

## Text S1

### *All or none regulation of gene expression from distant enhancers*

We propose that the Scl, Gata2 and Fli1 regulate gene expression by ratcheting the equilibrium between open (promoter site accessible) and closed (promoter site inaccessible) chromatin conformations. This dynamic equilibrium of wrapped and unwrapped nucleosomal DNA has also been discussed elsewhere [1]. Under our hypothesis the binding of TRs to the enhancer site increases gene expression by stabilizing the open conformation and thereby shifting the equilibrium towards it. This mechanism therefore allows the TRs to ratchet the spontaneous unwrapping of nucleosomal DNA and trap it in a state accessible to the transcriptional machinery. We illustrate this mechanism of the regulation of *Scl* expression by Gata2 and Fli1 in Figure S2. According to this mechanism binding of TRs only modulates the probability of gene expression and not the rate of transcription. Therefore, mutations of binding sites in the enhancers would affect the number of cells expressing the gene but the level of expression would be unaffected. To experimentally verify this all-or-none type of gene regulation embryonic stem cells containing the *Scl+19* enhancer-reporter constructs in Figure 1 were analyzed for reporter expression with flow cytometry (Figure S1A). Mutation of the binding sites significantly reduces the fraction of cells expressing  $\beta$ -galactosidase. However the mean  $\beta$ -galactosidase expression among cells that do show expression did not change thereby confirming our hypothesis about the mechanism of gene regulation by TR binding at distant enhancers. The bimodality of the data is obscured by the presence of several different cell types in the culture of ES cells. To highlight the all-or-none nature of gene regulation by the triad enhancers a similar experiment was carried out in 416B myeloid progenitor cells that were transfected with either SV/ $\beta$ -geo or SV/ $\beta$ -geo/*Scl+19* reporter constructs. Figure S2B shows that the inclusion of the *Scl+19* enhancer significantly enhances the number of cells expressing lacZ while the level of lacZ expression is unaffected. These results confirm our hypothesis about the all-or-none nature of gene regulation by enhancers within the Scl/Gata2/Fli1 triad.

### *Experimental Methods*

The wild type and mutated *Scl+19* enhancer elements previously described in Pimanda et al [2] and shown in Figure 1 were cloned downstream of an SV40LacZ reporter construct and inserted as a Not I blunt fragment into the HPRT targeting vector pMP8NEB $\Delta$ LacZ (a kind gift from Stephen Duncan) [3]. HPRT targeted ES cells were generated as previously described [4] using the HM-1 ES cell line [5] and differentiated in 90mm Petri dishes in IMDM supplemented with 15% FCS, 2mM L-Glutamine, 300ug/ml Transferrin, 4x10<sup>-4</sup>M MTG, 50ug/ml Ascorbic acid and 5% PFHM-II. At day 5 of differentiation embryoid bodies were disrupted using trypsin and the single-cell suspension was analysed for  $\beta$ -galactosidase activity using FDG (fluorescein di- $\beta$ -D-galactoside) [6]. Briefly, cells were incubated with 1mM FDG at 37°C for 1 min followed by the addition of 1ml ice cold PBS. The cells were then incubated on ice for 10 mins and the

reaction stopped using 1mM phenylethyl- $\beta$ -D-thiogalactoside. The fluorescent signal was then analysed on the FACS Calibur (Becton and Dickinson) in the FL-1 channel. The *Gata2-3* and *Fli1+12* enhancers have been characterized in [2]. The results for reporter expression from wild type and mutated enhancer elements for all three triad enhancers are shown in Table S1.

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