A Sox2-Fos-Socs3 gene regulatory network controls the self-renewal of Neural Stem Cells

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Abstract: The Sox2 gene encodes a transcription factor active in neural stem/progenitor cells (NSCs) in the developing vertebrate central nervous system (CNS). We previously demonstrated that Sox2 is important in the maintenance of NSCs self-renewal, in long-term *in vitro* cultures derived from P0 mouse forebrain, as well as *in vivo* (e.g. in hippocampus) (Favaro et al. 2009, PMID: 19734891; Bertolini et al. 2019 PMID: PMC6506828, Jagga et al. 2020, Nat. Commun. in press). In *in vitro* cultures, Sox2-ablated NSCs self-renew for several passages like the wild-type ones, but then undergo progressive exhaustion. We found that, upon differentiation, they also generate reduced numbers of neurons, with reduced arborization. Sox2 can regulate its targets by controlling long-range interactions between genes and distal enhancers, which regulate gene expression; indeed, many of these interactions are lost in Sox2-mutant cells. By RNAseq, we identified genes that are downregulated following Sox2 ablation. The most downregulated genes in mutant include Socs3 (Suppressor Of Cytokine Signalling 3) and key regulators of cell proliferation, like c-Fos, Jun and Egr2. Their overexpression into Sox2-deleted cells via lentiviral vectors rescues the ability of mutant cells to grow long-term, and partially rescues the neuronal differentiation defect. Fos alone rescues long-term proliferation and is required to maintain NSCs, as we observed reduction of NSC clones capable of long-term expansion following CRISPR/Cas9-mediated Fos inactivation, in the presence of wild-type Sox2. Further, pharmacological inhibition of the binding of the AP1 (FOS/JUN) complex to its targets reduced cell proliferation and expression of the Socs3 gene. Sox2 overexpression in Sox2-deleted cells reactivates Fos and Socs3 expression and also Fos overexpression reactivates Socs3 expression; the Socs3 promoter is directly bound by SOX2, FOS and JUN. These results demonstrated that there is a network of interactions between Sox2, Fos and Socs3, important for NSC proliferation and long-term maintenance and neural differentiation.