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insights into the phylogenetics of disease systems in general. Critically, neutrality is mediated not only by the accessibility of different structures to the pathogen but also by the hosts’ abilities to detect these differences. The phenotype of the pathogen thus depends on the specifics of the host response. The availability of different phenotypes under this framework may establish major differences between the phylogenetic characteristics of different diseases [e.g., influenza A and measles (3)] and the same disease in different hosts experiencing different ecologies (e.g., influenza A in swine, avian, and equine hosts). There is also evidence that the specificity of the host antibody response to influenza varies within a host population (45). This heterogeneity might have important consequences for the diffusion of strains along antigenically similar networks in genotype space.

This study highlights the necessity of coupling molecular evolution with population-level models to understand the basic aspects of a biological system. In the case of influenza, a more detailed understanding of the structural basis of antigenicity and the dynamics of immune recognition of viral epitopes is of utmost importance. The roles that neutrality and context dependency play in enabling phenotypic change (46) need to be addressed in virological studies. These efforts could help to identify the GP map of influenza’s HA and are critical for vaccine development, because antigenic distances between epidemic strains and vaccine strains determine vaccine efficacy (2). Further inquiries into population-level processes that affect influenza dynamics and evolution, such as the extent of epidemiological mixing and the global circulation of the virus in humans and other host species, are also necessary parts of this research. Integrating findings from these fields will be critical to understanding and managing influenza.

References and Notes

Structure of Dual Function Iron Regulatory Protein 1 Complexed with Ferritin IRE-RNA
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Iron regulatory protein 1 (IRP1) binds iron-responsive elements (IREs) in messenger RNAs (mRNAs), to repress translation or degradation, or binds an iron-sulfur cluster, to become a cytosolic aconitase enzyme. The 2.8 angstrom resolution crystal structure of the IRP1:Ferritin H IRE complex shows an open protein conformation compared with that of cytosolic aconitase. The extended, L-shaped IRP1 molecule embraces the IRE stem-loop through interactions at two sites separated by 6
Iron regulatory protein 1 (IRP1) is a cytosolic, RNA binding protein that regulates the translation or stability of mRNAs encoding proteins for iron transport, storage, and use. IRP1 has an alternate function as cytosolic (c)-aconitase when a [4Fe-4S]-cluster is bound (J−5).

The distribution of IRP1 between these mutually exclusive activities requires no new protein synthesis; iron excess or starvation promotes c-aconitase or RNA binding activity, respectively (6). Assembly and disassembly of the [4Fe-4S] cluster appears to be an effective mechanism for
binding proteins (7, 8). Nuclease protection analysis suggests that IRP1 binding spans the entire length of the IRE stem-loop (9, 10). The conserved nucleotides –CAGUG– (nucleotides 14 to 18 in the RNA used in this study) form a terminal –AGU– loop closed by a C14:G18 base pair, followed by an unpaired nucleotide (2, 11–14). The IRE stem is interrupted by the conserved, unpaired cytosine C8, which divides the helix into a 5–base pair upper stem and a variable length lower stem (2). Sequence and base pairing surrounding the C8 mid-helix distortion are variable among members of the IRE-RNA family (3, 15). A second iron regulatory protein, IRP2, binds the same family of IRE-mRNAs, but is a single-function protein lacking aconitase activity (16). IRP2 displays in vitro RNA binding that is more sensitive than IRP1 to distortions in the mid-helix (15). Variations in the IRE stem-loops among the mRNAs, exemplified by ferritin and mitochondrial aconitase IRE-RNAs, coincide with graded responses to iron signals in vivo (17) and different IRP binding stabilities in vitro (2, 15).

The recently elaborated crystal structure of c-aconitase showed a compact protein conformation, with the residues known to function in enzyme activity or IRE binding buried and relatively inaccessible (18). Clearly, the protein accesses different conformations during binding of alternate ligands, as demonstrated by marked changes in radius of gyration (19). Localized structural changes also have a significant effect. For example, [4Fe-4S] cluster binding or oxidation of Cys437 blocked RNA binding. By contrast, a serine substitution at this position had no effect (2, 20, 21). To determine the nature of the conformational changes underlying the bifunctionality of IRP1, we solved the crystal structure of the Cys437Ser/Cys503Ser double-mutant IRP1 in complex with ferritin H IRE [see Methods in supporting online material and (22)].

**Overall structure of IRP1:IRE complex.** The crystal structure of the IRP1:IRE complex shows...
that the protein binds a single IRE-RNA molecule through contacts at two spatially separate sites. The IRP1 molecule adopts an L-shape in the complex, with aconitase structural domains 3 and 4 extending perpendicularly from a central core composed of domains 1 and 2 (Fig. 1, A and B). The IRE stem-loop is bound across the concave opening of IRP1, with direct contacts from the RNA terminal loop and the RNA lower stem (Fig. 1, A and C). Exposed terminal loop residues A15 and G16 extend into a large protein cavity at the interface between domains 2 and 3. Bonds to A15, G16, and U17 provide specificity and stability to interactions at this site (Fig. 1C). Protein binding to the stem of the IRE is centered on the C8 nucleotide, which inserts into a pocket on the inner face of domain 4. A 31° bend in the RNA helix redirects the path of the lower stem along the same face of domain 4, which binds the side of the IRE lower stem farthest from the terminal loop (Fig. 1A). Mutagenesis and binding studies of IRP1:IRE interactions indicated that residues from each of the four structural domains of IRP1 contributed to IRE binding (23-27). Although the electron density for domain 1 residues 126 to 146 is unresolved, it suggests that some protein:RNA contacts here might be revealed with higher resolution data.

The high binding affinity and specificity of IRP1:IRE interactions are explained by the many protein:RNA contacts observed in the terminal loop and stem-binding regions (Fig. 1C). The interfaces cover ~1400 Å² for the terminal loop and ~1300 Å² for the stem, each comparable in area to individual binding sites for other protein:RNA complexes (28). The terminal loop and C8 of the IRE are separated by ~30 Å, held in their orientations by the 5–base-pair helix of the upper stem. Selectivity among different IREs may depend in part on helix parameters that reflect variations in the intersite distances, base pair sequences, and/or local distortions in the upper stem [e.g., (29)].

**IRP1 structure and comparison with c-aconitase.** The structural differences between IRP1 in the protein:RNA complex and c-aconitase are larger than those previously predicted from aconitase structures (18, 23). Although most of aconitase domains 1 and 2 form a single core in IRP1 with moderate changes relative to each other, there are extensive changes in domains 3 and 4. To a first approximation, these changes can be described as rigid body motions relative to the core (Fig. 2 and movie S1). Domain 4 rotates by 32° and translates ~14 Å. This eliminates the interactions between domains 3 and 4 that are present in c-aconitase. Instead, most of these surfaces are incorporated into the two separate RNA binding sites. Domain 4 is attached to domain 1 through strong hydrophobic contacts (18). As a result, residues 90 to 170 of domain 1 move with domain 4 during the conformational changes (Fig. 2 and movie S1).

Domain 3 displays the largest conformational difference between c-aconitase and the IRP1:IRE complex, with a rotation of 52° resulting in a translation of ~13 Å (Fig. 2 and movie S1). This difference was unexpected and contrasts with the predicted, and observed, movement of domain 4 (18, 23-26). Because the interface between domain 3 and the core domains 1 and 2 is only moderately buried in both conformations and is populated with more hydrogen-bonding groups than hydrophobic interactions, a major rearrangement is possible while adhesion is retained at the interface. A conformational change in the polypeptide linker joining domains 3 and 4 is central to their repositioning relative to the core. In c-aconitase, residues 593 to 614 in the linker comprise two helices, α21 and α22, separated by a bend preceding Proα20 (18). In the IRP1:IRE complex, formation of a single continuous helix over residues 593 to 614 facilitates the large domain shifts (movie S1).

**Regulation of IRE binding.** The large-scale domain rearrangements necessary for IRP1 to switch between c-aconitase and IRE-binding functions appear coupled to key internal reconfigurations of the protein, ultimately linked to the ligand-binding groups. The shift of domain 3 is accompanied by reconfiguration of two functionally important segments at the interface of domains 2 and 3, the 430 region (residues 436 to 442), and the 530 loop (residues 534 to 544). These segments play dual structural and functional roles by forming the ligand-binding environment for the Fe-S cluster in c-aconitase or by making IRE contacts in the complex. In c-aconitase, Cysα347 is one of the three iron-binding residues for the Fe-S cluster and is part of the tight, single-turn helix α14 [Fig. 3A and (18); Cysα503 and Cysα506 are the other cluster ligands]. In sharp contrast, α14 becomes an extended loop in the IRE-bound IRP1, which places residue 437, a serine in the mutant protein used here, in the terminal loop-binding cavity (Fig. 3B and movie S2). Serα373 has no direct RNA interactions, whereas Thrα318 and Asnα339 are in new positions and make direct contacts with the bound IRE.

The 530 loop contributes bonds to the RNA and occupies the space taken by the Fe-S cluster in c-aconitase. The configuration of the 530 loop in the protein:RNA complex is associated with the helix-to-loop change of α14 (Fig. 3 and movie S2). The 430 and 530 loops stabilize each other in the protein:RNA complex, forming a rim of the protein cavity that binds the RNA terminal loop. Mutation of Argα23 to either lysine or glutamine greatly reduces IRE binding affinity (27). This is rationalized by the network of hydrogen bonds that Argα451 makes to stabilize the entire 530 loop (movie S2). Replacing Argα536 with glutamine also decreases IRE binding (27), which is explained by the direct contact between Argα456 and the 2′OH of U20, over a distance too great to be spanned by a glutamine (Fig. 3B). Finally, a triple interaction between the 530 loop, the 430 region, and the IRE terminal loop stabilizes the protein:RNA complex, with Asnα535 establishing four hydrogen bonds: two to Thrα338 and two to the IRE.

The repositioning of domain 4 also occurs through key localized conformational changes. The pivot point for domain 4 movement is in domain 1 near Glyα90 of α4, ~13 Å away from the Fe-S cluster in c-aconitase. α4 has an unusual sequence with two helix-destabilizing residues (Glyα90 and Proα92) separated by a bend preceding Proα91 (18). In the IRP1:IRE complex, formation of a single continuous helix over residues 593 to 614 facilitates the large domain shifts (movie S1).

![Fig. 2. Differences in domain positions between c-aconitase and IRP1:IRE-RNA complex. The c-aconitase structure is from Dupuy et al. (18); Protein Data Bank 2B3X. Domains 1 and 2 of c-aconitase (left) correspond to the core in the IRP1:IRE complex (right). The two molecules are displayed in the same orientation to show the displacements of domains 3 and 4. The molecular surfaces are semitransparent to reveal the internal [4Fe-4S] cluster in c-aconitase, and the axes of rotation around which domains 3 and 4 pivot. The arcs show the motion of the centers of mass of domains 3 and 4. Domain 4 remains attached to domain 1 through hydrophobic contacts between the carboxy-terminal helix α31 (residues 879 to 889) and the buried helix α4 (residues 87 to 106), plus surrounding hydrogen-bonding interactions. As a result, the pivot axis for domain 4 extends through domain 1 near α4 and α31. Domain 3 pivots around the axis on the interface between it and the core. See also movie S1.](image-url)
to a β turn, and domain 4 has pivoted more than 30° with minimal internal distortion. The reversible disruption of α4 may be a mechanism linking the rotational freedom of domain 4 to the structural integrity of the Fe-S cluster.

**Overall structure of ferritin H IRE in the complex.** The ferritin IRE has 12 base pairs, all with Watson-Crick bonding, except for the wobble pair U5:G26 (Fig. 1C). Although the bound IRE retains the general stem-loop structure observed by nuclear magnetic resonance (NMR) spectroscopy (13, 14), there are several significant differences. The IRE −CAGUGU−terminal loop forms a pseudotriloop (30) both in the complex and in solution. The defining elements of the ferritin H IRE pseudotriloop are the −AGU− triplet, isolated by the conserved C14-G18 base pair, followed by the unpaired U19. Neither base in the C14-G18 bridge makes direct contact with the protein, which suggests that the bridge serves only a structural role for IRP1 recognition. In the NMR structures of free IREs, A15 is stacked on top of the C14-G18 pair; G16 and U17 are disordered (12–14). As mentioned above, the base of C14 occupies a pocket on the inner surface of domain 4, sandwiched between Arg713 and Arg780, two active site residues of c-aconitase (Fig. 4B). Arg780, whose mutation to asparagine abolishes IRE binding (27), is also in range for ionic interactions with the 3′ phosphate of C8 (Figs. 1C and 4B). The base of C8 has hydrogen bonds to Ser681, Pro682, Asp781, and Tyr782 (Fig. 4B). An unusual feature of the C8 pocket is that three of the four intrapocket bonds (the exception is Ser681) involve backbone atoms.

**Details of IRP1:IRE-RNA interactions.** The wide separation of the two IRE contact sites in IRP1 allows each to contribute individually to binding affinity and specificity. The terminal loop-binding cavity of IRP1 is generated by the displacement of domain 3 from domains 1 and 2 and, consequently, only exists in the IRE-binding conformation (movie S1). The IRP1:IRE terminal loop interactions are built around the main bonding features of the −AGU− pseudotriloop (Fig. 1C). As mentioned above, A15 and G16 extend into the terminal loop-binding cavity and make base-specific bonds with Ser577 and Lys579, respectively, and van der Waal’s contacts with residues lining the walls of the cavity (Figs. 1C and 4A). U17, the top stacked base of the upper stem, makes one base-specific bond with Arg609 at the cavity opening. Additional bonds to the RNA backbone of the terminal loop further strengthen the protein:RNA interaction (Figs. 1C and 4A). Differences between solution conformations of the IRE terminal loop and the conformation in the IRP1:IRE complex are consistent with a mutually induced fit mechanism of binding.

Conserved C8 is the focal point for IRE stem binding with IRP1 (Fig. 1C), consistent with mutagenesis studies that showed a preference for a cytidine at position 8 (2, 31). As mentioned above, the base of C8 occupies a pocket on the inner surface of domain 4, sandwiched between Arg713 and Arg780, two active site residues of c-aconitase (Fig. 4B). Arg780, whose mutation to asparagine abolishes IRE binding (27), is also in range for ionic interactions with the 3′ phosphate of C8 (Figs. 1C and 4B). The base of C8 has hydrogen bonds to Ser681, Pro682, Asp781, and Tyr782 (Fig. 4B). An unusual feature of the C8 pocket is that three of the four intrapocket bonds (the exception is Ser681) involve backbone atoms.

Specificity likely comes from the small volume of the C8 pocket and the bonding pattern. Substitution of C8 to G or A is sterically prohibited, whereas a U substitution would cause the loss of the H-donor capacity of the N4 amino group.

An IRP-specific subdomain (residues 680 to 730), predicted to be involved in RNA binding (18, 25), cradles the lower stem of the IRE, with residues 681 to 685 running along the minor groove of the lower stem of the ferritin H IRE. At the closest approach between the protein and RNA in this region, the side chain of Asn685
protrudes into the minor groove and binds to the N2 of G26 (Fig. 4B). Displacement of G26 by nonstandard pairing with U5 facilitates the interaction. The U5:G26 wobble pair is not conserved in IREs, and asparagine is replaced by serine at the position equivalent to 685 in IRP2 and all non-IRE-binding homologs of IRP1, such as c-aconitase in plants and Caenorhabditis elegans. The natural variations in the IRE lower stem and the 685 position in IRPs may also be important determinants for IRP:IRE selectivity.

Basis for IRE-RNA selectivity. The effect of binding a single IRE molecule through interactions at two separate binding sites essentially eliminates the possibility of significant interactions with nonspecific RNAs. Two-point recognition in the IRP1:IRE complex is analogous to the transfer RNA (tRNA):aminoacyl-tRNA synthetase complexes, where the individual RNA binding sites relate to the dual requirements of tRNA selectivity and aminoacylation (32). An advantage of two-point recognition in IRP:IRE interaction may be the quantitatively different responses observed for different IRE-mRNAs to the same biological signal (3, 4, 17). Conserved sequence and structure at the IRE terminal loop and C8 is coupled with variation in helix sequence and mid-helix distortion near C8, known to influence IRP binding (15, 33, 34). Such IRE-mRNA–specific variations may establish different protein:RNA interactions. The excess IRP1 binding capacity apparent in the IRP1:IRE complex supports such a model. Several amino acids are approximately in position for hydrogen bonding (e.g., Arg153, His207, Lys266, Gln377, Arg704, and Ser778), but too far (>3.7 Å) for direct contact with the ferritin H IRE. Thus, it is possible that in IRP1 (and, by inference, IRP2) alternate subsets of bonding groups are used for recognition of different IRE-RNAs. Such a combinatorial mode of binding would explain the graded response of different IRE-mRNAs to IRP regulation.

Conformational plasticity and dual functionality. The RNA-binding and enzyme active sites extensively overlap in IRP1, with many amino acids serving important, but different, roles in each functional state of the protein (Fig. 1C) (18). The functional plasticity of amino acids serving both catalytic and RNA binding roles reflects the conformational flexibility of the protein, particularly in the vicinity of the Fe-S and RNA binding sites. Given the high conservation of the RNA binding residues among c-aconitases, including those c-aconitases that lack IRE-binding ability (e.g., (35)), residues that confer the structural flexibility may be the key determinants of RNA binding.

Many of the details of IRP1:IRE interaction revealed in this work should also apply to IRP2, on the basis of the high sequence similarity and IRE-RNA binding preferences shared between the IRPs. What remains to be determined is the evolutionary origin and selective advantage of such dramatic conformational plasticity and dual functionality encoded in IRP1.

References and Notes
A Gaseous Metal Disk Around a White Dwarf


The destiny of planetary systems through the late evolution of their host stars is very uncertain. We report a metal-rich gas disk around a moderately hot and young white dwarf. A dynamical model of the double-peaked emission lines constrains the outer disk radius to just 1.2 solar radii. The likely origin of the disk is a tidally disrupted asteroid, which has been destabilized from its initial orbit at a distance of more than 1000 solar radii by the interaction with a relatively massive planetesimal object or a planet. The white dwarf mass of 0.77 solar mass implies that planetary systems may form around high-mass stars.

White dwarfs are the compact end products of stars with masses up to ~8 solar masses (M⊙) (1). Because of the low luminosity of white dwarfs, the detection of low-mass stellar companions (2) or planets (3) is much easier around white dwarfs than around main-sequence stars. During a search for cool companions to white dwarfs, an excess infrared flux was discovered around the white dwarf G29-38 (4). The atmosphere of G29-38 has been found to be enriched with metals, i.e., elements heavier than helium. The sedimentation time scales of heavy elements in the high-gravity atmospheres of white dwarfs are short compared to the evolutionary time scale of these stars (5) and, hence, the high metal abundances in G29-38 imply that this star is accreting at a relatively high rate (6).

Deep imaging and asteroseismological studies of G29-38 ruled out a brown dwarf companion (7, 8) and led to the hypothesis of a cool dust cloud around the white dwarf. The presence of dust near G29-38 has been verified by infrared observations with the Spitzer Space Telescope (9). Infrared surveys of white dwarfs exhibiting metal-enriched atmospheres recently led to the discovery of three other potential dust disks (10–12). A possible origin of such dust disks is the tidal disruption of either comets (13) or asteroids (14). Asteroids appear to be more likely candidates because they can explain the large amount of metals accreted by the white dwarfs from the dusty environment, as well as the absence of hydrogen or helium. Although the detection of asteroid debris around G29-38 and the other white dwarfs represents a possible link to the existence of planetary systems around their main-sequence progenitor stars, modeling the excess infrared luminosity provides no direct information on the geometric location and extension of the dust, impeding a more detailed understanding of the nature and origin of the circumstellar material (9). A concentration of dust in the equatorial plane around G29-38 has been suggested on the basis of the relative amplitudes of non-radial white dwarf pulsations observed in the optical and infrared wavebands (7).

We identified SDSS J122859.93+104032.9 (henceforth SDSS 1228+1040) as a moderately hot white dwarf in the Data Release 4 of the Sloan Digital Sky Survey (SDSS) (15), but noted very unusual emission lines of the Ca II 850- to 866-nm triplet, as well as weaker emission lines of Fe II at 502 and 517 nm. The line profiles of the Ca II triplet display a distinct double-peaked morphology, which is the hallmark of a gaseous, rotating disk (16, 17). Time-resolved spectroscopy (Fig. 1) and photometry do not reveal any radial velocity or brightness variations. These data exclude the possibility that SDSS 1228+1040 is an interacting white dwarf binary, in which an accretion disk around the white dwarf forms from material supplied by a nearby companion star. Furthermore, the absence of Balmer and helium emission lines implies that the gaseous disk around SDSS 1228+1040 must be extremely deficient in volatile elements, which independently rules out an interacting binary nature for this object.

Our detection of double-peaked metal emission lines from a circumstellar disk in SDSS 1228+1040 provides direct evidence for hydrogen- and helium-rich circumstellar disks around white dwarfs. Further studies will benefit from the ability of the future Laser Interferometer Space Antenna and other space-based instruments to resolve and measure the detailed structure of circumstellar disks around white dwarfs.