The role of REST/NRSF in adult neurogenesis

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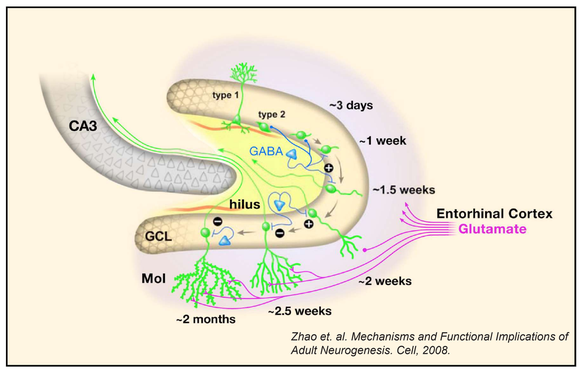
Introduction

REST/NRSF is a transcription factor traditionally known to act as a repressor of neuronal development. However, the role of REST is continually expanding, with studies now linking it to cancer, epilepsy, and Huntington's disease. Despite its role as a neuronal repressor, we and others have shown that REST is present in neural stem cells. Because most of these studies have only been performed in embryonic neural progenitor cells, we sought to ascertain whether there is a role for REST in hippocampal adult neurogenesis.

To best elucidate the presence of REST, we included both in vitro and in vivo approaches in our study. Our *in vitro* model utilized neural progenitor cells (NPCs) harvested from the adult rat hippocampus. REST was observed in adult hippocampal progenitor cells by immunofluorescence and we confirmed its presence with both Western blot and qRT-PCR. We then examined REST *in vivo* in C57BL/6 mice. We found REST to be expressed at significant levels in all areas of the hippocampus, including the dentate gyrus, CA1, CA2, and CA3.

Next, we examined a potential role for REST in the adult hippocampus. We are studying REST gain of function/loss of function in adult neurogenesis both in vitro and in vivo using retroviral and lentiviral delivery. Our constructs show over 80% knockdown *in vitro*, and preliminary results suggest that they function efficiently *in vivo* as well.



Results

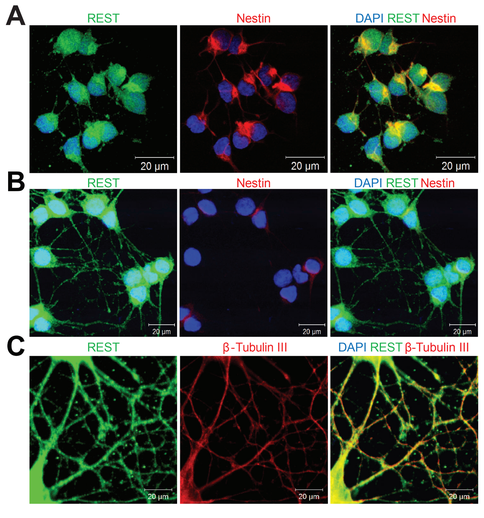


Figure 1 Rest is present in the cytoplasm of neural stem cells. (**A**) Immunofluorescent staining of Adult Hippocampal Progenitors (AHPs) cultured in FGF express REST. (**B**) AHPs differentiated in RA and FSK express REST after 6.5 days. (**C**) REST co-localizes with β-Tubulin III in AHPs differentiated in RA and FSK for 6.5 days. All stainings are as indicated.

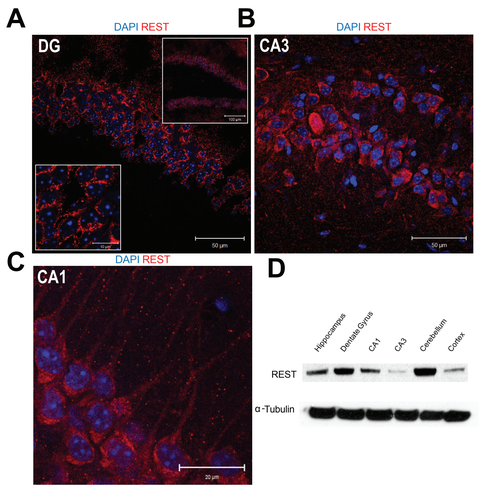


Figure 2 REST is present in the adult mouse hippocampus. (**A-C**) Immunohistochemistical staining of REST in (a) the dentate gyrus, (b) CA3, and (c) CA1. Stainings and regions are indicated. (**D**) Western blot of REST in various brain regions.

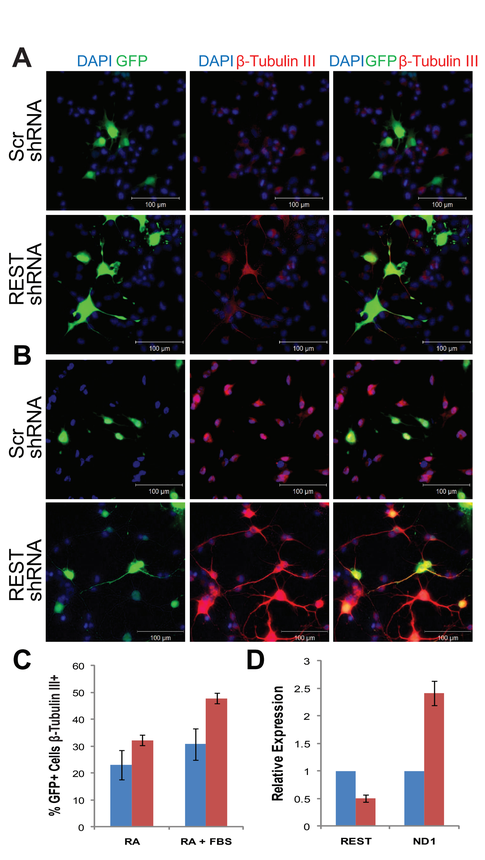


Figure 3 REST knockdown results in increased differentiation of AHPs in vitro. (**A-B**) Immunofluorescent staining of AHPs cultured in (a) FGF and (b) RA for four days and electroporated with scr shRNA and REST shRNA. (**C**) REST knockdown results in an increased percentage of GFP+/β-Tubulin III+ cells. (**D**) REST knockdown shows decreased levels of REST and increased levels of NeuroD1 by qRTPCR. Blue = control; red = shREST.

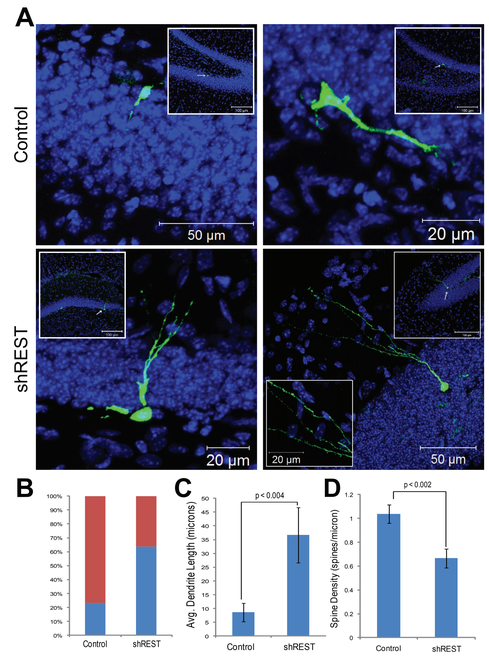


Figure 4 REST knockdown results in increased differentiation in vivo. (**A**) Representative images of control and shREST neurons 7 dpi. (**B**) Percent of neurons with dendrites for control (n = 30) and shREST tissue (n = 22) 7 dpi. Blue = % with dendrites; red = % without dendrites. (**C**) Average length of dendritic processes. (**D**) REST knockdown results in a statistically significant decrease in spine density in shREST (n=24) vs. control tissue (n=26).

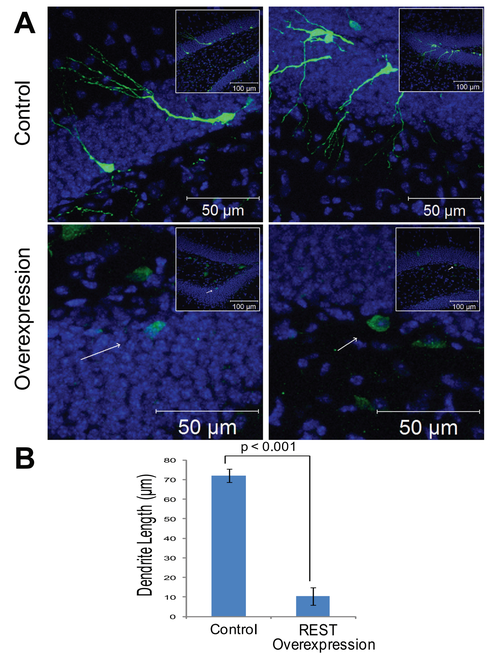


Figure 5 REST Overexpression results in decreased differentiation of AHPs in vitro and of newborn neurons in vivo. (**A**) REST overexpression results in decreased differentiation in vivo 14 dpi. (**B**) REST overexpression results in a statistically significant decrease in neurite length in control (n=33) vs. REST overexpression tissue (n=19).

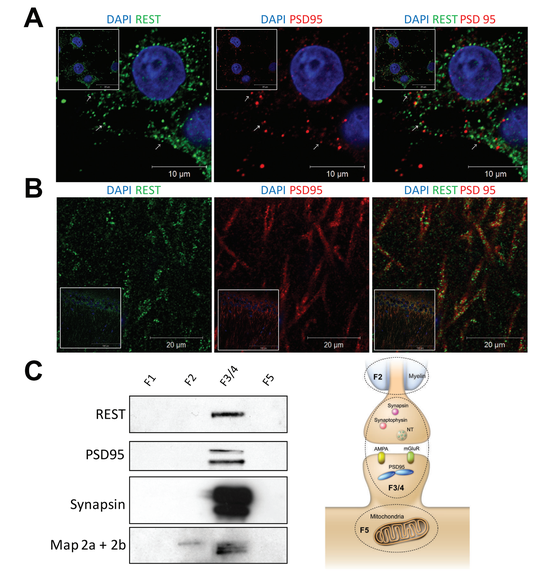


Figure 6 REST is Present in the Synapse. (**A**) REST co-localizes with PSD95 in AHPs differentiated in RA and FSK for 6.5 days. Stainings are as indicated. (**B**) REST colocalizes with PSD95 in the CA1 region of the hippocampus in vivo. (**C**) Synaptasome preparation from mouse hippocampi. Synaptasomes are in F3/4.

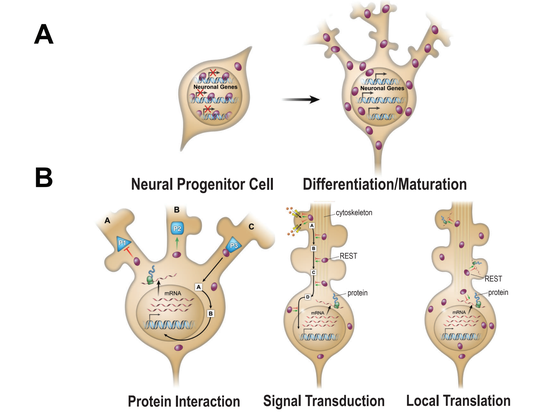


Figure 7 Possible mechanisms for a positive role for REST in adult hippocampal neurogenesis. (a) Decreases in nuclear REST levels as well as increases in cytoplasmic REST levels aid in neuronal differenation. (b) REST can aid neuronal differentiation via several potential mechanisms.

Conclusion

- REST is present in both the cytoplasm and nucleus of neural stem cells and differentiated neurons.
- REST knockdown in vitro is sufficient to induce neuronal differentiation, even in proliferating conditions.
- REST knockdown in vivo results in faster early differentiation but retardation indicated by decreased spine density at later stages.
- REST is present in the synapse of mature neurons.
- REST acts as a novel, positive, and possibly cytoplasmic mediator of adult neurogenesis beyond its traditional role as a negative regulator of neuronal development.

Acknowledgments

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