Genetics

# In sickness and in health: the importance of translational regulation

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## An important mechanism for regulating protein expression

In 2001, the first draft of the human genome was published.[1 2] Paediatricians from all disciplines can look forward to important and novel insights into the genetic basis for numerous diseases that affect children and their families. The surprising finding that our genome consists of 30–40 000 genes, only slightly larger than that of the plant *Arabidopsis thalania* (25 498 genes),[3] has now focused scientific study towards protein expression (termed *proteomics*), which is considerably more complex. It is also becoming apparent that defects in the mechanisms of protein expression are associated with human disease.

Since Watson and Crick described the structure of DNA in 1953, molecular biologists have done much to clarify the processes by which the genes contained in DNA are converted into proteins. Production of protein depends on transcription of the gene, which is controlled by a large number of factors interacting with various regulatory elements associated with the gene.[4] Pre-mRNA undergoes alternate splicing and other processing, before cytosolic translation of the messenger RNA (mRNA) results in protein. A number of diseases are known to be associated with abnormalities of transcriptional control (reviewed by Semenza[5]). However, increasingly we are realising that translational regulation is important both in physiological and pathological processes.

## TRANSLATIONAL REGULATION

Regulation can be global—that is, affecting the translation of all cellular mRNA, or specific to an individual mRNA. In the latter case the protein coding region is flanked by non-coding sequences—the untranslated regions (UTRs). Various regulatory molecules bind to specific sequences within the UTR, which may be on either side of the coding region, to either inhibit or enhance translation (fig 1). To add further complexity, different molecules can interact with the regulatory molecules to enhance or inhibit their effects. Control of protein expression from existing mRNA allows large increases in specific proteins and groups of proteins to be rapidly achieved. It appears to be important in up to 10% of all protein synthesis, particularly where a rapid, threshold, or a group response is required, for example, growth factors, cell cycle regulation, and immune response proteins.

### How iron homoeostasis is achieved in cells

The best known example of translational regulation by molecules binding to the mRNA is the intracellular control of iron concentrations. Iron is a "double edged sword" for cells. While it is essential, for example for DNA synthesis and respiration, it also catalyses the formation of highly toxic reactive oxygen intermediates. Cells have therefore evolved sophisticated control mechanisms to tightly control intracellular iron. The two key proteins, transferrin receptor and ferritin, undergo coordinated synthesis depending on the prevailing iron load, which is sensed by cytoplasmic molecules. Transferrin receptor expressed on the cell surface binds to transferrin to import iron into the cell. Ferritin has a counterbalancing role. It sequesters and stores excess iron within the cell, effectively removing it from taking part in cellular processes. Thus under conditions where intracellular iron is low, transferrin receptor expression is increased, and ferritin production is reduced, with the reverse occurring when intracellular iron is replete (fig 2)

This elegant coordination is achieved by regulating the translation of the mRNA of ferritin and transferrin receptor.[6 7] The protein coding region for ferritin mRNA is flanked by long, non-coding UTRs consisting of several hundred bases. Iron concentrations are sensed by cytoplasmic iron responsive proteins (IRPs), which bind to iron responsive elements (IREs) in the UTRs. IRPs bind to the IREs under conditions of low intracellular iron. In the case of ferritin mRNA this binding interferes with and therefore inhibits translation. However when the IRPs bind to transferrin receptor mRNA they stabilise it, allowing efficient translation. When iron is plentiful the IRPs do not bind, so ferritin is efficiently translated, but not transferrin receptor.

There is an important exception to this elegant and otherwise ubiquitous mechanism. Erythroid cells paradoxically maintain high transferrin mRNA and receptor concentrations despite high iron concentrations, and altered iron concentrations do not significantly alter mRNA concentrations.[8] It appears that they override normal IRE/IRP control of transferrin receptor, but interestingly they use distinct IRP–IRE interactions to regulate erythroid specific aminolaevulate synthase (ALA-S2), which is the rate limiting step in haem synthesis.

Translational regulation is widespread, with the regulatory sequences showing a high degree of evolutionary conservation. Some important examples include:

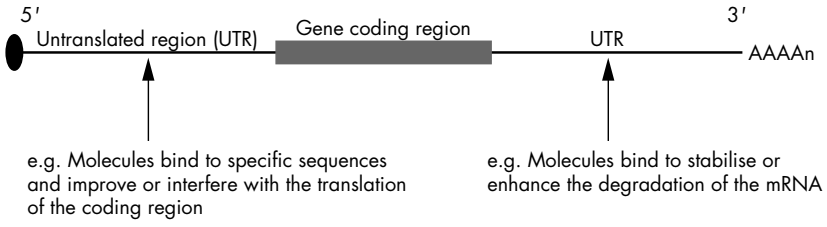


**Figure 1** Schematic representation of how mRNA translation can be controlled.

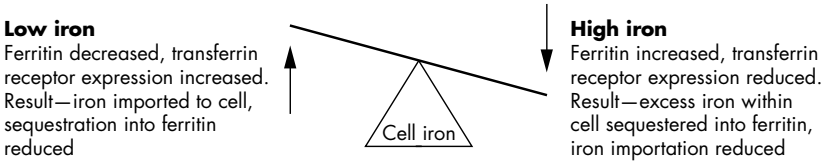


**Figure 2** The opposing coordinated translational regulation of ferritin and transferrin receptor

**Abbreviations:** FGF-2, fibroblast growth factor 2; FMRP, fragile X mental retardation protein; GM-CSF, granulocyte macrophage colony stimulating factor; IGF, insulin like growth factor; IL, interleukin; IRE, iron responsive element; IRP, iron responsive protein; TGFβ, transforming growth factor β; TNFα, tumour necrosis factor α; UTR, untranslated region; VEGF, vascular endothelial growth factor

- Cell growth and division in the zygote—there is barely any detectable transcription in early embryogenesis, and translational regulation of pre-existing mRNA accounts for the complex protein expression that directs the very rapid and precise growth of the developing embryo.
- Many cytokines, including tumour necrosis factor α (TNFα), interleukin 1 (IL-1), IL-6, and IL-8, and growth factors such as granulocyte macrophage colony stimulating factor (GM-CSF), transforming growth factor β (TGFβ), insulin like growth factor 2 (IGF-2), and vascular endothelial growth factor (VEGF) show translational regulation. Through sharing similar sequences in the UTRs, different mRNAs can be coordinately regulated while taking advantage of the rapid response inherent to translational control mechanisms.
- Infecting viruses manipulate normal cell translational control mechanisms to ensure their own highly efficient translation. A key response by the cell is to globally inhibit its translational apparatus through the protein kinase PKR,[9] thereby restricting viral replication. This results in self sacrifice by the cell as PKR has widespread involvement in restricting cell growth, and is an activator of apoptosis.

## TRANSLATIONAL DYSREGULATION

Perhaps unsurprisingly the more sophisticated the mechanism, the more opportunities there are for things to go wrong. It is now clear that mutations in the UTR sequences or defects in the molecules that bind to them can cause disease (termed translational pathophysiology[10]). While an exhaustive list is outside the scope of this article (and would be unable to keep pace with the rapid progress currently being made), I have selected some examples that are not only illustrative, but also of relevance to paediatricians.

### Hereditary hyperferritinaemia cataract syndrome

This disease was the first condition for which the mechanism of translational dysregulation was established.[11] It is a rare autosomal dominant condition characterised by raised serum ferritin with normal serum iron concentrations. High intralenticular ferritin causes cataract formation, which commences in early childhood. Various mutations exist in the IRE of the L-ferritin gene, such that IRP binding is reduced and ferritin production remains increased. The severity of the disease corresponds to the degree to which a mutation affects the ability of the IRP to bind.

### Hereditary thrombocythaemia

This autosomal dominant condition presents in early childhood, and is characterised by a high platelet count with thrombosis and/or haemorrhages caused by excessive thrombopoietin. Several different mutations have now been described in regulatory sequences of the UTR.[12] Reduced binding of regulatory molecules results in the uncontrolled overproduction of thrombopoietin.

### Fragile X syndrome

This is characterised by loss of the fragile X mental retardation protein (FMRP) coded for by the FMR1 gene. FMRP appears to be important in the development of neuronal elements, and normally acts as a translation inhibitor. Affected individuals may not be able to translate FMR1 mRNA,[13] possibly as a result of excessive self inhibition.[14]

### Oncology

Translational dysregulation of numerous growth and transcription factors, receptors, enzymes, and cell cycle regulators have been described in a broad range of malignancies. Well known oncogenes are regulated through UTR interactions, for example, BCL-2, c-mos, and c-*myc*.[15] Abnormal translational control of c-*myc* appears to be important in Bloom's syndrome. Fibroblast growth factor 2 (FGF-2) enhances epithelial cell proliferation, and translational dysregulation resulting in overexpression of FGF-2 has been implicated in breast cancer.[16] It is interesting that high cellular PKR (involved in translational repression, see above) is often (not always) associated with a more favourable prognosis for cancers.

## FUTURE DEVELOPMENTS AND SUMMARY

The scientific, clinical and commercial potential of this field is immense. To consider some aspects:

- *Screening*. Genome screening for as yet undescribed UTRs will undoubtedly lead to the mechanisms of numerous diseases to be elucidated or even redefined. Current microarray technology allows rapid diagnostic screening to be performed. Genetic polymorphisms in our translational control mechanisms might go some way to explaining our individual susceptibilities to viruses and other infectious agents, cancers, and inflammatory conditions.
- *Pharmacological*. Drugs that act on translational control mechanisms already exist. Dexamethasone destabilises the mRNA of TNFα and cyclin-D3 (a critical cell growth factor) among others, which may partially account for the broad effects of glucocorticoids. Experimentally tristetraprolin can regulate TNFα and GM-CSF mRNA stability.[17]

Looking further ahead, the use of expensive recombinants such as GM-CSF or thrombopoietin might be reduced if endogenous mRNA translation could be stabilised/enhanced. Therapeutic mimicry using peptides or chemicals, or gene therapy to correct defects of UTR binding should be possible. Cancer is clearly one area where novel treatments targeting abnormal protein production offers exciting potential. Antiviral strategies might inhibit the use of cellular translational apparatus by infecting viruses. For some translational pathophysiologies protein expression might be blocked or enhanced, for example, from an unaffected allele in an autosomal dominant disorder.

## Conclusions

The new age of genomics, proteomics, and bioinformatics offers much hype but also much hope for new diagnostic, imaging, and therapeutic agents, many of which will target diseases that affect children. Translational regulation is critical for the coordinated and controlled expression of many important regulatory proteins. Translational dysregulation is a novel, interesting, and increasingly implicated pathophysiological mechanism. It's an old cliché, but watch this space.

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