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Role of Essential Metal Ions in AAV Vector Biology

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Metal elements are essential components of approximately half of all cellular proteins, and approximately one-third of all known enzymes thus far are metallo-enzymes. Although a number of cellular proteins and enzymes undoubtedly impact the transduction efficiency of recombinant AAV vectors, the precise role of metal ions in this process has not been studied to any significant extent. We have initiated a systematic study in which we have evaluated the role of all essential metal ions on the transduction efficiency of AAV vectors. In the initial set of experiments, HeLa cells were transduced in triplicates with scAAV2-EGFP vectors at an MOI of 200 vgs/cell, followed by addition of various concentrations of magnesium chloride, zinc chloride, cobalt chloride, and nickel chloride. Transgene expression was determined 48 hrs post-transduction using fluorescent microscopy, and images were analyzed using the ImageJ software. The results showed a dose-dependent increase in transgene expression, with maximal increase with 100 μg/ml of magnesium chloride (~2-fold), with 30 μg/ml of zinc chloride (~8-fold), with 25 μg/ml of cobalt chloride (~2-fold), and 20 μg/ml of nickel chloride (~3-fold). Interestingly, when cells were treated with a combination of zinc chloride with any one of the other metal ions, an increase in the transduction efficiency was observed to be more than additive. The following three combinations (zinc chloride + cobalt chloride, zinc chloride + nickel chloride, and zinc chloride + magnesium chloride) led to ~15-fold increase in transgene expression. Similar levels of augmentation in the transduction efficiency by these combinations were observed with AAV1, AAV3, AAV4, AAV5, and AAV6 serotype vectors. Studies are currently underway to evaluate the role of all essential metal ions and their various permutaions and combinations in primary human cells *in vitro* and in a murine model *in vivo*. Our results suggest that this simple strategy of essential metal ions-mediated enhancement may have important implications in the optimal use of AAV serotype vectors in human gene therapy.