FEBS Letters 389 (1996) 40–43 FEBS 17183

Minireview

Iron-sulphur clusters as genetic regulatory switches: the bifunctional iron regulatory protein-1

Efrosyni Paraskeva, Matthias W. Hentze*

Gene Expression Programme, European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany

Received 10 May 1996

Abstract In the eighties, iron regulatory protein-1 (IRP-1) was identified as a cytoplasmic mRNA-binding protein that regulates vertebrate cell iron metabolism. More recently, IRP-1 was found to represent the functional cytoplasmic homologue of mitochondrial aconitase, a citric acid cycle enzyme. Its two functions are mutually exclusive and depend on the status of an Fe-S cluster: the (cluster-less) apoIRP-1 binds to RNA, while the incorporation of a cubane 4Fe-4S cluster is required for enzymatic activity. Cellular signals including iron levels, nitric oxide and oxidative stress can regulate between the two activities posttranslationally and reversibly via the Fe-S cluster. Recent reports suggest that other regulatory proteins may be controlled by similar mechanisms.

Key words: Iron responsive element; Aconitase; Iron metabolism; RNA/protein interactions

1. Introduction

In recent years, a new class of bifunctional proteins made its appearance: enzymes that perform seemingly unrelated second functions as nucleic acid binding proteins. The list includes (amongst others) thymidylate synthase, dihydrofolate reductase, iron regulatory protein-1, glyceraldehyde-3-phosphate dehydrogenase and glutamate dehydrogenase. The physiological role of RNA binding remains obscure or incompletely characterised for the majority of these proteins [1], with iron regulatory protein-1 (IRP-1) representing a notable exception: the interaction of IRP-1 with its target mRNAs regulates key aspects of cellular iron metabolism. Its second identity is that of cytoplasmic aconitase, converting citrate to isocitrate (for recent reviews see [2,3]). In this minireview, we will illustrate the two functions of IRP-1 and discuss the novel mechanism by which they are regulated.

2. IRP-1: an RNA binding protein that regulates iron homeostasis

IRP-1 (previously termed IRF, IRE-BP, FRP) is a 98 kDa cytoplasmic protein. It was first identified in mammalian cells as an RNA-binding protein that recognises a specific RNA hairpin motif, the iron-responsive element (IRE). An IRE is a ~30 nucleotide structure formed by two RNA helices that are separated by a bulged cytosine residue, and by a six-nucleotide loop of the sequence 5′CAGUGN3′ (N is usually a pyrimidine). The sequence of the loop and the bulged nucleotide is conserved, as is the length of the RNA helix between the two (5 base pairs).

Functional IREs have been identified in the 5′ untranslated regions (UTR) of ferritin H-chain, ferritin L-chain, erythroid aminolevulinate synthase (eALAS), mitochondrial (mt)-aconitase and succinate dehydrogenase (SDH) mRNAs, as well as the 3′ UTR of transferrin receptor (TfR) mRNA [2–11]. Binding of IRP-1 to a single IRE in the 5′ UTR inhibits translation by preventing the stable association of the small ribosomal subunit with the mRNA [12], probably by a steric mechanism that does not require additional co-factors or cellular proteins [13]. When it binds to the 3′ UTR of TfR mRNA, IRP-1 stabilises the message by binding to five IREs [10,11]. The molecular mechanism underlying this protection of TfR mRNA from degradation is complex and not yet well understood.

IRP-1 binds to IREs with high affinity when cellular iron levels are low. As a result, ferritin, eALAS, mt-aconitase and SDH synthesis is repressed, while TfR mRNA is stabilised. When cells are iron replete, the affinity of IRP-1 for IRE is drastically diminished [2,3]. Translational repression of the target mRNAs is relieved, while TfR mRNA is rapidly degraded. In this way the expression of proteins involved in iron uptake (TfR), storage (ferritin) and utilisation (eALAS is the first enzyme in the heme biosynthetic pathway, mt-aconitase and SDH are iron containing enzymes) is co-ordinated by the regulation of the affinity of IRP-1 for its IRE binding sites.

3. IRP-1 is a cytoplasmic aconitase

Following the purification of IRP-1 and the cloning of its cDNA, computer-based comparisons of the deduced amino acid sequence revealed the homology between IRP-1 and mitochondrial aconitase [14,15]. Mitochondrial aconitase is a 4Fe-4S cluster-containing protein which catalyses the conversion of citrate to isocitrate within the mitochondrial citric acid cycle. The two proteins display ~31% identity and ~56% similarity along their entire length. A cytoplasmic homologue of mitochondrial aconitase was long known to exist [16], but its role in cell metabolism was not well understood. Both IRP-1 and c-aconitase are known to be localised on human chromosome 9, and the homology between IRP-1 and mt-aconitase, particularly the 100% identity of amino acids involved in the formation of the enzyme's catalytic core, suggested the possibility that IRP-1 and cytoplasmic aconitase might represent two forms of the same protein [14,15]. A number of studies have experimentally corroborated this hypothesis,

*Corresponding author. Fax: (49) (6221) 387 518.
E-mail: Hentze@EMBL-Heidelberg.DE

PII S0014-5793(96)00574-1

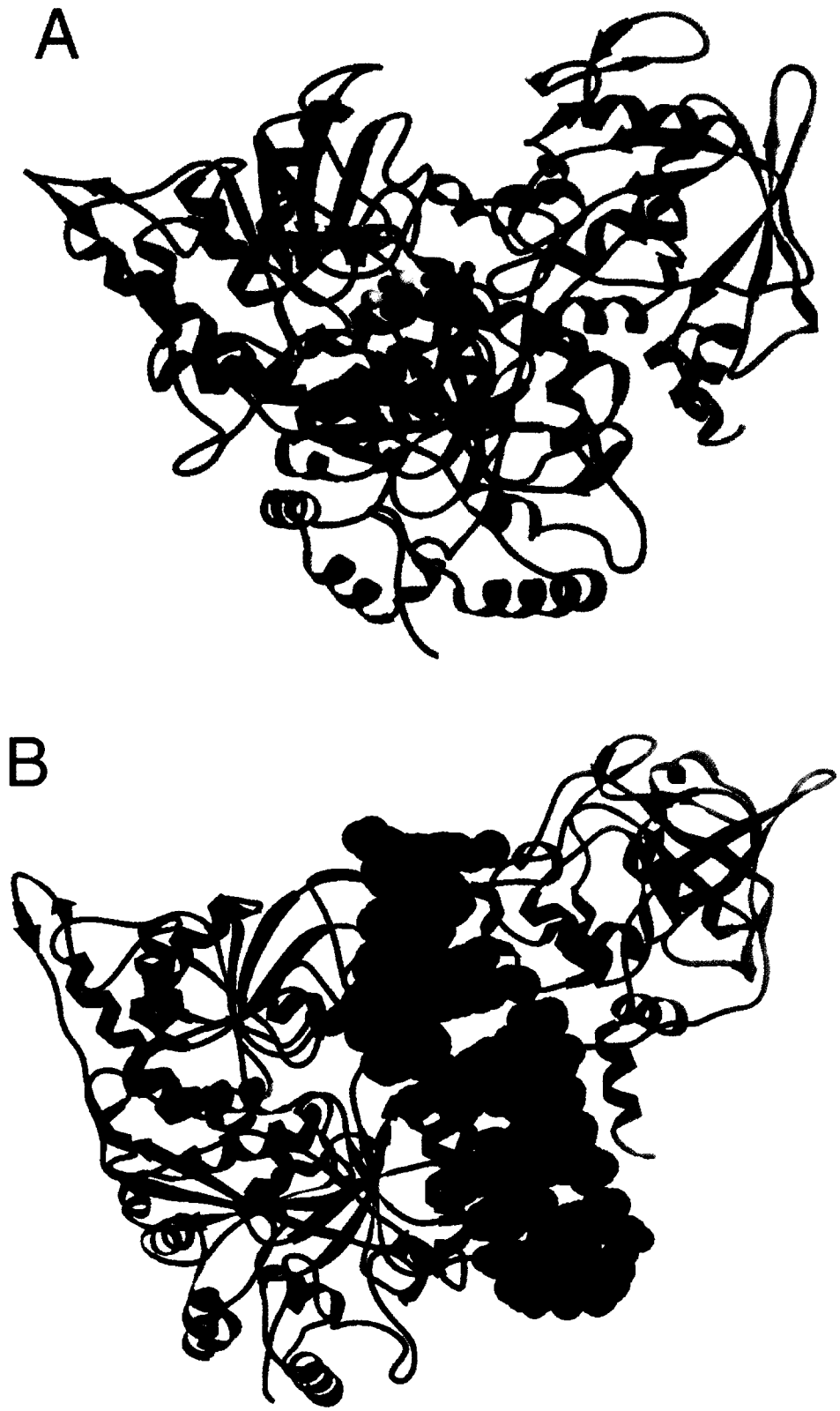

Fig. 1. Mitochondrial aconitase as a structural model for IRP-1, in its role as cytoplasmic aconitase (A) and IRE-binding protein (B). Based on the structure of mt-aconitase [25,26], two forms have been drawn without making allowances for differences between mt-aconitase and IRP-1. (A) In the 4Fe-4S cluster containing enzyme, domains 1–3 (coloured in green) and 4 (coloured in blue) form a narrow cleft (closed form), where the Fe-S cluster (Fe atoms coloured in blue, S atoms coloured in yellow) is found, linked to three cysteines (coloured in orange) of the protein backbone. The substrate, citrate (coloured in red), is situated within the cleft and interacts with both the cluster and the protein backbone (R536, R541, R699, R780 coloured in magenta). In this conformation, the narrow cleft prohibits IRE binding. (B) The apoprotein form may adopt a more open conformation, via the hinge linker. Domains 1–3 and 4 may separate enough to accommodate the IRE (coloured in red). In this form, the IRE may contact amino acids 121–130 (coloured in magenta) and the region close to C437 (coloured in orange). The aconitase structure [25,26] is depicted using RIBBONS [49] and WHAT IF [50] for modeling.

demonstrating that purified cytosolic aconitase and purified as well as recombinant IRP-1 display IRE-binding and aconitase activity, and that the amino acid sequence of purified bovine cytosolic aconitase closely matches that of human IRP-1 [17–20].

cDNAs encoding a protein with ~60% identity and ~80% similarity to IRP-1 have also been cloned. This protein (IRP-2), which will not be discussed further here, is also an IRE-binding protein and thus participates in the control of cellular iron metabolism (for a review see [3]). The regulation of its RNA binding activity occurs by a different mechanism, and IRP-2 does not exhibit aconitase activity [21–27].

4. Switching between the two functions

Much information is available concerning the biochemistry and structure of mt-aconitase [28–30]. Mt-aconitase is composed of four domains. The three N-terminal domains form a compact core, while the fourth domain is linked to this core by a flexible hinge (Fig. 1A). Domains 1–3 (coloured in green) and 4 (coloured in blue) form a narrow cleft between them, which leads to the active site of the enzyme. A cubane 4Fe-4S cluster (coloured in blue and yellow) is situated within this cleft, with three of the four Fe atoms linked to three cysteines of the protein backbone. The fourth Fe atom of the cluster can interact with the substrate, citrate (coloured in red). On the basis of the similarities in amino acid composition and enzymatic function, the structure of mt-aconitase may provide a suitable model for considering structural features of IRP-1.

Three forms of IRP-1 can be purified from cells. The 4Fe-4S cluster containing protein is the predominant form within iron replete cells. It does not bind to IREs, binds citrate and displays aconitase activity. In addition to its interaction with the Fe of the cluster, citrate also interacts with four arginines (R536, R541, R699, R780 in the human IRP-1 sequence) which contributes to the stabilisation of the protein structure and narrowing of the cleft. Under these circumstances, the narrow cleft may prohibit IRE binding. Since a fully assembled cluster is required for enzymatic activity, a 3Fe-4S cluster containing IRP-1 (although able to bind citrate) is enzymatically inactive. In addition, the 3Fe-4S form of the protein exhibits no IRE binding activity, presumably because the Fe-S cluster (and citrate?) maintain the cleft in a closed state. It is not clear whether this 3Fe-4S form may represent a physiological intermediate in cluster disassembly of IRP-1. The apoprotein form of IRP-1 is the RNA binding form that is present in iron-starved cells. It shows no aconitase activity and little citrate binding. The lack of the cluster may allow IRP-1 to adopt a more open conformation between domains 1–3 and 4 which permits access and binding of the IRE, as suggested on the basis of the mt-aconitase structure depected in Fig. 1B.

Thus, the two functions of IRP-1 are mutually exclusive and regulated via the iron-sulphur cluster. Mutational analyses show that serine replacement of any of the three active site cysteine residues (C437, C503, C506) involved in cluster binding results in complete loss of aconitase activity and constitutive RNA binding [31]. Alkylation of C437 with a bulky compound (*N*-ethylmaleimide) causes loss of RNA binding, while alkylation with a smaller group (iodoacetamide) does not affect IRE binding [32]. When modeled according to the structure of mt-aconitase, C437 is not far from amino acids 121–130, to which the IRE has been crosslinked using ultraviolet light [33]. Furthermore, three of the four arginines that participate in substrate binding (R536, R541, R780) may also contribute to RNA binding [34]. Taken together, these findings suggest that the IRE binding site(s) is in close proximity to the catalytic center of the protein, and that a significant rearrangement of the protein is required to permit access of the IRE to what would otherwise be hidden within the narrow cleft. Such a rearrangement may be the opening of the cleft via the flexible hinge linker, as suggested in Fig. 1B. Unexpectedly, multiple sequence alignment of several members of the Fe-S isomerase family has identified additional, highly conserved residues that were previously unrecognised. These amino acids could also prove to be important for the structural/functional organisation of IRP-1 [35].

The molecular mechanisms by which 4Fe-4S cluster assembly and disassembly are achieved in response to cellular iron levels remain to be defined. It is not known whether these processes are 'spontaneous' or whether cellular factors facilitate cluster formation and/or removal. Interestingly, the bacterial protein NifS has been shown to be required for the in vivo formation of 4Fe-4S centers in nitrogenase [36] and to accelerate 2Fe-2S cluster formation in vitro in the *E. coli* transcription factor SoxR [37]. The role of NifS appears to be the mobilisation of inorganic sulphide from L-cysteine [38], and it is tempting to speculate that mammalian homologues of NifS may exist and be involved in iron regulation of IRP-1. Independently of iron, nitric oxide (NO) and hydrogen peroxide (H_2O_2) regulate the aconitase and IRE-binding activities of IRP-1 [39–44]. Biochemical evidence suggests that NO and H_2O_2 also induce cluster removal [42,43], but a definitive answer will require the application of more direct analytical methods. Although both are small, diffusible, reactive molecules, NO and H_2O_2 regulate IRP-1 via different pathways: while NO regulation is kinetically slow (as is iron regulation), H_2O_2-mediated activation of IRE binding occurs within 30–60 min [45]. The regulation of IRP-1 by NO and H_2O_2 strongly suggests that iron-sulphur clusters can serve as regulatory modules in response to stress signals. This notion is corroborated by the recent implication that iron-sulphur clusters may play a role in the stress regulation of two bacterial transcription factors, Fnr [46] and SoxR [47]. Thus, iron-sulphur clusters are beginning to emerge as a new class of switches suited to execute regulatory responses to stimuli such as NO, oxidative stress and oxygen tension.

5. Why two functions?

The role of iron regulated IRE-binding of IRP-1 for the homeostatic, posttranscriptional control of iron storage, uptake and utilisation is easily understandable. Less clear is the physiological role of IRP-1 as a cytoplasmic aconitase. Aconitase activity is not a necessary requirement for the regulation of IRE binding, because mutation of a critical serine (S778) to an alanine causes apparent loss of catalytic activity without affecting iron regulation of IRE binding in vivo [34]. Recent results suggest that the expression of mt-aconitase is translationally regulated by IRP-1 binding to an IRE in the 5′ untranslated region of mt-aconitase mRNA [9]. Taken together with the posttranslational regulation of cytosolic aconitase activity, the translational regulation of mt-aconitase may reflect a metabolic advantage for co-regulation of both

aconitases in response to cellular iron levels, perhaps to affect the catalytic turnover of citrate. Since citrate can bind iron and may serve as an intracellular iron transporter [48], both functions of IRP-1 might conspire to regulate intracellular iron transport. Clearly, this model calls for experimental testing.

Acknowledgements: We thank Gert Vriend for the generation of Fig. 1, Eric Westhof for help with this figure and members of the laboratory for comments on the manuscript. E.P. was the recipient of a HCM EU fellowship.

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