Widespread evasion of posttranscriptional regulation associated with proliferation

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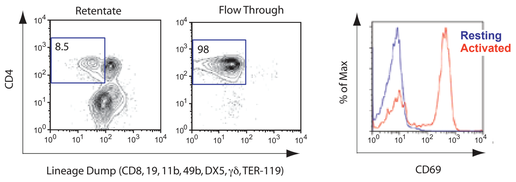
Abstract

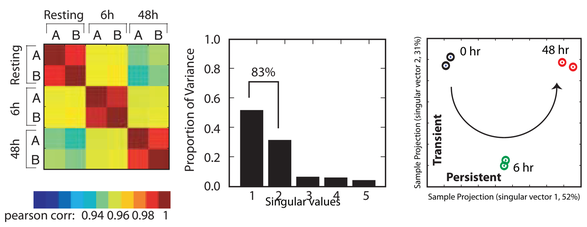
The stability, localization and translation of mammalian mRNAs are largely determined by sequences in the 3' untranslated region (UTR). Here, we describe a conserved program of increased upstream polyadenylation site usage following activation of primary murine CD4$^+$ T lymphocytes. This program, resulting in shorter 3' UTRs, is a characteristic of immune cell activation and strongly correlates with proliferation across diverse cell types and tissues. Enforced expression of full-length 3' UTRs confers differences in protein expression that can in some cases be eliminated by deletion of predicted microRNA target sites in the variably included region. Together, our data indicate that polyadenylation site usage is coordinately regulated such that states of increased proliferation are associated with widespread reduction of 3' UTR-based regulation.

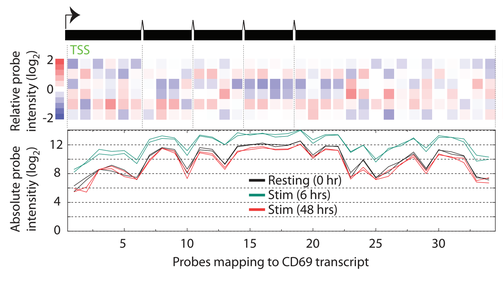
CD4$^+$ T lymphocyte activation



CD4$^+$ T-cells (routinely >90% pure) were isolated from murine lymph nodes and spleens by negative selection. Activation confirmed by assaying CD69 expression via flow cytometry

Biological replicates are highly correlated in direct comparison and SVD analysis

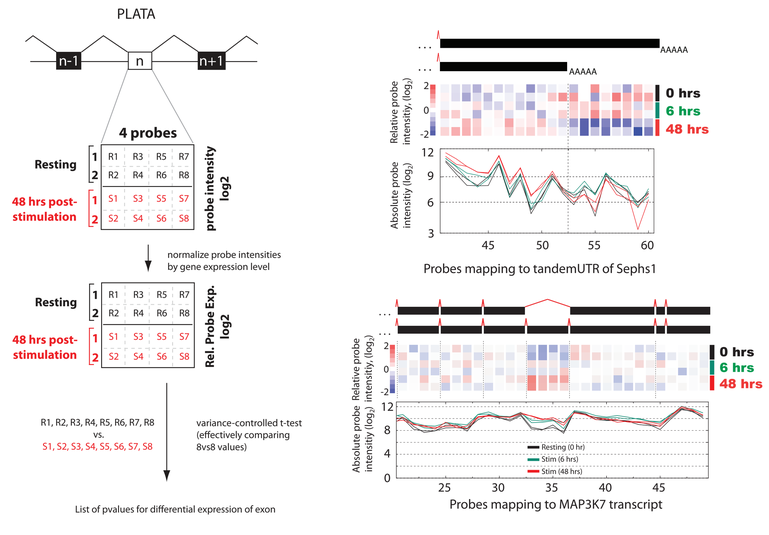




Probe-level visualization of exon array data for activation marker CD69. Note that absolute intensities vary greatly (up to 64-fold), but relative increases are fairly consistent

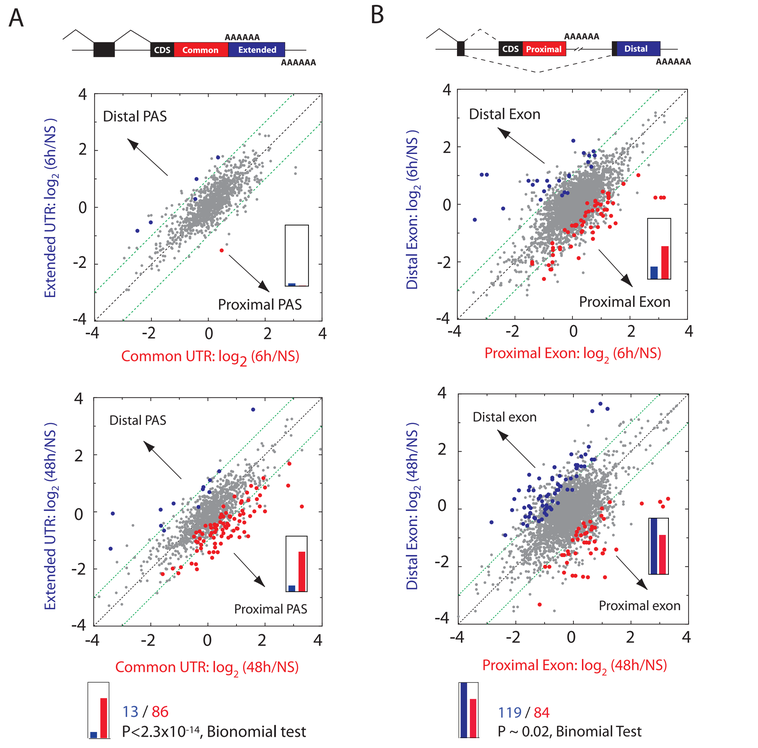
Probe-level exon array analysis

key insight: use relative probe intensities from individual probes (do not average probes for an exon as done in most analysis methods, e.g. MiDAS)



3′ UTR expression dynamics in activated T lymphocytes

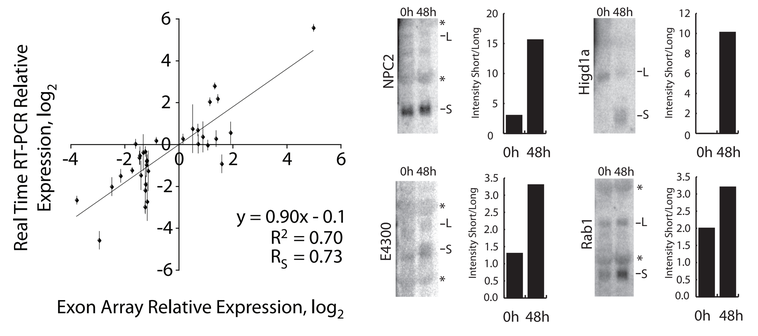
Both tandem UTRs (A) and mutually exclusive UTRs (B) are differentially utilized following T lymphocyte activation. Tandem UTR events are directional and only observed at late stages of activation (significant changes are colored):



13 / 86
P<2.3x10^{-14}, Bionomial test

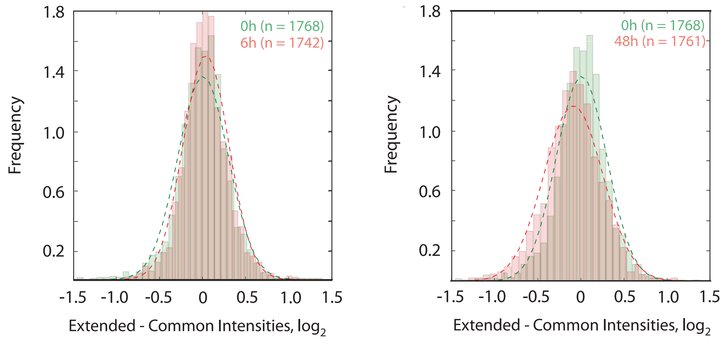
119 / 84
P ~ 0.02, Binomial Test

Real-time qRT-PCR and Northern analysis validate 3′ UTR swiching and shortening events



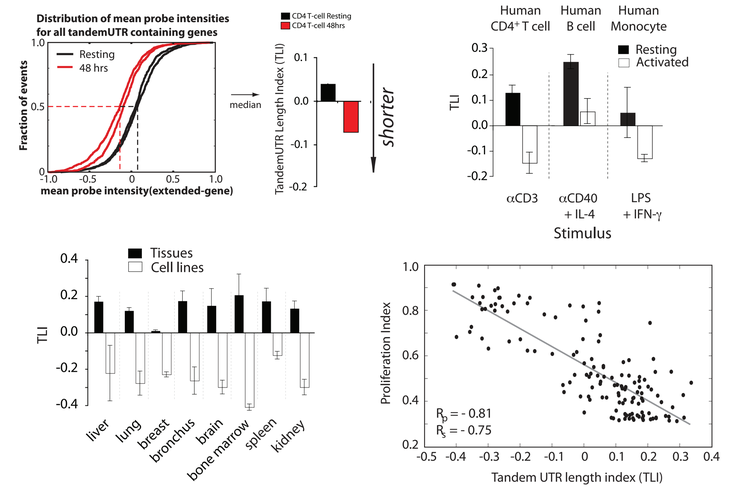
Global shortening of 3′ UTR elements at late stages of T lymphocyte activation

Examination of the aggregate of genes with tandem UTRs reveals decreased intensity of probes in the extended region at late, but not early stages of activation (P < 6.6x10^{-19}).



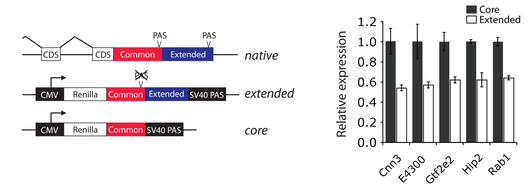
Shortening is conserved and correlates with levels of proliferation

To extend this analysis , we developed a Tandem UTR Length Index (TLI) to measure relative tandem UTR expression in publicly available array data:



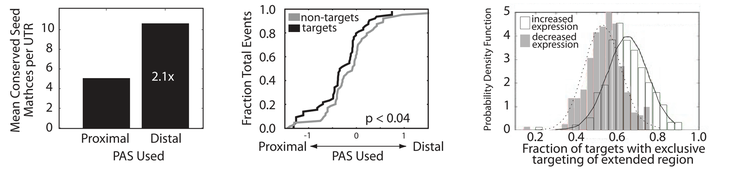
Evasion of regulatory elements

Enforced expression of the longer 3′ UTR element adversely affects protein expression in activated T cells

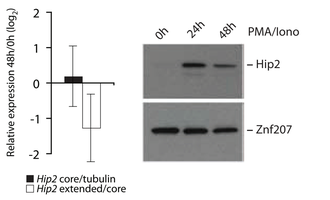


Dual luciferase assay in PMA/ionomycin stimulated primary mouse CD4$^+$ T cells

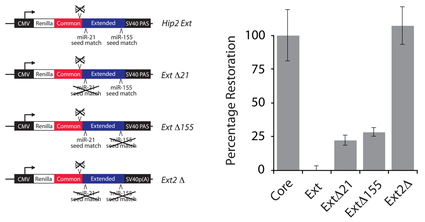
Shortening of 3′ UTRs removes roughly half of predicted microRNA target sites



Huntingtin interacting protein 2 RNA and protein expression:



Deletion of miRNA target sites in extended UTR of Hip2 restores protein expression:



Conclusions

Activation and proliferation programs are correlated with a global increase in expression of transcript isoforms with shorter 3′ UTRs

This shortening removed microRNA target sites and and other recognition motifs from the mRNAs.

These observations suggest a previously unrecognized layer of gene regulation that is both widespread and coordinated.

Acknowledgements

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