Cell Research (2014) 24:1383-1384.

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RESEARCH HIGHLIGHT

# Mixed messages: Re-initiation factors regulate translation

*Cell Research* (2014) **24**:1383-1384. doi:10.1038/cr.2014.114; published online 22 August 2014

**When ribosomes encounter upstream open reading frames (uORFs) during scanning of the 5′ untranslated region (5′ UTR), translation of the downstream ORF requires re-initiation. In a recent paper in *Nature*, Schleich *et al*. describe metazoan factors which specifically promote re-initiation.**

Translation of a message into a protein can be initiated when the 43S complex, while scanning the message from 5′ to 3′, encounters an initiation codon. Recently, it has become clear that metazoan mRNAs very often contain small ORFs upstream of the main start codon (uORFs) [1]. For example, almost half of mammalian mRNAs contain uORFs, which are also frequently translated as suggested by ribosomal footprinting data [2]. Overall, uORFs currently emerge as key elements of translational control mechanisms, and are implicated in a growing number of human diseases [3].

What is the specific impact of uORFs on translation? Generally, uORFs are thought to repress translation of the main message by sequestering initiation events. However, since the 1980s it has been thought that after termination, the 40S ribosomal subunit can remain bound to the mRNA and re-initiate at a downstream ORF. It is believed that the efficiency of re-initiation depends on the time that the ribosome spends in translating the uORF and scanning downstream intervening regions, since some initiation factors remain transiently associated with the ribosome while others need to be re-recruited. Thus, re-initiation is particularly efficient after short ORFs such as uORFs.

Several factors have been previously implied to be involved in re-initiation [4]. In the paper by the Teleman and Duncan labs [5], Schleich and colleagues describe a combination of *in vitro* and *in vivo* experiments in *Drosophila* which strongly suggest that DENR and MCT-1 (a well-known oncogene) can specifically function to regulate re-initiation after translation of a uORF. These two proteins form a complex combining N- and C-terminal domains of the functionally analogous initiation factor eIF2D, and promote translation initiation [6, 7]. The groups first studied DENR function *in vivo*. Knockout flies die as adults and show a variety of phenotypes, which are indicative of impaired translation of mRNAs specifically involved in cell proliferation and signaling. The authors then switch to S2 cell lines and use polysome profiling and metabolic labeling to study the role of DENR in translation. Indeed, DENR knockdown leads to reduced polysome/monosome ratios and lower rates of protein synthesis; however, the DENR dependence is only observed in proliferating but not in quiescent cells, and translation repression is most pronounced for mRNAs containing many uORFs with strong Kozak sequence context.

How did the authors arrive at this intriguing result? They initially focused on *mbc*, one of the genes most depleted from DENR-knockdown polysomes. Luciferase reporter assays suggested that DENR dependence of the *mbc* 5′ UTR is conferred by a region containing 3 uORFs with strong Kozak context. It does not depend on flanking *cis*-acting sequences or, incidentally, on the uORF coding sequence itself. However, it requires a stop codon, suggesting that DENR indeed promotes downstream re-initiation rather than preventing uORF translation. Accordingly, such DENR dependence could be conferred to synthetic reporters when uORFs with strong Kozak context were introduced. As expected, multiple uORFs and longer uORFs decreased DENR-dependent re-initiation at the main start codon. Further, these features correlate with reduced translation seen in polysome profiling assays on a transcriptome-wide scale, which was supported by additional reporter assays with 5′ UTRs of predicted DENR-dependent genes.

The authors then related these findings back to the *in vivo* context. First, flies with DENR knockout had reduced expression and signaling of insulin and ecdysone receptors. Both genes had been used to confirm DENR dependence of their 5′ UTRs. Second, they further investigated why impairment of DENR seems to have a more pronounced effect in proliferating compared to quiescent cells, although DENR mRNA and protein are present in non-proliferating cells. The authors made transgenic flies which expressed fluorescent reporters designed to reflect DENR activity. By imaging larval tissues, they confirmed that DENR regulation is indeed more pronounced in proliferating tissues such as brain and wing disc than in non-proliferating ones such as salivary glands or fat bodies. Although it remains unclear which factors regulate DENR activity, the authors provide evidence that phosphorylation of conserved residues in MCT-1 might be involved.

Taken together, Schleich *et al*. uncover metazoan factors which specifically regulate re-initiation downstream of uORFs with strong Kozak context in a specific cellular context. While it cannot be ruled out that DENR/MCT-1 also have more widespread and potentially indirect effects on translation, their role in re-initiation contrasts with a well-studied response to cell stress in which initiation at uORFs and downstream re-initiation are globally coupled [3].

Importantly, not all effects of uORFs on gene expression are connected to re-initiation. Protein output from the main ORF is reduced when initiation at the uORF leads to ribosomal stalling during elongation or termination, dissociation of both ribosomal subunits from the mRNA, or even degradation of the mRNA itself [3]. Further, little is known about factors that could regulate uORF effects in a sequence-specific manner. One of the few examples is Sex lethal, which binds upstream of the main start codon and promotes initiation at a uORF [8]. Also, expression of different 5′ UTR isoforms can influence uORF regulation [3]. In some cases, a uORF-encoded small peptide can interfere with translation *in cis* or *in trans* [3]. In fact, many small ORFs in the 5′ or 3′ UTRs of mRNAs are not only translated but also give rise to detectable small peptides [9, 10]. Some even show clear evolutionary signatures of negative selection on the encoded amino acid sequence, suggesting functionality of the peptide product.

Intriguingly, using methods that we originally applied in vertebrates [10], we noticed that several uORFs with DENR-dependent effects on translation are conserved in this sense. Some reside on mRNAs encoding important transcription factors like cryptocephal or gemini. In such cases, the distinction between a *cis*-regulatory uORF and an ORF encoding a small peptide with functions *in trans* is no longer clear-cut. In the future, it might therefore be very interesting to investigate re-initiation as a mechanism to regulate relative expression from different ORFs on the same "mixed" message.

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## References

1 Calvo SE, Pagliarini DJ, Mootha VK. *Proc Natl Acad Sci USA* 2009; **106**:7507-7512.
2 Ingolia NT, Lareau LF, Weissman JS. *Cell* 2011; **147**:789-802.
3 Somers J, Pöyry T, Willis AE. *Int J Biochem Cell Biol* 2013; **45**:1690-1700.
4 Skabkin MA, Skabkina OV, Hellen CU, *et al*. *Mol Cell* 2013; **51**:249-264.
5 Schleich S, Strassburger K, Janiesch PC, *et al*. *Nature* 2014; 512:208-212.
6 Dmitriev SE, Terenin IM, Andreev DE, *et al*. *J Biol Chem* 2010; **285**:26779-26787.
7 Skabkin MA, Skabkina OV, Dhote V, *et al*. *Genes Dev* 2010; **24**:1787-1801.
8 Medenbach J, Seiler M, Hentze MW. *Cell* 2011; **145**:902-913.
9 Slavoff SA, Mitchell AJ, Schwaid AG, *et al*. *Nat Chem Biol* 2013; **9**:59-64.
10 Bazzini AA, Johnstone TG, Christiano R, *et al*. *EMBO J* 2014; **33**:981-993.