Dispensable iron-sulfur clusters: the interconversion of aconitase with the RNA-binding protein, IRE-BP

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The unexpected convergence of one or more seemingly disparate lines of research is increasingly common and exciting. Recently, studies have revealed that the genetic regulation of proteins involved in iron metabolism is closely interconnected with cytoplasmic citrate metabolism [1]. In most eukaryotic cells, the level of iron uptake is determined by the expression of the transferrin receptor (TfR). Inside the cell, iron is used in metabolism or sequestered by cytoplasmic ferritin. The levels of expression of TfR and ferritin are reciprocally regulated; excess iron results in increased TfR synthesis and decreased ferritin synthesis. Both effects are mediated at the mRNA level; regulation of the TfR is achieved through changes in the half-life of the mRNA, while ferritin is controlled at the level of translation initiation. The regulation involves *cis*-acting RNA stem-loop structures contained in each of these transcripts, which are known as iron-responsive elements (IREs).

A cytosolic *trans*-acting factor, the iron-responsive element binding protein (IRE-BP), binds with high affinity to IREs. The ability of the IRE-BP to bind to its target RNA is determined by a post-translational modification that registers changes in iron levels. The nature of this modification became apparent when the sequence of IRE-BP was found to be strikingly similar to that of mitochondrial aconitase (mAco), an enzyme that converts citrate to isocitrate, and identical to that of cytosolic aconitase (cAco). The mAco protein contains an iron-sulfur [4Fe-4S] cluster which is required for enzymatic activity; when the predicted [4Fe-4S] cluster is assembled in IRE-BP *in vitro*, the protein gains aconitase activity and loses RNA-binding capacity. This reciprocal regulation involves the complete assembly/disassembly of the [4Fe-4S] cluster.

The mAco protein has four domains; the first three are compactly folded, and the link to the fourth domain appears to be flexible (see Fig. 1). The Fe-S cluster is integral to the active site and is buried in a cleft between the fourth domain and the rest of the protein. In cAco, which is likely to have the same fundamental structure as mAco, a peptide involved in RNA binding has been identified whose predicted position is within the active site. Thus, the RNA-binding form must have a significantly more open structure than the aconitase form (see Fig. 1, right panel), since an RNA stem loop would not otherwise fit within the cleft.

Perhaps the most surprising feature of this system is that not only is IRE-BP/cAco bifunctional, but the two functions, aconitase and RNA binding, are reciprocally regulated. Understanding why the cell co-regulates cytosolic citrate metabolism with iron metabolism will require re-examination of the consequences of changes in cAco activity. The use of a biologically labile Fe-S cluster as an environmentally sensitive switch is not likely to be unique to the IRE-BP/cAco system. How the cell uses such switches will depend on how the clusters are disassembled and reassembled. Fe-S clusters are sensitive to oxidants, and new observations that the RNA-binding form of IRE-BP can be activated in macrophages by nitric oxide suggests that this switch might also sense oxidative stress [2,3]. The *Escherichia coli* dihydroxy acid dehydratase has recently been shown to undergo a reversible disassembly/reassembly of its [4Fe-4S] cluster in response to oxygen stress [4], and the SoxSR regulatory system also appears to use such a switch (see Storz, G., page xvi this issue). Further, the rate-limiting enzyme in purine biosynthesis, glutamine phosphoribosyl-pyrophosphate amido transferase, may be regulated by oxidative stress via a sensitive Fe-S cluster [5].

There are many examples of biological molecules that have evolved to serve two purposes. In IRE-BP/cAco, we have a dramatic disparity in function in a protein that is both an enzyme and a regulator of cytosolic gene expression. Furthermore, the nucleic acid binding site overlaps with the highly conserved enzymatic active site. The ability of the cell to reversibly disassemble and assemble an Fe-S cluster contained within the active site is central to this particular regulatory system. Elucidating the mechanisms by which the cell alters these ubiquitous bio-inorganic structures provides an interesting challenge at the interface of biology and inorganic chemistry.

References

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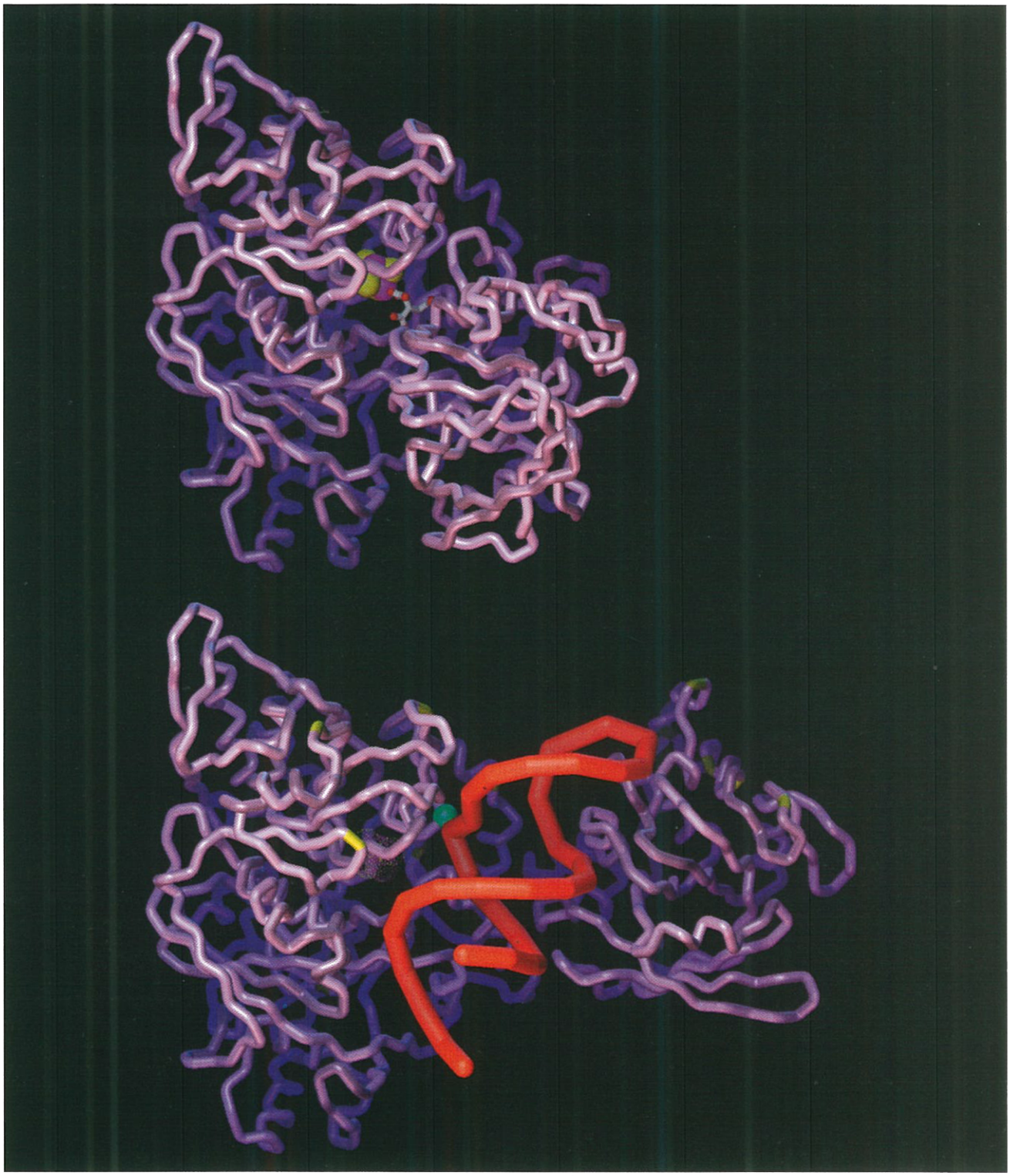

Fig. 1. Model for the interaction of the IRE with cAco (IRE-BP). The cAco model is based on the crystal structure of the homologous mitochondrial aconitase (mAco), which is shown at the top. The [4Fe-4S] cluster is shown as solid spheres adjacent to bound substrate (isocitrate). In this view the active site cleft is oriented vertically. The cAco model shown on the bottom is in the same orientation as the mAco structure. The consensus IRE sequence (red) is represented by phosphorous positions of an A-form RNA helix with six base pairs, a bulge U, one base pair, a bulge C, five base pairs and a six residue loop. To accomodate the binding of RNA, the mAco active site cleft is opened via extension of a 25 amino-acid hinge/linker which separates the fourth domain (right) from the first three (left). The site of cross-linking to the IRE is shown as a green sphere. The positions of 15 insertions greater than two amino-acids in length required to align the cAco sequence onto mAco are indicated by yellow bars. Except for one insertion adjacent to a conserved active site residue Asp125 (Asp100 in mAco), it can be seen that all insertions map onto the surface of the structure. The position of the [4Fe-4S] cluster in mAco, which is not present in the RNA-binding form of cAco, is indicated as a dot surface. (Graphics by Mike Pique.)