binding of factor A to the 5S RNA gene comes from inhibition studies with metal chelators. Both EDTA and 1,10-phenanthroline inhibit the specific binding as determined by the DNase I footprinting method. The concentrations required for a complete inhibition are 2 m*M* for EDTA and 0.15 m*M* for 1,10-phenanthroline. Inhibition of the specific binding of factor A to the 5S RNA gene is the result of Zn chelation from factor A by metal chelators. A direct proof for this conclusion is provided by the observation that the specific binding ability of EDTA-treated factor A can be restored by the addition of exogenous Zn ions (15 μM) prior to the footprinting reaction. In contrast to the specific binding, EDTA and 1,10-phenanthroline do not inhibit nonspecific binding of factor A to DNA as measured by nitrocellulose filter binding assay. These differential effects suggest that Zn may play a more specific role in the binding of factor A to the 5S RNA gene (*e.g.*, recognition of nucleotide bases in the control region) than simply providing a charge bridge between protein and DNA.

In an *in vitro* transcription system which synthesizes 5S RNA as well as tRNA, both syntheses are inhibited by high concentrations (>1 m*M*) of 1,10-phenanthroline, possibly reflecting the necessity of Zn in transcription reactions in general. An interesting finding is that at a low concentration (0.25 m*M*), 1,10-phenanthroline completely inhibits 5S RNA synthesis whereas tRNA synthesis is scarcely affected. Since factor A is the only addition necessary for 5S RNA synthesis in this system, the specific inhibition on 5S RNA synthesis is likely due to the failure of factor A binding to the 5S RNA gene via Zn chelation by 1,10-phenanthroline.

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The Catalytically Competent Coordination Environment of the Active Site Metal Ion of Liver Alcohol Dehydrogenase

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The coordination environment of the active site metal ion of horse liver alcohol dehydrogenase is investigated by EPR and steady-state kinetic methods with use of the native (ZnLADH) and the active site specific Co^{2+}-reconstituted enzyme (CoLADH) described by Zeppezauer and coworkers [1]. The pH dependence of kinetic parameters for the oxidation of benzylalcohol reveals two ionizations ($pK_1 \sim 6.7$; $pK_2 \sim 10.6$) that govern k_{cat} and belong to the ternary enzyme-NAD^+-alcohol complex and two ionizations ($pK'_1 \sim 7.5$; $pK'_2 \sim 8.9$) that govern k_{cat}/K_m and belong to the binary enzyme-NAD^+ complex. Only pK_2 and pK'_2 are substantially influenced by metal substitution. Comparable results are observed for the oxidation of isopropanol. We attribute these ionizations to metal-bound water that occur separately at the ternary and binary complex level in the course of alcohol oxidation.

In parallel studies from this laboratory [2, 3], we have demonstrated that the magnitude of the zero-field splitting (ZFS) of the high-spin Co^{2+} ion falls into three ranges according to coordination number: 0–13 cm^{-1} for tetracoordinate; 20–60 cm^{-1} for penta-coordinate; and 90–310 cm^{-1} for hexa-coordinate environments. We have determined the ZFS