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IRESes: new potential drug targets? ▼

Several laboratories are today creating expanded libraries of different compounds to selectively target RNA structures[1]. It is envisioned that RNA-binding drugs might produce effects that cannot be achieved by conventional protein-binding drugs. Internal ribosomal entry segments (IRESes) are distinct RNA structures with important regulatory functions, which might prove to be ideal as drug targets.

Translation of mRNA in eukaryotic cells can be 'cap-dependent' or 'cap-independent'. In cap-independent initiation (Fig. 1), the ribosome complex recognizes an IRES, which is a defined structural element located in the 5′ untranslated region (UTR) of the mRNA. IRES-mediated translation has been thoroughly explored with respect to several viral transcripts. Until recently, only a few examples existed that showed that this mechanism is also used by eukaryotic cells. During the past few years, a large number of eukaryotic IRESes have been identified and it has been shown that these sequence elements can function independently. For example, IRES-modules have been identified within the 5′ UTRs of transcripts for fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), oestrogen receptor and c-myc[2]. It is evident that IRES-mediated initiation can be cell-type specific. For instance, it has been demonstrated that IRES activity in the 5′ UTR of FGF-2 mRNA is low in most adult human organs, but is exceptionally high in the brain[3]. Another example is provided by Chappell and coworkers, who have shown that in cell lines derived from patients with multiple myeloma, the translational regulation of c-myc is disturbed and that this correlates with a mutation from cytosine to thymidine in an IRES located in the 5′ UTR of the c-myc mRNA[4]. This single mutation in the c-myc-IRES is sufficient to cause enhanced initiation of translation via internal ribosome entry and represents a novel mechanism of oncogenesis.

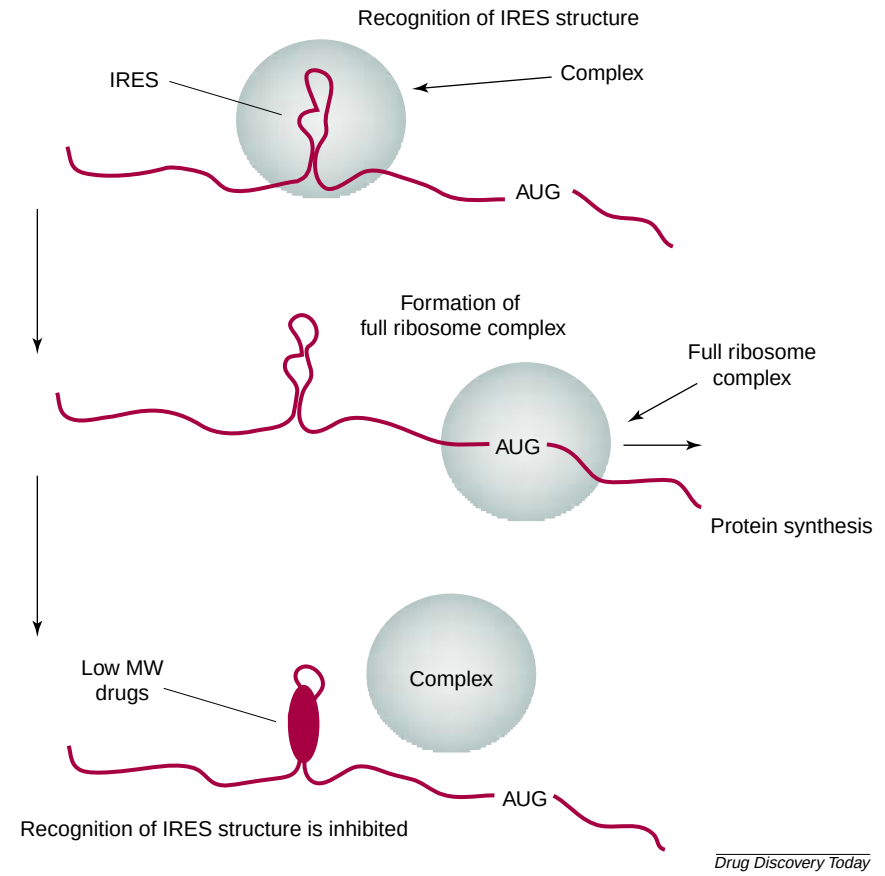

Figure 1. In cap-independent translation an internal ribosomal entry segment (IRES)-structure in the 5′ untranslated region (UTR) of the mRNA is recognized by a protein complex. This results in binding to the initiation codon, formation of a full ribosome complex and the start of protein synthesis. Low molecular-weight (MW) drugs that bind to the IRES might interfere with protein recognition of the IRES and thereby inhibit the protein synthesis of the transcript.

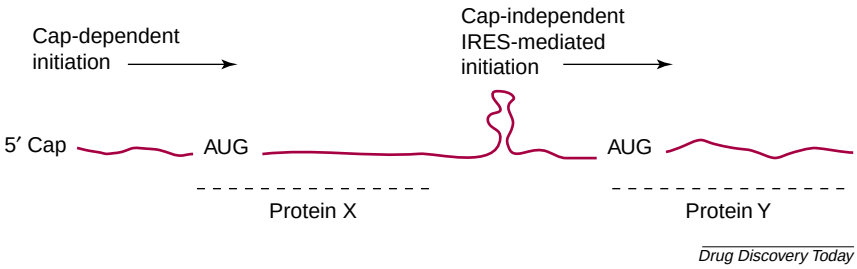

Figure 2. A dicistronic reporter gene contains two open reading frames, typically encoding two different reporter proteins (X and Y). The translation of one of these proteins is initiated by a cap-dependent mechanism. An internal ribosomal entry segment (IRES) could be inserted upstream of the start codon of the second reading frame, that is, in the intercistronic spacer region. Accordingly, the translation of this protein can be mediated by the IRES, that is, be initiated by a cap-independent mechanism.

Therefore, targeting IRES structures would enable the modulation of translation of distinct mRNAs in specific cell types without affecting protein expression in other tissues. This provides an additional level of drug specificity that is generally not achieved by targeting proteins.

For many years it has been known that aminoglycosides and other antibiotics act by binding to microbial RNA. However, it was not until recently that the concept of screening for small molecules with high affinity for a given RNA structure has been recognized by the pharmaceutical industry. Antisense oligonucleotides provide a valid concept for RNA modulation. However, such macromolecules possess several disadvantages as therapeutic drugs, not least in terms of bioavailability. Today, several pharmaceutical and biopharmaceutical companies are developing strategies to perform HTS for mRNA modulators. Several companies have already reached the stage of clinical trials with drugs that interfere with the translation of viral transcripts, for example, HIV and hepatitis C.

In a conventional HTS campaign, the resource-consuming procedures typically involve the production and purification of functional target protein and/or assay development. Translation reporter assays represent a generic concept and the assay set-up can be completed in a few days. A large number of reporter genes are available on the market, for example, growth hormone, luciferase and β-galactosidase. Transiently transfected cells can readily be used for HTS of low molecular-weight compounds and it is possible to generate highly reproducible data (J. Ekblom *et al.*, unpublished).

Dicistronic reporter genes typically encode a transcript that can be started at two different sites – a classical cap-dependent start site in addition to a cap-independent site initiated by an IRES (Fig. 2). The transcript will thus yield two recombinant proteins. Accordingly, the level of IRES-mediated translation can be expressed as a ratio to the cap-dependent translation. Dicistronic plasmid vectors encoding, for example, the enhanced blue and green fluorescent proteins (EBFP and EGFP, respectively), can be delivered into mammalian cells by transient or stable transfection and used as a translation reporter system. The cap-dependent translation will provide an excellent 'internal control signal', that is, it will detect non-specific inhibitors of translation. Translation reporter assays could, for instance, be used to screen for small ligands that specifically interfere with IRES-mediated c-myc translation in IRES-mutants in multiple myeloma cells, without affecting expression of this multi-functional protein in other cells.

It is likely that a large number of IRESes will be discovered and characterized in the next few years because of the development of powerful experimental tools for the identification of IRESes and the publication of the entire human genome sequence. Moreover, it can be assumed that the accumulating data on genetic variability will reveal mutations that cause disease in humans by interfering with translational regulation, such as in the case with c-myc. These RNA sub-domains could prove to be of great value as pharmaceutical drug targets.

References

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Antisense technology: inaccessibility and non-specificity ▼

Despite the simplicity and the elegance of the concept, antisense methods remain largely empirical. In principle, only the gene sequence is required for targeting with antisense reagents. In practice, it is far more demanding than this and almost every step in the application of these methods poses a problem.

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