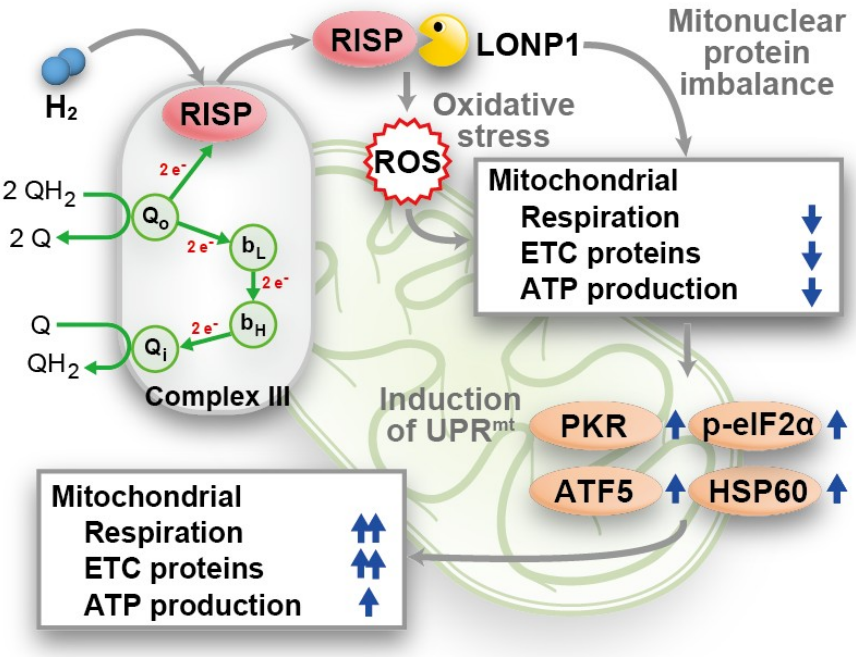
News Release

Molecular hydrogen primarily targets the Rieske iron-sulfur protein -Mitohormesis is the identity of the therapeutic effects of molecular hydrogen

Key Points

- H_2 primarily targets the Rieske iron-sulfur protein (RISP) in the mitochondrial electron transport chain (ETC) complex III.
- The targeting initiates LONP1-mediated degradation of RISP.
- Mitonuclear imbalance induces mitochondrial unfolded protein response (UPR^{mt}).
- The consequent mitohormesis accounts for the therapeutic effects of H_2.
- Paradoxical and pleiotropic effects of H_2 on redox and inflammatory markers have been explored.



Summary

The mechanisms underlying the biomedical effects of molecular hydrogen (H_2) remain poorly understood and are often attributed to its selective reduction of hydroxyl radicals, based on the long-held notion that H_2 is biologically inert. Drs. Shuto Negishi, Mikako Ito, and Kinji Ohno at Nagoya University Graduate School of Medicine, and Dr. Tyler W. LeBaron at Southern Utah University and Molecular Hydrogen Institute demonstrates that H_2 is biologically active, specifically targeting the Rieske iron-sulfur protein (RISP). The research team first observed that

H_2 induces the mitochondrial unfolded protein response (UPR^{mt}) in cultured cells exposed to H_2 and in mouse liver after H_2 water administration. H_2 suppressed electron transport chain complex III activity in mouse liver homogenates to 78.5% within 2 min. Given the evolutionary link with hydrogenases, they examined RISP as a potential target of H_2. They found that H_2 promotes RISP degradation within one hour in cultured cells by activating mitochondrial Lon peptidase 1 (LONP1). Loss of RISP and subsequent UPR^{mt} induction may explain the pleiotropic and paradoxical effects of H_2. Professor Ohno, the team leader, says that these findings identify RISP as a primary target of H_2, demonstrating that H_2 is biologically active as a signaling molecule.

This study was published online in the *Redox Biology* on December 2, 2025.

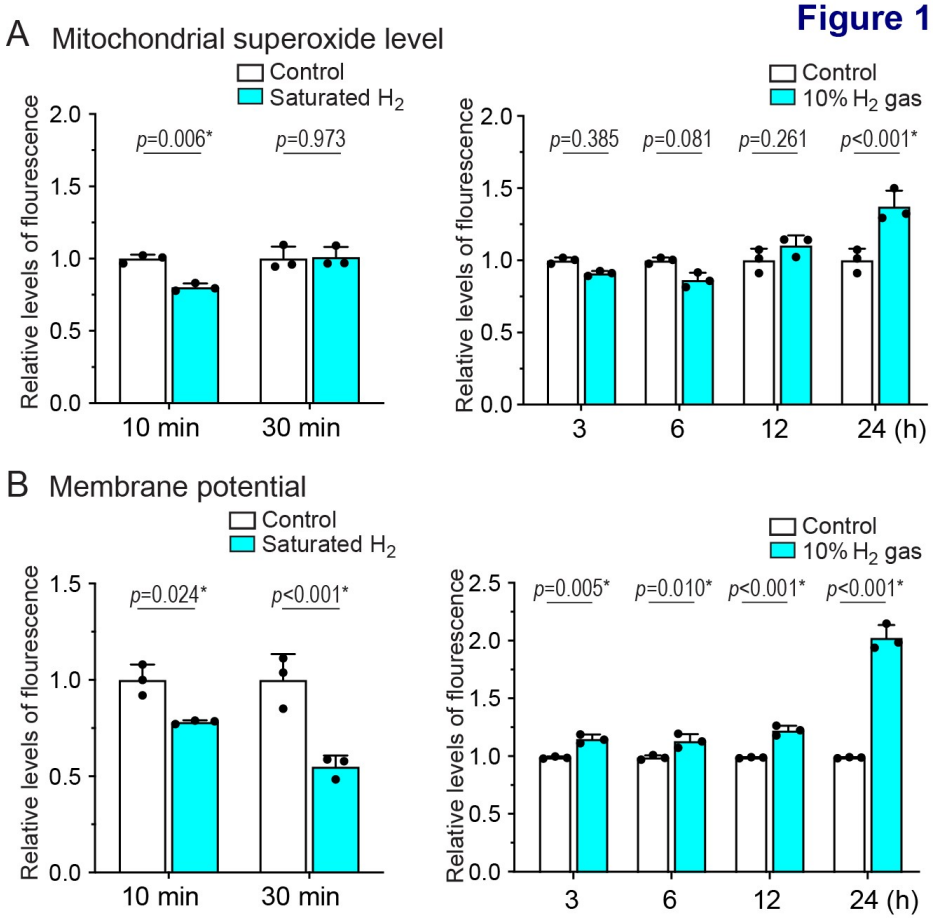
Research Background

Molecular hydrogen (H_2) has historically been considered biologically inert due to its small, nonpolar nature and the absence of hydrogenase enzymes in humans, which justified its use in deep-sea diving. However, emerging evidence, including its association with improved health and extended lifespan, challenged this view. A pivotal shift occurred with studies in 1975 and 2007, suggesting H_2's therapeutic effects against tumors and brain damage, initially attributed to its function as a hydroxyl radical (•OH) scavenger. This antioxidant mechanism is now challenged by H_2's slow reaction rates and low concentrations *in vivo*. While recent work shows H_2 can interact with Fe-porphyrin, these effects are likely too minimal to explain the observed prolonged biological protection. Given these limitations, the research teams hypothesized that H_2 might interact with an evolutionarily conserved hydrogenase-like protein like the Rieske iron–sulfur protein (RISP) in mitochondrial electron transport chain complex III, which contains a [2Fe-2S] cluster as observed in the catalytic center of hydrogenases.

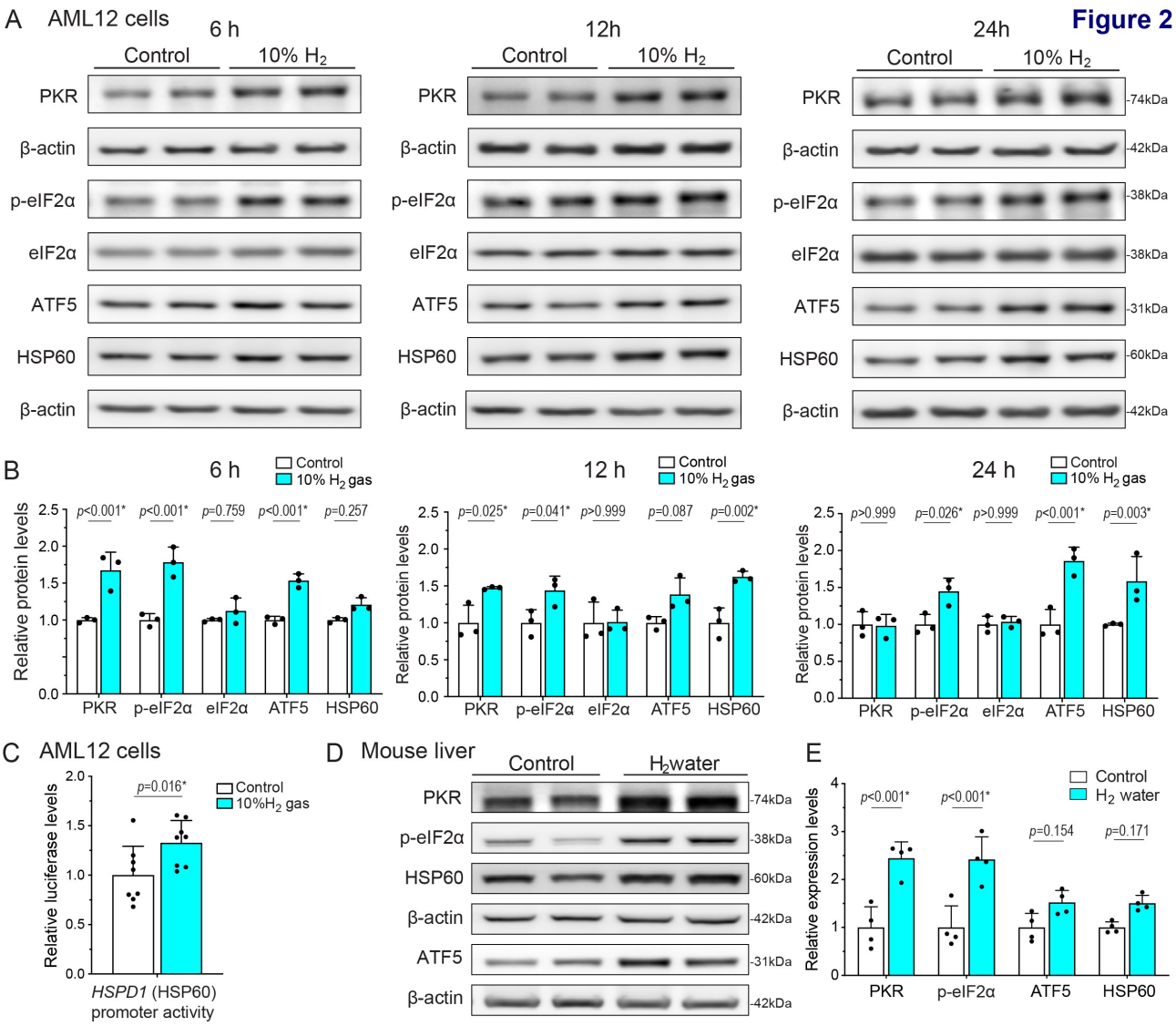
Research Results

H_2 initially decreases and subsequently increases the mitochondrial superoxide level and the mitochondrial membrane potential in cultured mammalian cells: Mouse liver-derived AML12 cells cultured under 10% H_2 decreased the mitochondrial superoxide level and the mitochondrial membrane potential in 10–30 min compared to control cells cultured under 10% N_2 (**Figure 1**). Both mitochondrial markers were subsequently upregulated. The early decrease and late increase of superoxide production and membrane potential suggest inhibition of the mitochondrial ETC and subsequent induction of mitohormesis.

Figure 1

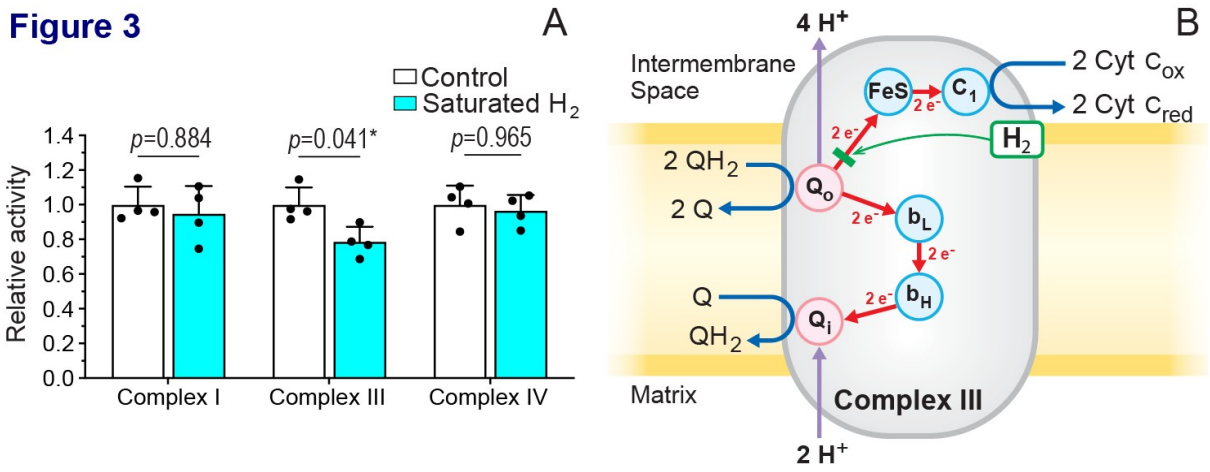


H_2 induces UPR^{mt} in cultured mammalian cells and wild-type mouse liver: To determine the involvement of mitohormesis, the research team examined whether H_2 induces UPR^{mt} in AML12 cells and in wild-type mouse liver. Exposure of AML12 cells to 10% H_2 gas for six hours or longer increased the levels of UPR^{mt}-related proteins (PKR, p-eIF2α, ATF5, and HSP60) (**Figure 2AB**) and increased the promoter activity of *Hspd1* encoding HSP60 (**Figure 2C**). Similarly, wild-type mice that were administered H_2-rich water *ad libitum* for four weeks exhibited elevated levels of UPR^{mt}-related proteins (PKR, p-eIF2α, ATF5, and HSP60) in the liver (**Figure 2DE**).



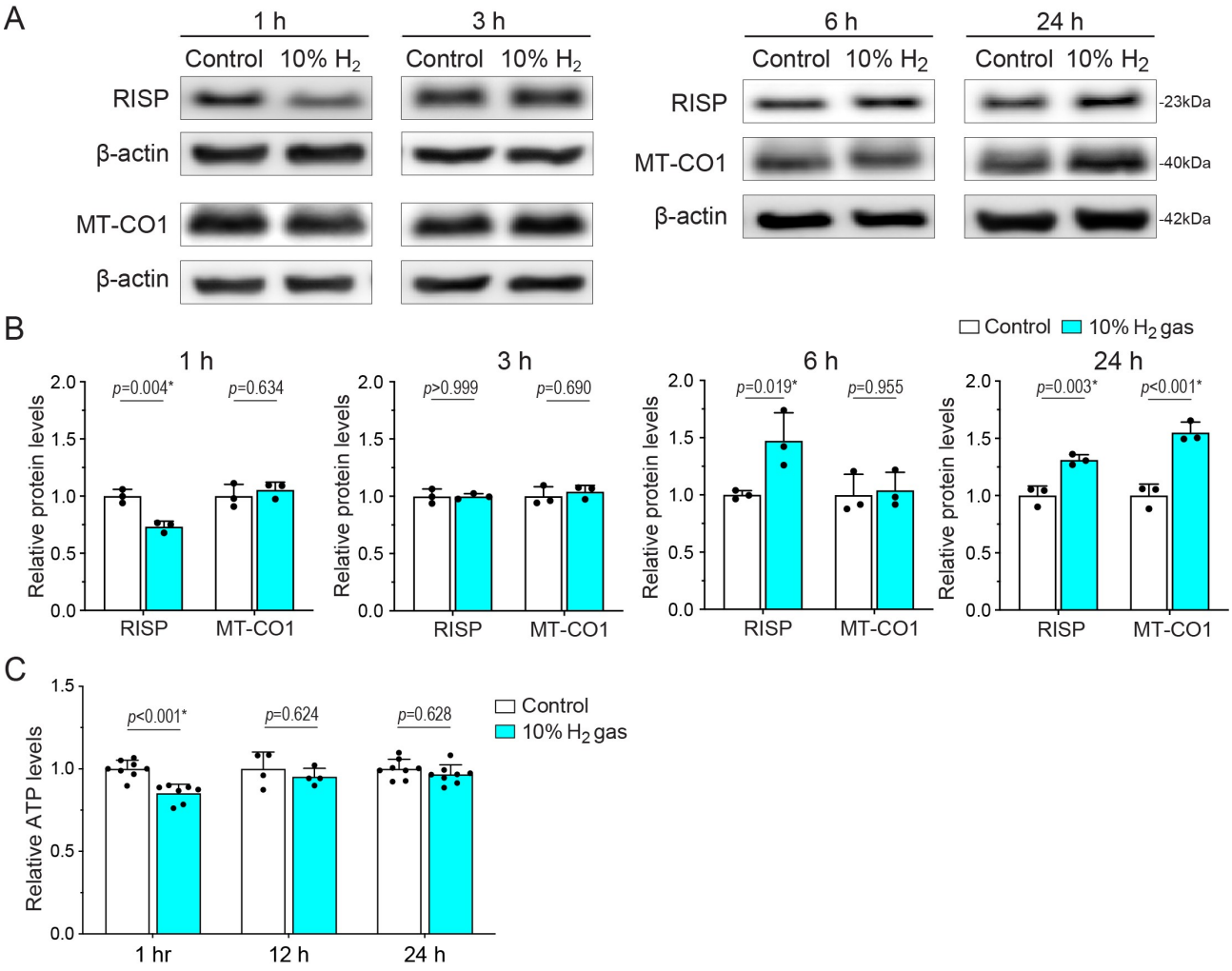
H_2 blocks electron flow in ETC Complex III in the FeS-*c1* pathway: The research team next examined the effects of H_2 on the activities of mitochondrial ETC complexes I, III, and IV, which together constitute the major pathway to generate ATP. Exposure of mitochondria isolated from wild-type mouse liver to H_2-rich buffer for as little as two minutes suppressed the enzymatic activity of ETC Complex III by 78.5%, but not those of Complex I or IV (**Figure 3A**). Further dissection reveals that the target of H_2 is likely to be in the FeS-*c1* pathway, where FeS is a core component of the RISP (**Figure 3B**).

Figure 3



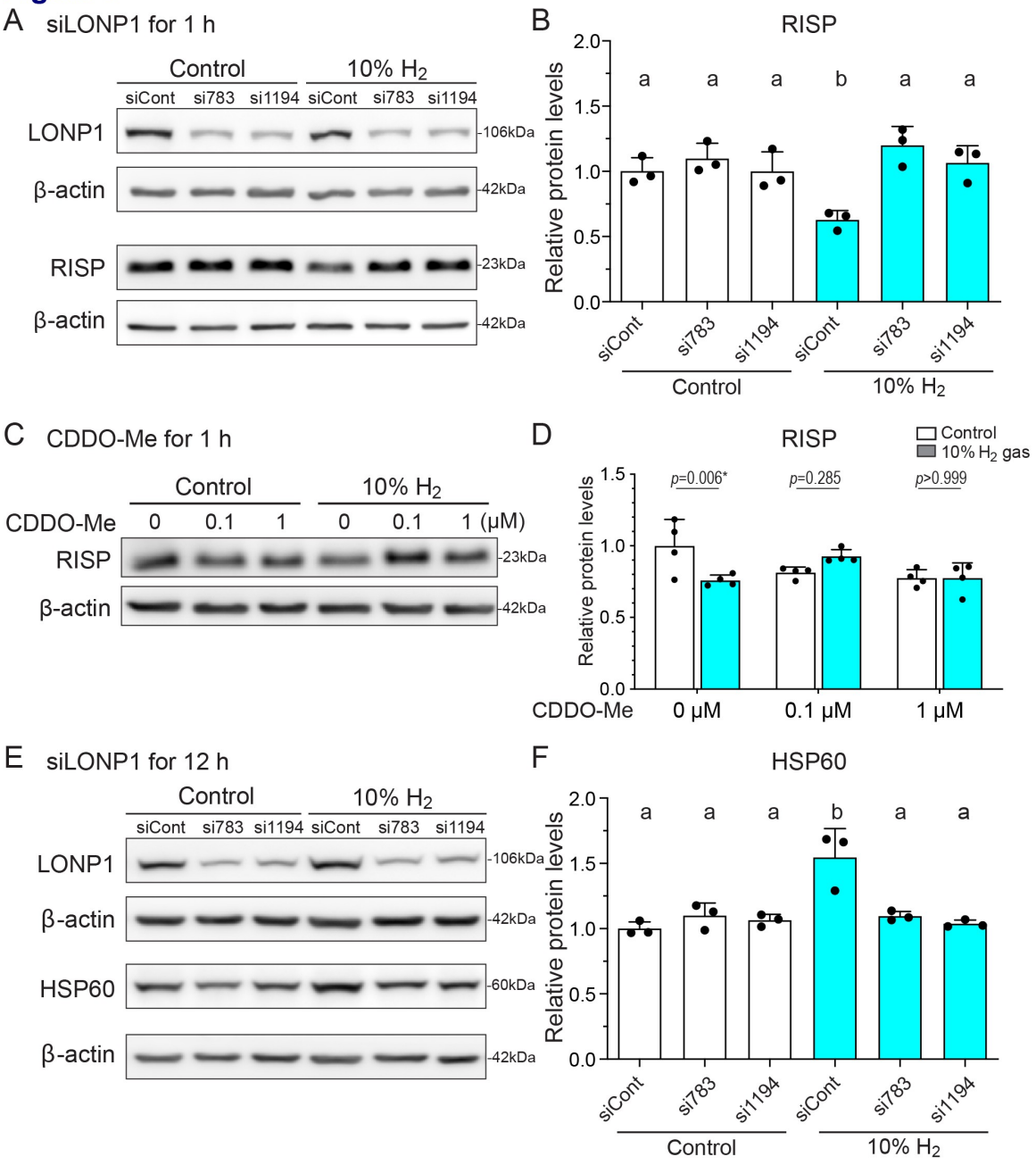
H_2 targets and decreases RISP: Hydrogenases that directly react with H_2 as substrate contain an iron-sulfur cluster (Fe-S cluster). RISP is the only component in Complex III containing an iron-sulfur cluster, and directly accepts electrons at the Q_0 site in the FeS-c_1 pathway (**Figure 3B**). The research team found that exposure to H_2 gas for one hour decreased RISP in AML12 cells (**Figure 4AB**). The RISP level resumed to baseline at three hours and increased further at six and 24 hours (**Figure 4AB**). In accordance with the decrease of RISP, H_2 decreased the ATP level to at one hour, but resumed it to the basal level at 12 and 24 hours (**Figure 4C**). In contrast, the level of cytochrome *c* oxidase subunit I in complex IV that is encoded by *MT-CO1* on mitochondrial DNA remained unchanged up to six hours but was increased to at 24 hours (**Figure 4AB**). As mitonuclear protein imbalance is one of the major causes inducing UPR^{mt}, transient reduction of RISP at one hour likely initiated UPR^{mt}, which subsequently led to a compensatory increase in both RISP and MT-CO1 in 24 hours.

Figure 4



Mitochondrial Lon peptidase 1 (LONP1) mediates the H_2-induced degradation of RISP: LONP1 is a major mitochondrial protease that selectively degrades misfolded, unassembled, or damaged polypeptides in mitochondria, and plays a substantial role in the induction of UPR^{mt}. As expected, knockdown of LONP1 nullified the effects of H_2 on the decrease of RISP in 1 hour (**Figure 5AB**). Similarly, a LONP1 inhibitor, CDDO-Me, cancelled the effects of H_2 on the decrease of RISP in 1 hour (**Figure 5CD**). In addition, LONP1 knockdown cancelled the increase of HSP60 expression by H2 gas in 12 hours (**Figure 5EF**).

Figure 5



Research Summary and Future Perspective

The research team demonstrates that molecular hydrogen (H_2) is biologically active, specifically targeting the Rieske iron-sulfur protein (RISP) within mitochondrial electron transport chain (ETC) Complex III, thereby challenging the long-held notion that H_2 is biologically inert. H_2 promotes the degradation of RISP in cultured cells within one hour via activation of mitochondrial Lon peptidase 1 (LONP1). This initial effect causes the suppression of ETC Complex III activity, which was reduced to 78.5% within 2 minutes in mouse liver homogenates. The subsequent transient reduction of RISP leads to a mitonuclear protein imbalance that induces the mitochondrial unfolded protein response (UPR^{mt}). This cycle

of stress induction and adaptive recovery exemplifies a hormetic interaction, which readily accounts for the temporally diverse and seemingly paradoxical effects of H_2 on various redox and inflammatory markers.

Elucidating this precise mechanism provides critical insights to guide the design and interpretation of clinical trial protocols aimed at optimizing the therapeutic potential of H_2. Nonetheless, further research is needed to determine the exact conformational changes in RISP induced by H_2 and the specific mechanism by which LONP1 recognizes the H_2-exposed RISP for degradation.

Glossary

Rieske iron–sulfur protein (RISP): RISP is a crucial component of Complex III (also known as cytochrome *bc1* complex) in the mitochondrial electron transport chain (ETC). Its primary function is to facilitate the transfer of electrons and contribute to the generation of the proton gradient necessary for ATP synthesis.

Mitochondrial electron transport chain (ETC): The mitochondrial ETC, also known as the respiratory chain, is a critical series of protein complexes and electron carriers embedded in the inner mitochondrial membrane. Its primary purpose is to produce the vast majority of the cell's energy in the form of adenosine triphosphate (ATP), a process called oxidative phosphorylation (OXPHOS).

Lon Peptidase 1 (LONP1): LONP1 is a highly conserved, ATP-dependent serine protease located primarily in the mitochondrial matrix. Its main functions are:

1. Proteolytic activity: It selectively degrades damaged, misfolded, or aggregated proteins, preventing their accumulation and preserving mitochondrial function.
2. Chaperone activity: It assists in the proper folding and assembly of newly synthesized and imported mitochondrial proteins.
3. UPR^{mt} mediator: As a key component of the UPR^{mt}, its upregulation and activity are crucial for clearing stress-damaged proteins, thereby promoting cellular adaptation and survival (mitohormesis).

In essence, LONP1 maintains mitochondrial protein homeostasis (proteostasis) and integrity.

Reactive Oxygen Species (ROS): ROS are a group of highly reactive molecules containing oxygen, formed as a natural byproduct of oxygen metabolism. They are extremely unstable and readily react with other molecules inside a cell. This group includes both free radicals (molecules with at least one unpaired electron, like the hydroxyl radical (•OH) and superoxide anion ($O_2^{\bullet -}$)) and non-radical species (like hydrogen peroxide

(H_2O_2)).

Mitochondrial unfolded protein response (UPR^{mt}): The UPR^{mt} is an evolutionarily conserved adaptive signaling pathway that is activated when mitochondria experience stress or dysfunction. Its primary role is to act as a quality control system to restore protein homeostasis (proteostasis) within the mitochondria and, by extension, maintain overall cellular health.

Mitohormesis: Mitohormesis is a biological phenomenon where the induction of a mild, sublethal mitochondrial stress triggers a coordinated adaptive response that ultimately leads to an enhancement of health, stress resistance, and viability for the cell or organism. The term combines ”mito” (referring to mitochondria) and ”hormesis” (the dose-response concept where a small dose of a stressor is beneficial, while a large dose is harmful). It’s essentially the idea that “what doesn’t kill the mitochondria makes them stronger.” The UPR^{mt} is one of the primary and most significant molecular mechanisms by which mitohormesis is achieved in the cell.

Publication

Title: The Rieske iron-sulfur protein is a primary target of molecular hydrogen
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