C/EBP β : lost beyond translation

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The EMBO Journal (2011) 30, 3663–3664. doi:10.1038/emboj.2011.288

A/U-rich elements (AREs) are short sequences in the 3'UTRs of genes, acting *in cis* to regulate mRNA decay and translation. In this issue of *The EMBO Journal*, Basu *et al* (2011) describe a new function for AREs, in the context of the C/EBP β transcription factor. Specifically, they show that the C/EBP β ARE is responsible for sequestering the corresponding protein from subcellular compartments in which kinases reside to derepress C/EBP β . As a result, the transcription factor is unable to execute its cytostatic function in the face of an oncogenic insult. These results reveal a new mode of regulation of an already carefully controlled transcription factor. Given the widespread occurrence of AREs in genes, they also predict that this process, termed '3'UTR regulation of protein activity' (UPA), may have a more common role in controlling protein activities.

The transcription factor C/EBP β (for CCAAT/enhancer-binding protein) is involved in a plethora of physiological and pathophysiological processes, including cancer. It is a basic leucine zipper DNA-binding domain protein regulating proliferation and differentiation of a wide range of cell types. It has several relatives and through homodimerization and heterodimerization C/EBP β can be recruited to DNA to regulate gene transcription. Consistent with its prevalent expression and diverse functions, it is regulated by a complex array of mechanisms. For example, the C/EBP β transcript is translated into several isoforms, including a truncated form (LIP, for Liver Inhibitory Protein) and a longer polypeptide (LAP, for Liver Activating Protein). Further, the C/EBP β messenger contains a small upstream open reading frame governing the balanced expression of C/EBP β isoforms in response to changing protein translation conditions (Wethmar *et al*, 2010). Adding to this multifactorial regulation, C/EBP β is also subject to activation from a latent form, involving auto-inhibitory elements that suppress DNA binding and transactivation (Kowenz-Leutz *et al*, 1994; Lee *et al*, 2010).

A key signal for derepression can be provided by an active RAS pathway, particularly through the RAF-MEK-ERK module, thereby enhancing C/EBP β 's cytostatic function, for example, during oncogene-induced senescence (OIS; Sebastian *et al*, 2005; Kuilman *et al*, 2008). Intriguingly, there is also a pro-oncogenic role associated with this transcription factor, as conversely, C/EBP β -deficient mice are refractory to skin tumour development driven by oncogenic RAS (Zhu *et al*, 2002).

In this issue of *The EMBO Journal*, Peter Johnson and colleagues reveal yet another facet of C/EBP β regulation,

and it is a most original one (Basu *et al*, 2011). The investigators asked whether the 3'UTR of the C/EBP β transcript contributes to the downregulation of its resultant protein product by oncogenic RAS, an observation they made earlier (Sebastian and Johnson, 2009). While expecting an miRNA-dependent silencing mechanism, they observed that the

Figure 1 Model displaying a new mode of control for C/EBP β , called 3'UTR regulation of protein activity (UPA). Previously established roles for 3'UTRs include regulation of mRNA stability (top) and mRNA translation (middle). Basu *et al* (2011) show, for the transcription factor C/EBP β , that an A/U-rich element (ARE) within the 3'UTR of its messenger recruits RNA-binding proteins including HuR (bottom). HuR, and possibly other factors, direct C/EBP β to peripheral areas in the cytoplasm, sequestering it from kinases necessary for activation. This process is operational only in neoplastic cells, presumably because untransformed cells have a reduced cytoplasmic pool of HuR.

C/EBP β transcript containing an intact 3'UTR displayed a compromised cytostatic activity compared with the corresponding mRNA lacking this region. As this could not be explained by differential mRNA or protein expression, Basu *et al* (2011) dug further and found that, unexpectedly, the 3'UTR regulated C/EBP β function by blocking its post-translational activation. This effect was also seen for C/EBP α and was mapped to a so-called ARE (for A/U-rich element).

AREs like those encoded by C/EBP β are short *cis*-acting sequences that can recruit several RNA-binding proteins, including HuR. They are known to manage post-transcriptional control by regulating mRNA decay and translation (Khabar, 2010). RNAi depletion of HuR largely relieved 3'UTR inhibition of C/EBP β , converting it into a potent growth inhibitor in the context of active RAS. The authors went on to show that the UTR was responsible for directing the C/EBP β transcript to peripheral cytoplasmic regions, in a HuR-dependent fashion. In these subcellular compartments, C/EBP β is sequestered from its activating kinases, such as p-ERK1/2, which reside mostly in a perinuclear region, explaining why RAS-induced phosphorylations were diminished in C/EBP β -UTR-encoded protein (Figure 1). Correspondingly, whereas active C/EBP β lacking the 3'UTR stimulated the senescence-associated secretome as expected (Kuilman and Peeper, 2009), the full-length messenger failed to do so. Lastly, Basu *et al* (2011) show that this phenomenon is specific for immortalized and oncogenically transformed cells, as primary cells with a reduced cytoplasmic HuR pool apparently abrogate the negative effects of the 3'UTR, allowing for OIS to occur.

These results not only add a new dimension to C/EBP β regulation, but also uncover a new role for 3'UTRs. In addition to their established functions in governing mRNA stability and translation, this paper suggests that 3'UTRs can also modulate the activation of the protein encoded by the corresponding transcript, a mechanism that the authors dub 3'UTR regula-

tion of protein activity (UPA). Of course, this begs the question as to how general this mechanism is, beyond the two C/EBP family members studied here. The fact that up to 8% of human genes contain ARE-like regions (Bakheet *et al*, 2003) predicts that indeed many genes may be subject to this mode of post-translational control.

Another question emerging from this work is how the subcellular distribution of HuR is controlled differentially in normal and cancer cells, and how it suppresses C/EBP β activity in the latter. This is particularly interesting because of the observation that increased HuR levels can occur in human malignancies (Yuan *et al*, 2010). Also deserving further study is the nature and role of the peripheral cytoplasmic areas to which the 3'UTR directs the C/EBP β messenger. For example, are they so-called 'stress granules' to which some mRNAs localize when exposed to shock conditions? HuR and other RNA-binding proteins are known to accumulate in these foci (von Roretz and Gallouzi, 2008).

C/EBP β is already known to be regulated at various levels, and the current paper adds yet another layer of complexity. Conceivably, this is related to the diverse functions this transcription factor has in proliferation and differentiation. In particular, the apparently opposing functions of C/EBP β in RAS-induced senescence versus RAS-induced skin tumorigenesis may arise from the UPA mechanism.

Last but not least, a typical way to study gene function is to drive expression from a UTR-less cDNA cassette. One wonders how many properties thus ascribed to genes need reevaluation, now that it is becoming increasingly clear that non-coding regions harbour critical regulatory functions controlling translation and, as shown here, post-translational activation.

Conflict of interest

The author declares that he has no conflict of interest.

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