EDITORIAL

The short and the long of UTRs

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One of the revelations of the post-genomics era has been that that much more of the genome is transcribed than was previously imagined, and that ncRNAs rival protein coding transcripts in genomic abundance. A pre-mRNA splices out much non-coding – though not necessarily non-regulatory – RNA sequence, yet the mature messenger RNA often still retains a significant non-protein-coding RNA sequence that contains regulatory information relevant to the protein's proper translation. Whilst the open reading frame contains a dense specification of protein function, the 5' and 3' untranslated regions (UTRs) may contain highly conserved sequences that interact with the cellular regulatory milieu to provide context specific regulation of translation.

Since the elucidation of the lin-4/lin-14 regulatory relationship, miRNA target site prediction has largely focused upon complementary base pairing within the 3'UTR sequence of a mRNA transcript, with conservation of binding to the miRNA 5' 'seed' region providing the most rigorous means for identification of conserved miRNA binding sites. However, siRNAs and miRNAs have been reported to repress translation of mRNAs via non-conserved sequences within the 3' and 5'UTRs and the coding region in some experimental conditions (Lytle et al, 2007; Duursma et al, 2008). One might predict that mRNA coding sequences will be subject to remodeling by ribosome read-through in a manner dependent upon ribosome activity on a transcript, whereas 3'UTRs would be spared remodeling due to ribosome drop-off at the stop codon. Such small RNAs binding to the coding region may have a smaller window of repression, dependent on their means of action, i.e. partial site translational repression versus perfect cleavage. Massively parallel sequencing efforts have revealed that many cell types express a small number of miRNAs at high level (2-10,000 copies per cell), and a broader range of miRNAs at low abundance (<500 copies per cell) (Calabrese et al, 2007). Low abundance miRNAs may be transcribed at a low levels, or derive from small populations of cells expressing the miRNA within the larger pool. Reporter assays generally indicate that highly expressed miRNAs exert a more reliable translational repression than those of low abundance. The role of low copy number miRNAs may be restricted to specific target interactions that are overwhelmed by higher copy reporter mRNAs, and analogously, reporters of miRNA activity may be responsive to si/miRNA over-expression even though an interaction is not biologically relevant at endogenous expression levels.

A number of studies have demonstrated interactions between 5' and 3' UTR binding factors, and a model in which circularized mRNAs allow 3' UTR bound factors to modulate events at the 5' end of the mRNA, such as ribosome initiation, is reasonably established. Average 3'UTR length has been correlated with species complexity, even within vertebrates (Mazumder et al, 2003). Oocyte 3'UTRs have been noted to be shorter on average than genes expressed in somatic tissues, and brain mRNAs on average are on average the longest in adult tissues (Beaudoing et al, 2007). It is tempting to speculate that longer UTRs may contain regulatory motifs necessary to specify complex temporal and spatial translational programmes in complex cells. A range of 3'UTR regulatory motifs had been reported and characterized prior the discovery of miRNAs, but the interaction of cis- and trans- regulators has provided a new layer of complexity to understanding translational regulation. A number of reports, notably two from the Steitz lab at Yale, have described the unexpected observation that miRNAs may not repress translation in all cellular contexts, and in serum starved HeLa cells may actually enhance translation of their targets. AU-rich elements (AREs) alter mRNA stability and translation in many conditions, the ARE of TNFalpha cooperating with mir-16 to destabilize mRNAs, yet on cell cycle arrest the TNFalpha ARE enhances translation relative to mRNA levels upon serum starvation

in quiescent cells (Mori et al, 2000; Vasudevan and Steitz, 2007, Vasudevan et al, 2007). Experiments from Polesskaya et al that RNA binding proteins associate in a sequence specific manner with the UTR of IGF2 mRNA in muscle and enhance translation of this mRNA at low abundance (Polesskaya et al, 2007). Bhattacharyya et al identified elements of the cationic amino acid transporter 1 (CAT-1) 3'UTR that confer susceptibility to repression by miR-122 and derepression by interaction with the Hu-R protein (Bhattacharyya et al, 2006). Hu-D also binds to AU-rich sequences within the Neuroserpin UTR to enhance mRNA half-life and protein translation (Cuadrado et al, 2002). In all, these results point towards a Gordian interplay between a range of cis and trans acting 3'UTR factors that finely tune translation to cell specific contexts.

Given the regulatory capacity of 3'UTRs, on might also predict that factors defining 3'UTR length, such as alternative polyadenylation, might alter the regulatory sequence within an mRNA. Researchers at MIT (Joel Neilson, personal communication) have observed dynamic regulation of 3'UTR length during T-cell activation. Alternative 3' UTR splicing and polyadenylation events favour shorter or alternative UTRs of mRNAs upregulated during T-cell activation, perhaps to allow their expression without attracting regulatory factors concurrently expressed within the cell. Furthermore, their analysis has been extended to other cell types, and suggests that UTR length correlates with cellular proliferation - shorter UTRs are observed in cell lines and tumor cells relative to untransformed tissue.

Additional support for the broader relevance of these observations comes from Chen and colleagues at the University of Colorado. A short isoform of Cyclin D1 (CCND1) mRNA, commonly over-expressed in mantle cell lymphoma (MCL), is associated with poor prognosis. Chen et al demonstrate that the truncation eliminates mir16-1 binding sites within the 3'UTR, and thus that avoidance of mir16-1 repression might play a role in development of this cancer (Chen et al, 2008). These observations fit neatly with previously reported experiments that disruption of cellular miRNA biogenesis machinery enhances tumor forming propensity in mice, and that miRNAs are commonly downregulated in tumor samples (Kumar et al, 2007; Lu et al, 2005). These lines of evidence point to a model in which evading the cellular 3'UTR regulatory milieu is a key step in tumor development.

It will be greatly informative to identify further examples of 3'UTR shortening in diverse models to understand how widely cells employ this mechanism during dynamic gene expression. Uncovering signaling pathways that lead to alternative UTR formation may provide further insight into disease pathogenesis. For geneticists, a new emphasis upon functionality within 3'UTR may provide insight into how non-coding SNPs and repeat polymorphisms contribute to human disease. For miRNA biologists though there remain significant challenges in understanding the quantitative regulation of gene expression by miRNAs. At a fundamental level though, systems biology techniques to understand how miRNAs alter proteome expression are in their infancy and predicting and validating miRNA targets is still an unsatisfactory process, with a number of reports providing contradictory data in cell culture and in animal models. It may be that key findings in that forward the understanding of miRNA translation regulation come from wider analyses of RNA binding proteins in unique regulatory contexts in parallel to global studies of miRNA activity using by advanced proteomics techniques.

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