Investigation of Ing4 on Developmental Hematopoiesis

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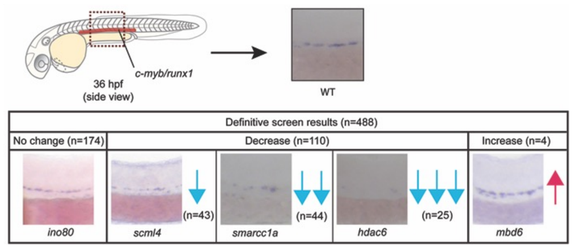
Abstract

During development, hematopoietic stem cells (HSCs) are specified from the hemogenic endothelium. Through this process, HSCs self-renew, proliferate and differentiate to form mature blood cells. Regulation of this process is in part achieved by factors that orchestrate chromatin structure and establish an epigenetic code for hematopoiesis. One of these such factors is the Inhibition of Growth 4 (Ing4) gene, which contains chromatin remodeling complex activity and codes for a tumor suppressor protein. This protein interacts with specific complexes, such as the protein complex Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), to regulate the immune response and encourage proper development. Consequently, it has been shown that inhibition of the Ing4 gene affects the hemogenic endothelium and the production of HSCs. The extent to which the hemogenic endothelium was affected was evaluated via the performance of *in situ* Hybridization of zebrafish embryos to determine the rate of expression of specific factors. These factors include the *fli*1 gene which is involved in vessel formation, the *eph2b* gene which assists in artery specification, and the *scl* gene which plays a role in HSC specification. The results of this experiment suggest that via the inhibition of Ing4 there is a decrease in the expression of the *fli*1 gene and in the specification of HSCs, but artery specification is not necessarily affected by this process. This hints that vessel formation could be affected by Ing4 inhibition due to the harmful residual effects on the hemogenic endothelium. Future experimentation will examine the interactions between NF-κB and Ing4 in a drug screening process and attempt to rescue HSC production in Ing4 morphant zebrafish.

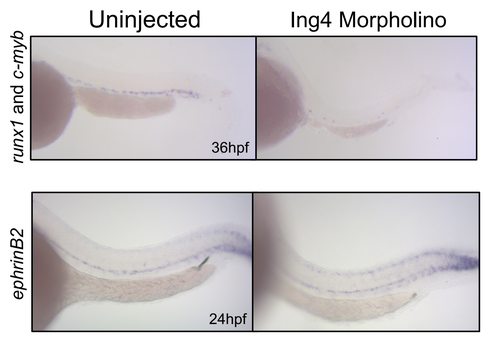
Background Information

Chromatin Factor Screen Summary



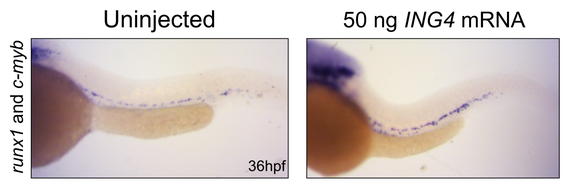
36hpf embryos, staining for *c-myb* and *runx1*

Loss of Ing4 does not alter artery specification



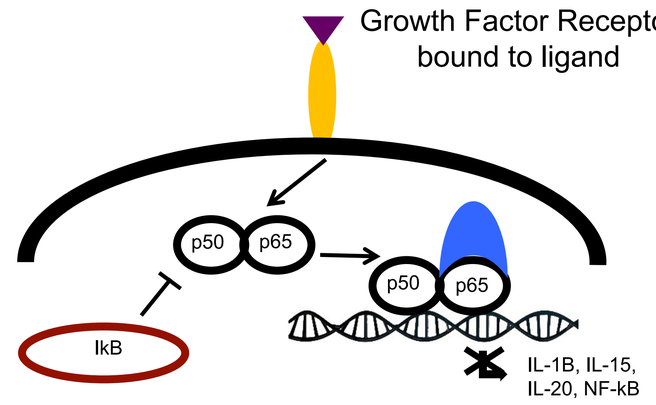
Embryos were injected with Ing4 morpholino and stained for either *c-myb* and *runx1* expression at 36hpf or ephrinB2 at 24hpf by *in situ* hybridization.

Overexpression of Ing4 causes an increase in HSPCs

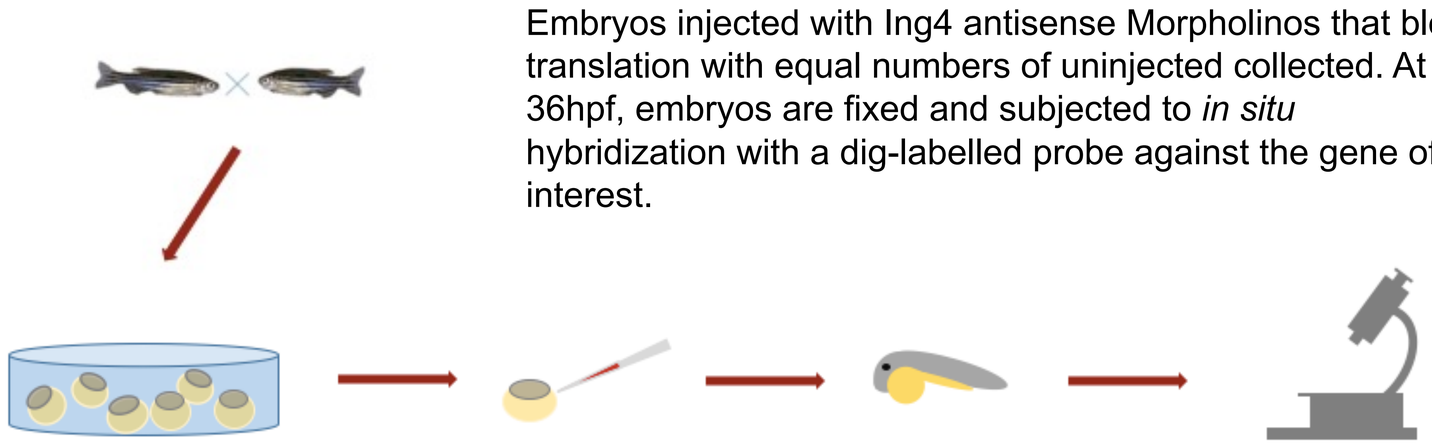


Embryos were injected with human ING4 mRNA and stained for *c-myb* and *runx1* expression at 36hpf *in situ* hybridization.

Ing4 negatively regulates NF-kB (p50/p56)

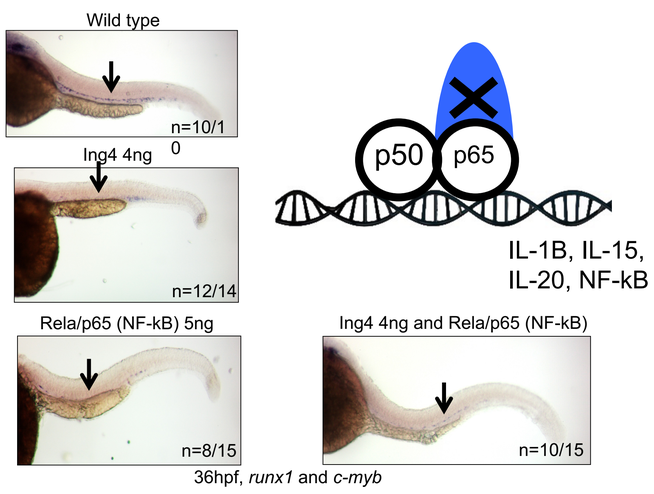


Methodology



Embryos injected with Ing4 antisense Morpholinos that block translation with equal numbers of uninjected collected. At 36hpf, embryos are fixed and subjected to *in situ* hybridization with a dig-labelled probe against the gene of interest.

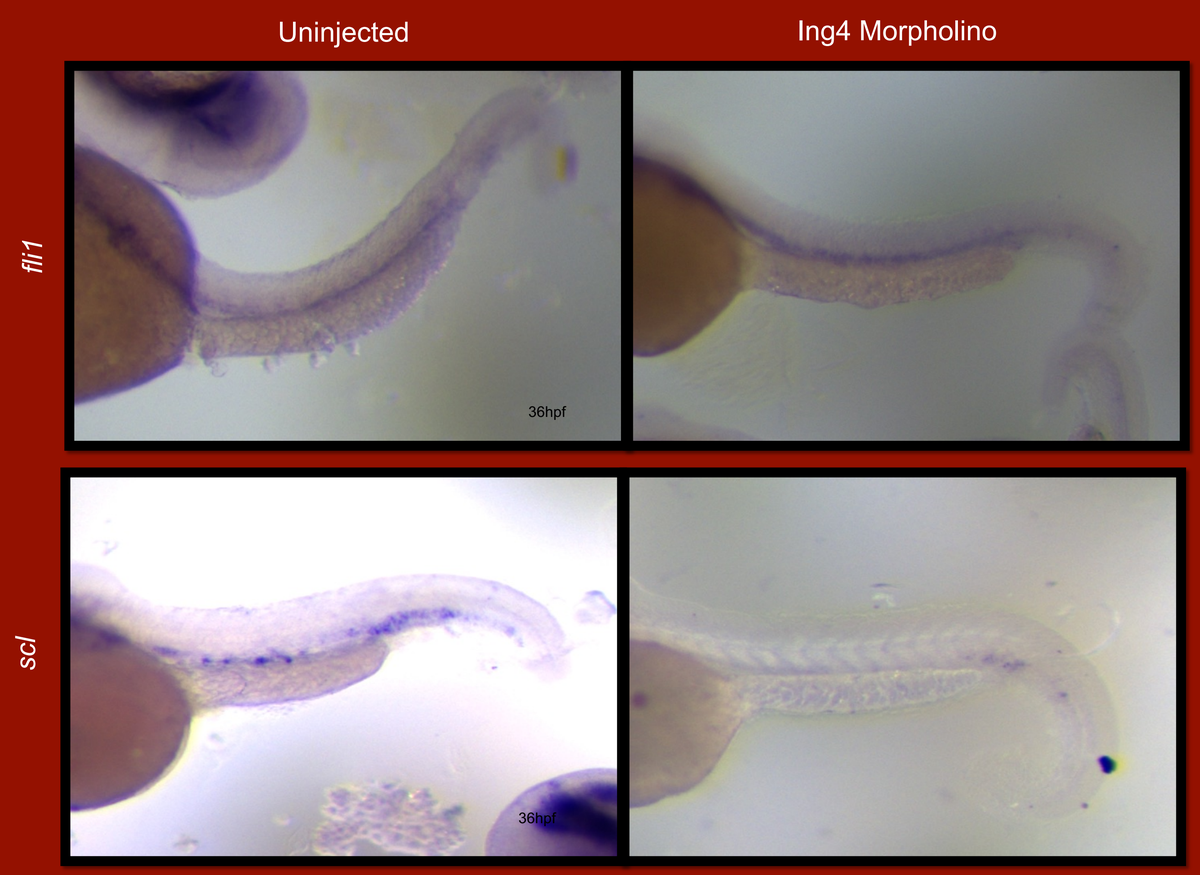
Loss of NF-kB rescues HSPCs in Ing4 morphants



36hpf, *runx1* and *c-myb*

Results

Ing4 deficiency results in a decrease in HSC formation and the knockdown of *fli*1 expression



Embryos were injected with Ing4 morpholino and stained for either *fli*1 at 36hpf or *scl* expression at 36hpf by *in situ* hybridization.