The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule

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AUTHOR SUMMARY

It is estimated that up to one-third of all proteins synthesized by eukaryotic cells are initially trafficked through the specialized environment of the endoplasmic reticulum (ER). In this subcellular compartment, conditions are optimized for protein folding and entry to the ER represents the first commitment step toward the secretory pathway. Diseases as diverse as cancer, neurodegeneration, and metabolic disorders such as insulin resistance and type II diabetes mellitus are associated and often caused or exacerbated by the failure of proteins to fold correctly in the ER. The evolutionary response to this potential toxicity is a tight regulation of components of this cellular compartment. Perturbances in the protein-folding environment of the ER are detected and corrected by a cellular stress response known as the unfolded protein response, the most ancient component of which is initiated by an ER-localized transmembrane protein called IRE1 (1). IRE1 has an ER luminal domain that senses unfolded proteins and transmits a signal across the ER membrane to the effector domain of the protein in the cytosol. This effector domain is endowed with two enzymatic activities: a protein kinase and a RNase, both of which are activated in response to the accumulation of unfolded proteins in the ER (so-called ER stress).

In animals, IRE1 activity splices the latent messenger RNA (mRNA) of unconventional mRNA splicing by an enzyme. Structure shows human IRE1 protomer (Protein Data Bank ID code 3P23, Left) and the detail (Lower Right, residues 870 to 939) shows computationally docked 4μ8C (green) at K907, with residues F900, Y902, N908, K907, and H910 highlighted.

Fig. P1. Targeted inhibition of IRE1. 8-formyl-7-hydroxy-4-methylcoumarin (abbreviated 4μ8C, see inset to graph), identified by high-throughput screening, was found to inhibit the endonuclease activity of mammalian IRE1 with high selectivity in both an in vitro FRET-derepression assay (see graph) and cultured cells. The compound binds to a critical lysine in the endonuclease active site of IRE1 by formation of an unusually stable Schiff base. IRE1 K907-4μ8C modification constrains the flexibility of the endonuclease site by formation of stacking interactions with F900 and interjects between essential catalytic residues, inactivating the enzyme. Structure shows human IRE1 protomer (Protein Data Bank ID code 3P23, Left) and the detail (Lower Right, residues 870 to 939) shows computationally docked 4μ8C (green) at K907, with residues F900, Y902, N908, K907, and H910 highlighted.

It is estimated that up to one-third of all proteins synthesized by eukaryotic cells are initially trafficked through the specialized environment of the endoplasmic reticulum (ER). In this subcellular compartment, conditions are optimized for protein folding and entry to the ER represents the first commitment step toward the secretory pathway. Diseases as diverse as cancer, neurodegeneration, and metabolic disorders such as insulin resistance and type II diabetes mellitus are associated and often caused or exacerbated by the failure of proteins to fold correctly in the ER. The evolutionary response to this potential toxicity is a tight regulation of components of this cellular compartment. Perturbances in the protein-folding environment of the ER are detected and corrected by a cellular stress response known as the unfolded protein response, the most ancient component of which is initiated by an ER-localized transmembrane protein called IRE1 (1). IRE1 has an ER luminal domain that senses unfolded proteins and transmits a signal across the ER membrane to the effector domain of the protein in the cytosol. This effector domain is endowed with two enzymatic activities: a protein kinase and a RNase, both of which are activated in response to the accumulation of unfolded proteins in the ER (so-called ER stress).

In animals, IRE1 activity splices the latent messenger RNA (mRNA) that encodes XBPI. This triggers a cascade of events that activate XBPI, a potent transcription factor that upregulates genes that will enhance the ability of the ER to cope with unfolded proteins and upregulate secretory capacity (2, 3). Thus, IRE1 activity results in rectifying gene expression changes that include the enhanced expression of specialist protein folding enzymes and degradation components to clear misfolded proteins, expanding the ER apparatus to cope with the protein folding load. In animal cells, IRE1 has also been linked to the promiscuous degradation of ER-localized mRNAs in a process known as regulated IRE1-dependent degradation (or RIDD), but the mechanistic basis and functional consequences of this process are presently incompletely understood (4).

Although the unfolded protein response in simple eukaryotes comprises only IRE1, in animals two other regulatory pathways arise from the ER when proteins fail to fold in this compartment. The translation initiation factor 2α (eIF2α) kinase PERK constitutes a second branch and acts to attenuate ER load by inhibiting protein synthesis, and a third branch is mediated through transcriptional regulation by ATF6. For many years, redundancy between the long-term effects mediated by the three arms of the unfolded protein response has obscured the interpretation of genetic experiments to ascertain the unique role of each of these three components, IRE1, PERK, and ATF6 (1). These considerations, among others, have generated an interest in selective inhibitors of unfolded protein response


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components, both as tools for fundamental research and as potential anticancer, antiinflammatory, and antiviral therapeutic agents.

In this study, we used a fluorescence-based high-throughput screening program adapted from our studies of the yeast enzyme (5) to search for small molecule inhibitors of the mammalian IRE1. We found and characterized 8-formyl-7-hydroxy-4-methylcoumarin (abbreviated herein as 4μ8C) as a potent and selective inhibitor of IRE1. Using the unique spectroscopic properties of 4μ8C to trace its interaction with IRE1 in vitro, we discovered that the compound reacts covalently with the enzyme to form a stable Schiff base at two critical lysines in both the kinase and RNase pockets of the molecule. In cells this modification is limited to the RNase site at Lysine 907, dissecting the catalytic moieties of the IRE RNase and inactivating the enzyme (see Fig. P1).

We demonstrate that Lysine 907 is the target of all extant IRE inhibitors and explored this unifying mechanism for IRE1 inhibition with docking and molecular dynamics simulations. In contrast to other lysine residues on the molecule, Lysine 907 is buried in the enzyme active site. The inhibitor is held in place by hydrophobic and stacking interactions with protein side chains in the active site to specify an unusually stable Schiff base at Lysine 907 and accounting for the surprising selectivity of the drug for IRE1.

We used the inhibitor to unequivocally link the IRE1 RNase to the phenomenon of ER stress-induced mRNA decay, because 4μ8C rapidly blocks this process both in vitro and in cell culture. Moreover, our studies revealed that in mammalian cells, unlike in yeast and worms, IRE1 has no measureable role in cell survival during acute ER stress. Rather, loss of IRE1 function following 4μ8C treatment caused a pronounced block in ER expansion and secretory output and attenuated the growth of multiple myeloma cells. These observations suggest that in animals the IRE1 branch of the unfolded protein response has specialized to adapt cells to a heavy secretory load and is no longer important in day-to-day ER protein folding homeostasis.

This study introduces a tool for studying the unfolded protein response and suggests that IRE1 inhibitors may find their greatest clinical utility in circumstances of on-going differentiation in pathogenic secretory cells, exemplified by malignant plasma cells in multiple myeloma or mucous-producing cells in chronic obstructive pulmonary disease.