Activity-dependent transcriptional dynamics in mouse primary cortical and human iPS - derived neurons

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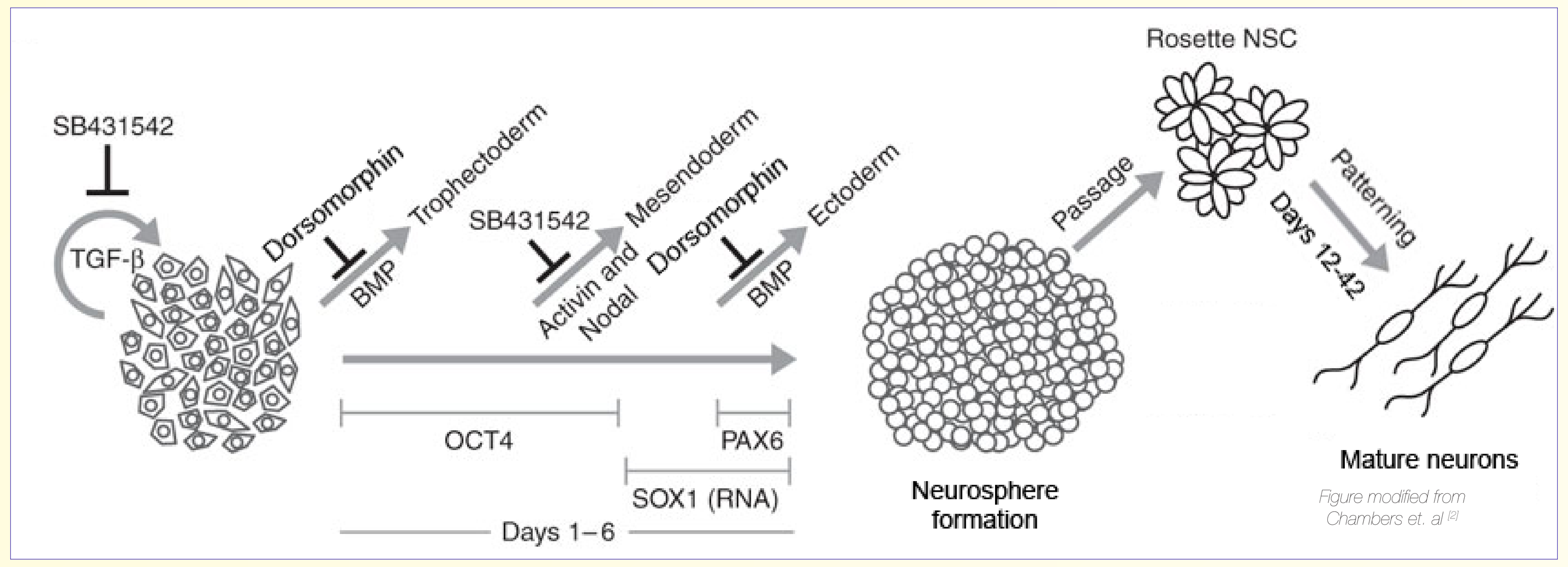
Background

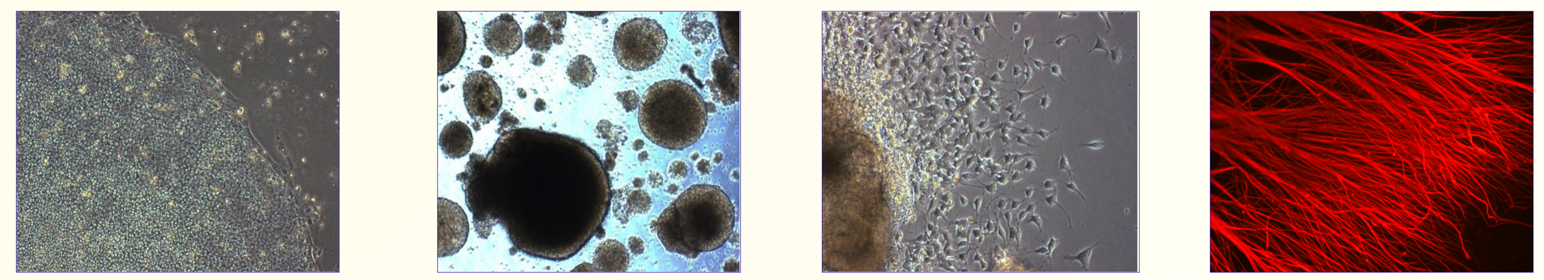
A handful of non-coding RNA have been found to be involved in various aspects of nervous system function: maintenance of pluripotency, lineage specification, neurogenesis in the embryo and adult, and higher cognitive functions, including memory formation[1]. However, the global extent of transcription in response to neuronal activation remains unknown.

Objectives

To characterize changes in the coding and non-coding RNA in the cell that occur in response to neuronal activity, including differential gene expression analysis, alternative splicing and RNA editing

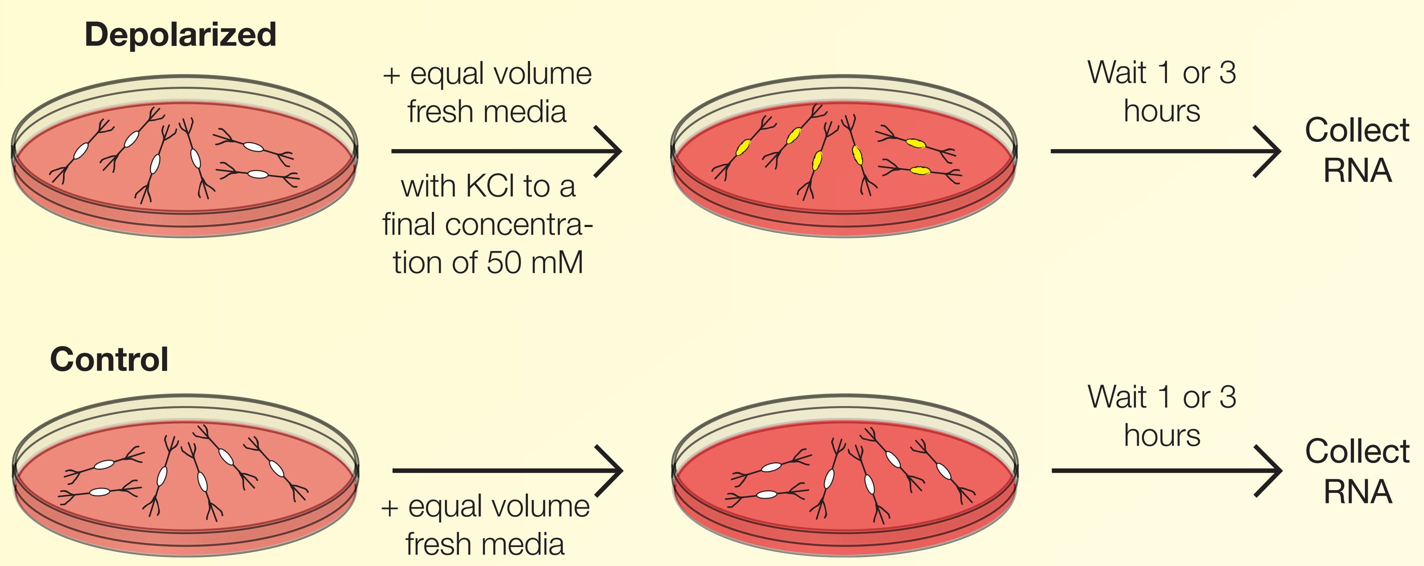
iPS-derived neuronal model system



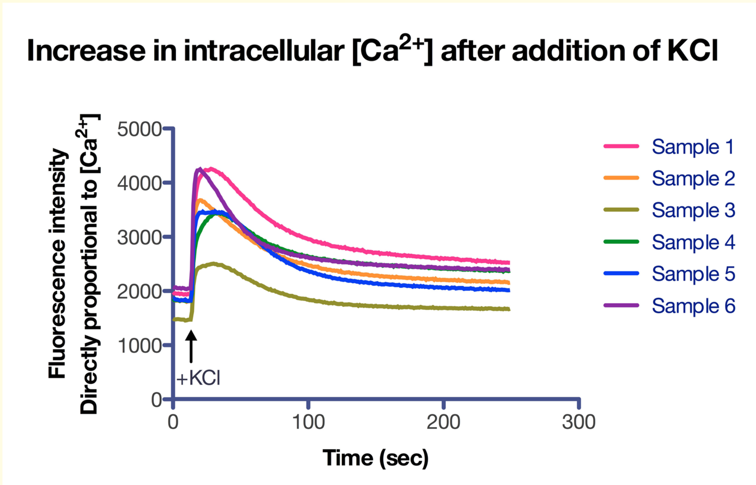


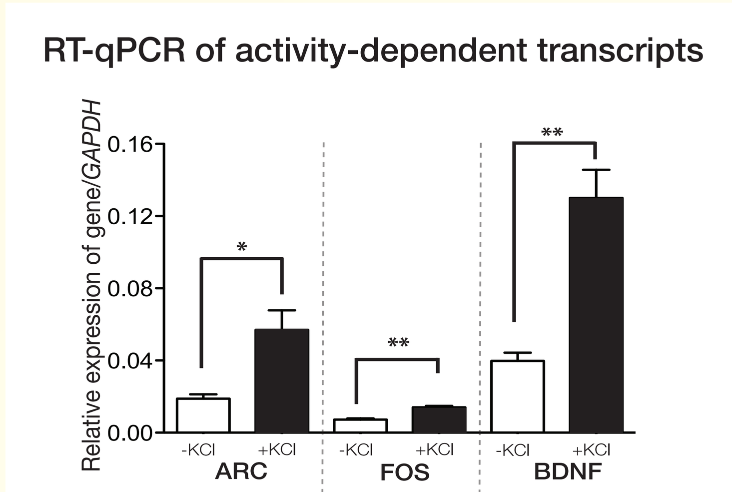
Neurons were differentiated from iPS cells using a modification of the 2-dimensional neural differentiation protocol developed in the Studer laboratory[2], based on dual SMAD inhibition using Noggin and SB431542, which mimics the *in vivo* transition of undifferentiated hESCs to FGF5+ epiblast-like cells through to PAX6+ cells competent of neural rosette formation. We have adapted this protocol by replacing Noggin with the small molecule dorsomorphin and routinely obtain greater than 90% PAX6+ cells from iPS cells within 6 days. These neuron progenitors are then propagated for a further four weeks.

Methodological overview



1. Human iPS-derived neurons show normal activity responses

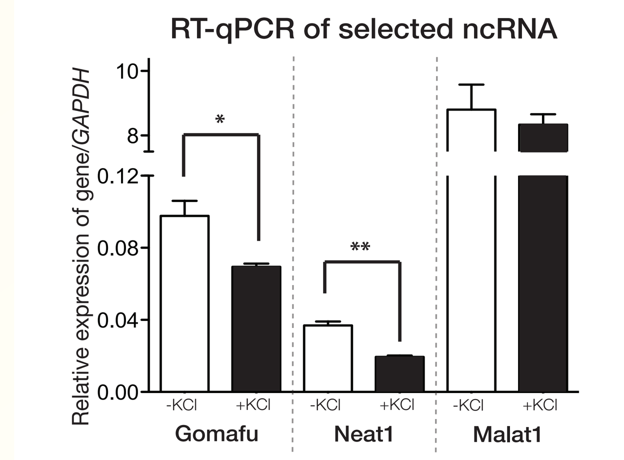




iPS-derived neurons may express markers characteristic of mature nerve cells, but may not be physiologically active, for example as a result of lack of assembled receptor complexes at the synapse. We used the FLIPR Tetra system to assess changes in cytoplasmic Ca^{2+} concentration (left), which normally increases dramatically in response to activity, and RT-qPCR to determine expression levels of known activity-dependent transcripts (right).

2. Many ncRNA are expressed in an activity-dependent manner in human iPS-derived neurons

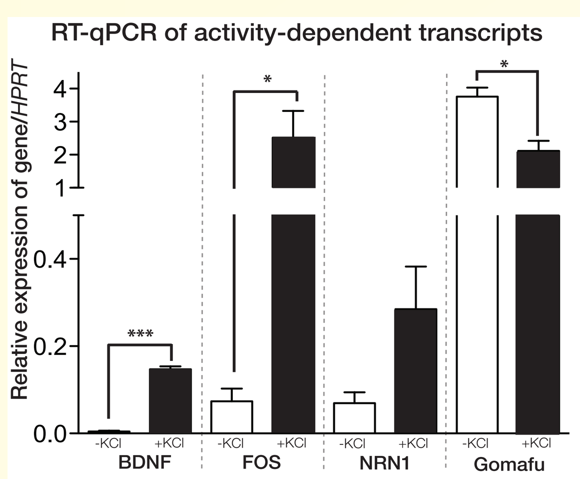
	Coding targets	Non-coding targets
Upregulated after 3 hours KCl treatment	26	16
Downregulated after 3 hours KCl treatment	44	11



RNA from human iPS-derived neurons was hybridized to the human NCode microarray, which contains probes for over 17 000 putative lncRNAs and protein-coding genes. Values in the table represent significantly differentially expressed probes (B-value > 3, log_2-fold change > 0.5 or <-0.5). qPCR validation of two ncRNAs down-regulated in response to activity (Gomafu, Neat1) is shown at right, with levels of the ncRNA Malat1 remaining unchanged.

3. Many ncRNA are expressed in an activity-dependent manner in mouse primary cortical neurons

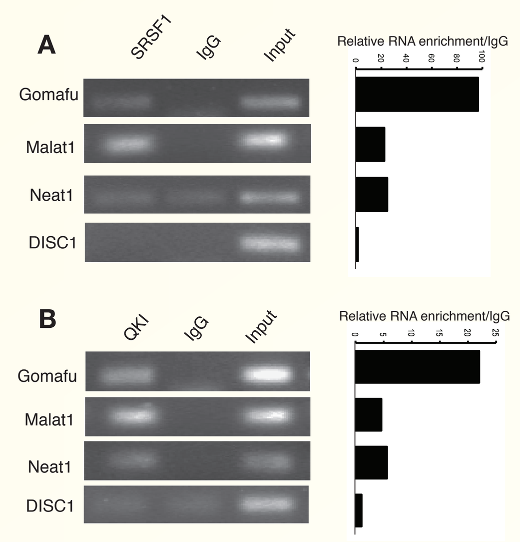
	Coding targets	Non-coding targets
Upregulated after 1 hour KCl treatment	23	13
Downregulated after 1 hour KCl treatment	5	19
Upregulated after 3 hours KCl treatment	148	13
Downregulated after 3 hours KCl treatment	142	15



RNA from mouse primary cortical neurons was hybridized to the mouse NCode microarray, which contains probes for ~10 000 putative lncRNAs and protein-coding genes. Values in the table represent significantly differentially expressed probes (B-value > 3, log_2-fold change > 0.5 or < -0.5).
qPCR validation of selected targets is shown on the right.

4. The activity-dependent ncRNA Gomafu binds directly to multiple splicing factors

Gomafu is a ncRNA expressed predominately in the nervous system, dynamically regulated during retinal development[3], differentiation of neural stem cells into oligodendrocytes[4] and in mouse embryonic stem cell differentiation[5,6]. Gomafu has been shown to bind the splicing factor SF1[7].
We found that Gomafu was down-regulated in response to activity in both the human and mouse model systems, and using RNA immunoprecipitation validated preliminary evidence from the Blackshaw lab[8] which suggested that this ncRNA may bind other splicing factors such as QKI (quaking homolog, KH domain containing) and SRSF1 (serine/arginine-rich splicing factor 1).
The RNA immunoprecipitation experiments shown at right demonstrate that antibodies to human SRSF1 (A) and QKI (B) pull down Gomafu and to a lesser extent Malat1 and Neat1 in extract from the human neuroblastoma-derived SH-SY5Y cell line.



Summary

1. Similar to coding transcripts, a significant number of ncRNA are differentially expressed in response to activity in both mouse primary cortical neurons and in human iPS cell derived neurons.

2. The ncRNA Gomafu is down-regulated as a result of activity, and binds to several splicing factors, including QKI and SRSF1.

Future directions

Having validated that human iPS-derived neurons respond to activity as a normal neuronal population, we plan to use high-throughput sequencing to characterize changes in the human coding and non-coding transcriptome as a result of activity.

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For additional information about the work conducted please follow the link:

References

[1] Salta, E., & De Strooper, B. (2012). Non-coding RNAs with essential roles in neurodegenerative disorders. The Lancet Neurology, 11(2), 189–200, and references therein **[2]** Chambers, S. M., Fasano, C. A., Papapetrou, E. P., Tomishima, M., Sadelain, M., & Studer, L. (2009). Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nature Biotechnology, 27(3), 275–280. **[3]** Blackshaw, S., Harpavat, S., Trimarchi, J., Cai, L., Huang, H., Kuo, W. P., Weber, G., et al. (2004). Genomic Analysis of Mouse Retinal Development. PLoS Biology, 2(9), e247. **[4]** Mercer, T. R., Qureshi, I. A., Gokhan, S., Dinger, M. E., Li, G., Mattick, J. S., & Mehler, M. F. (2010). Long noncoding RNAs in neuronal-glial fate specification and oligodendrocyte lineage maturation. BMC Neuroscience, 11(1), 14. **[5]** Sheik Mohamed, J., Gaughwin, P. M., Lim, B., Robson, P., & Lipovich, L. (2010). Conserved long noncoding RNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells. RNA, 16(2), 324–337. **[6]** Dinger, M. E., Amaral, P. P., Mercer, T. R., Pang, K. C., Bruce, S. J., Gardiner, B. B., Askarian-Amiri, M. E., et al. (2008). Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Genome Research, 18(9), 1433–1445. **[7]** Tsuiji, H., Yoshimoto, R., Hasegawa, Y., Furuno, M., Yoshida, M., & Nakagawa, S. (2011). Competition between a noncoding exon and introns: Gomafu contains tandem UACUAAC repeats and associates with splicing factor-1. Genes to Cells, Competition between exons and introns, 16(5), 479–490. **[8]** S. Blackshaw, E. Poth, The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine - personal communication.