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Identification of protein complexes maintaining Oct4 expression in mouse ES cells

Octamer binding transcription factor-4 (Oct4) is one of the key factors controlling the fate of embryonic stem (ES) cells. Oct4 expression at a specific level is required to maintain the ES cells' capability for self-renewal, i.e. ability to replicate indefinitely without loss of pluripotency. Whereas numerous studies focused on the target genes or direct protein interactors of Oct4, the regulation of Oct4 expression itself is less explored.

Our work aims at finding the genes and protein complexes involved in the regulation of Oct4 expression. The study is based on two independent genome-wide siRNA screens [1, 2] conducted in the mouse ES cell line, Oct4-GiP, which allows to measure the change of Oct4 expression upon siRNA knock-down of query genes.

Direct comparison of the results from both screens at the gene level did not show a statistically significant consistency between the screens. Possible causes of this disagreement include variations in the experimental setup (different siRNA libraries), variability related to high-throughput experiments and drawbacks of siRNA screening methodology (false discoveries resulting e.g. from off-target effects). We reasoned that incorporation of additional orthogonal information in the analysis might remove noise and improve the consistency between screens. We therefore mapped the genes tested in siRNA screens to known protein complexes, assuming that genes participating in the same complex should cause similar phenotypes. To identify complexes enriched with high-scoring genes, we tested several set enrichment methods (hypergeometric test, weighted Kolmogorov-Smirnov statistic, Bayesian network and regularized linear regression). The resulting scoring of protein complexes showed considerably greater consistency between screens than the original gene scores. Subsequently we combined the results from both screens in order to obtain a single set of high-confidence complexes enriched for genes causing Oct4-related phenotypes. Thereby we obtained several complexes with known functions related to cell-cycle or stem cell maintenance. Importantly, these complexes contain many genes that were not identified as significant "hit genes" in the original screens.

The performed analysis reveals that combining results of siRNA screens and adding external data helps to extract more comprehensive information from the experiments. Our analysis identifies a catalogue of protein complexes critically involved in the regulation of Oct4 expression and thus important for ES cells maintenance.

References

[1] L. Ding *et al.*, *A genome-scale RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity* Cell Stem Cell. **4(5)** 403–415.
[2] G. Hu *et al.*, *A genome-wide RNAi screen identifies a new transcriptional module required for self-renewal* Genes Dev. **23(7)** 837–848.