

## Activation of the rainbow trout metallothionein-A promoter by silver and zinc.

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### Abstract

In fish, the synthesis of metallothionein (MT) is increased by a number of heavy metals. The rainbow trout MT-A gene promoter region contains six known metal responsive elements (MREs), that mediate promoter activation by metals. In the present study, two fish cell lines differing in their ability to produce MT, RTG-2 (produce MT protein) and CHSE-214 (produce no detectable MT protein), were used to help elucidate the roles of Zn, Ag and MT in the activation of the MT promoter. The hypothesis tested was that Ag activates the MT-A promoter indirectly by displacing Zn from pre-existing Zn-MT and that this liberated Zn subsequently induces MT synthesis. Both cell lines were transfected with a luciferase reporter gene construct containing the rainbow trout MT-A promoter, exposed to various concentrations of Zn or Ag, and assayed for luciferase activity. CHSE-214 cells showed five times greater production of luciferase than RTG-2 cells when exposed to identical concentrations of Ag. Thus, Ag can likely induce MT transcription without displacing Zn from pre-existing Zn-MT. Furthermore, Ag activated the MT promoter at concentrations 100-fold lower than those required for Zn to initiate transcription, suggesting that zinc displaced from other sites by such low concentrations of Ag would not be sufficient to initiate MT transcription. This interpretation was further supported by radiotracer studies indicating that Ag did not cause a redistribution of <sup>65</sup>Zn within either of

the two cell types. These combined results indicate that Ag may be a direct inducer of MT.

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