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COMMENTARY

Sulfide metabolism and the mechanism of torpor

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ABSTRACT

Hibernation is a powerful response of a number of mammalian species to reduce energy during the cold winter season, when food is scarce. Mammalian hibernators survive winter by spending most of the time in a state of torpor, where basal metabolic rate is strongly suppressed and body temperature comes closer to ambient temperature. These torpor bouts are regularly interrupted by short arousals, where metabolic rate and body temperature spontaneously return to normal levels. The mechanisms underlying these changes, and in particular the strong metabolic suppression of torpor, have long remained elusive. As summarized in this Commentary, increasing evidence points to a potential key role for hydrogen sulfide (H_2S) in the suppression of mitochondrial respiration during torpor. The idea that H_2S could be involved in hibernation originated in some early studies, where exogenous H_2S gas was found to induce a torpor-like state in mice, and despite some controversy, the idea persisted. H_2S is a widespread signaling molecule capable of inhibiting mitochondrial respiration *in vitro* and studies found significant *in vivo* changes in endogenous H_2S metabolites associated with hibernation or torpor. Along with increased expression of H_2S-synthesizing enzymes during torpor, H_2S degradation catalyzed by the mitochondrial sulfide:quinone oxidoreductase (SQR) appears to have a key role in controlling H_2S availability for inhibiting respiration. Specifically, in thirteen-lined squirrels, SQR is highly expressed and inhibited in torpor, possibly by acetylation, thereby limiting H_2S oxidation and causing inhibition of respiration. H_2S may also control other aspects associated with hibernation, such as synthesis of antioxidant enzymes and of SQR itself.

KEY WORDS: H_2S, Hypometabolism, Mitochondria, Sulfide:quinone oxidoreductase, Hibernation

Introduction

Hibernation is a seasonal physiological state of a number of mammalian species, where metabolic rate and body temperature are depressed for several days, which enables energy saving during winter (Heldmaier et al., 2004; Staples, 2014). During winter, small hibernators, such as the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*), reduce their energy requirement by fasting and spend most of their time in a state of deep metabolic depression named torpor, where metabolic rate decreases by ∼95% and body temperature drops down to ∼5°C (Heldmaier et al., 2004; Muleme et al., 2006; Staples, 2016). These torpor bouts are periodically interrupted by short arousals of few hours (∼12–24 h), named interbout euthermia (IBE), where metabolic rate and body temperature are restored (Fig. 1A). Compared with smaller hibernators, bears have already low mass-specific basal metabolic rates and exhibit a less marked suppression of basal metabolism during torpor (Heldmaier et al., 2004). Large hibernators such as bears maintain a fairly high body temperature (∼30°C) during torpor and do not cycle between states of torpor and IBE as regularly as squirrels, but still experience considerable decreases in metabolic rate of ∼75% (Tøien et al., 2011) (Fig. 1B). Thus, the metabolic depression of torpor is actively regulated, i.e. it does not follow body temperature passively (Heldmaier et al., 2004; Staples, 2014). For all hibernators, the changes in metabolic rate are paralleled by changes in ventilation rate and heart rate (MacCannell et al., 2018; Milsom and Jackson, 2011) to match oxygen supply with consumption, reaching a minimum level during torpor (Heldmaier et al., 2004). Thus, metabolism is essentially aerobic during hibernation and sustained by lipid catabolism of fat tissue (Hindle et al., 2014) without lactate buildup (Ma et al., 2005; Revsbech et al., 2013; Serkova et al., 2007). However, the molecular mechanisms underlying the metabolic rate suppression of torpor appear complex and are not yet fully understood (Giroud et al., 2021). In this Commentary, we highlight some recent advances indicating that hydrogen sulfide (H_2S), a widespread signaling molecule, plays a potentially key role in the suppression of metabolic rate during torpor.

The gasotransmitter H_2S is a signaling molecule continuously synthesized and degraded *in vivo* that can modify the function of target proteins and enzymes. It is also stored in tissues as protein-bound persulfide and polysulfide (Kolluru et al., 2013) and transported in blood (Bianco et al., 2018; Jensen and Fago, 2020). Bound and unbound H_2S are in equilibrium *in vivo*, with measured biological levels of free H_2S in the nanomolar to micromolar range, depending on the method used (Shen et al., 2015; Ditrói et al., 2019). *In vivo* levels of H_2S are also tightly controlled by several factors, including redox buffer capacity of protein cysteines reacting with H_2S (generating a pool of protein-bound sulfide, from which H_2S can be regenerated), local pH determining the ratio of H_2S/HS^- and the availability of oxygen for its oxidation and removal (Kolluru et al., 2013). At concentrations exceeding the low micromolar physiological levels, H_2S is a highly toxic molecule, and inhalation of >250–500 ppm ambient H_2S can be lethal for humans (Olson, 2011). This is due to the inhibitory effect of H_2S on cytochrome *c* oxidase, the O_2-consuming complex IV of the mitochondrial electron transport system (ETS) (Collman et al., 2009; Cooper and Brown, 2008; Nicholls et al., 2013). However, two studies found that inhalation of low levels of exogenous H_2S (80 ppm) induced a reversible torpor-like state in mice during hypoxia (Blackstone et al., 2005; Volpato et al., 2008), suggesting that H_2S suppressed metabolic rate and body temperature. These results had a strong impact but also raised some controversy, as inhaled H_2S had no effect on large mammals such as sheep and piglets (Haouzi et al., 2008; Li et al., 2008) and it was also argued that hypoxia itself could cause torpor and facilitate H_2S effects (Hemelrijk et al., 2018). In reality, many of these studies were performed under varying experimental conditions (summarized in Table 1), including different exposure protocols, ambient temperature, fasting and use

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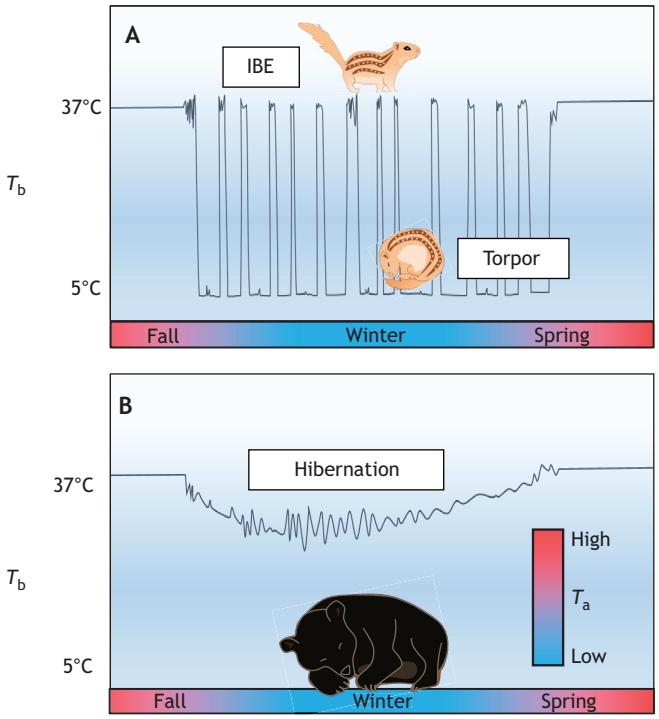

Fig. 1. Hibernation patterns in thirteen-lined ground squirrels and bears. Schematic diagrams showing (A) regular patterns of body temperature (T_b) changes between torpor bouts and arousals to interbout euthermia (IBE) during winter hibernation for a thirteen-lined ground squirrel and (B) multiday cycles of T_b oscillations in a hibernating black bear. T_a is the ambient temperature. Traces are free-hand drawings inspired by data from Staples and Brown (2008) and Tøien et al. (2011).

of anesthesia, making it difficult to assess under which conditions externally supplied H_2S gas may work. A recent study (Marutani et al., 2021) demonstrates that prior exposures to 80 ppm H_2S gas ('sulfide preconditioning') eliminate the inhibitory effects on metabolic rate in mice, which might have been the case in some earlier data.

To understand the possible role of H_2S in torpor, other studies have focused on endogenous rather than exogenous H_2S. Supporting a role for H_2S in hibernation, significant *in vivo* changes in the levels and composition of H_2S metabolites have been detected in the plasma and liver of thirteen-lined ground squirrels (D'Alessandro et al., 2017; Jensen et al., 2021), in the plasma of brown bears (Revsbech et al., 2014) and in the lung of Syrian hamsters (Talaei et al., 2012) during winter hibernation (bears) or torpor (squirrels and hamsters). In addition, emerging evidence of increased enzymatic synthesis of H_2S (Talaei et al., 2012; D'Alessandro et al., 2017) and decreased degradation (Jensen et al., 2021) during torpor point towards a potential role of H_2S as a key physiological signaling molecule in controlling torpor *in vivo*. Here, we provide an overview of recent studies on how *in vivo* H_2S production and degradation may change during hibernation and suppress metabolic rate during torpor.

H_2S production during torpor

H_2S is ubiquitously produced from sulfur-containing substrates including cysteine, homocysteine and cystathionine by the cytosolic enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (Fig. 2). Moreover, the enzyme 3-mercaptopyruvate sulfurtransferase (MST), found in both the mitochondria and cytosol, can synthesize H_2S from 3-mercaptopyruvate in the presence of reducing co-substrates. Enzymatic H_2S production depends on several factors, such as enzyme expression and localization, substrate availability and the presence of various modulators (Giuffrè and Vicente, 2018; Kabil et al., 2011; Talaei et al., 2011). A metabolomics study on thirteen-lined ground squirrels found that substrates cysteine and cystathionine peak in the plasma during late torpor, suggesting increased H_2S production and complex IV inhibition (D'Alessandro et al., 2017). That study also proposed a model whereby CBS is activated by a nitric-oxide-dependent mechanism, suggesting the existence of a series of events in triggering and sustaining H_2S-based torpor. However, in studies by our group, we detected higher levels of plasma H_2S in summer compared with late torpor and IBE in thirteen-lined ground squirrels (Jensen et al., 2021) and in summer-active brown bears compared with winter-hibernating ones (Revsbech et al., 2014). These findings indicate that increased availability of plasma substrate for enzymatic H_2S synthesis may not necessarily reflect increased plasma H_2S levels. Plasma H_2S may derive from several sources, including extracellular enzymatic production by CSE and CBS secreted by endothelial cells and the liver (Bearden et al., 2010) and H_2S produced in the red blood cells (Revsbech et al., 2014; Vitvitsky et al., 2015) or carried by hemoglobin (Bianco et al., 2018; Jensen and Fago, 2018, 2020). Thus, an increased enzymatic production of H_2S in tissues during torpor may be difficult to detect in plasma. Also, circulating plasma cysteine in winter-hibernating

Table 1. Overview of published values of body temperature (T_b) and metabolic rate decrease after exposure to H_2S gas under varying experimental protocols

Inhaled H_2S (ppm)	Inhaled O_2 (%)	Duration	Species	Anesthesia	Fasted	T_a (°C)	T_b (°C)	% Metabolic rate at end of exposure	Reference
80	17.5	6 h	Mouse	No	n.r.	25–13	13	10%	Blackstone et al. (2005)
80	17.5	6 h	Mouse	No	No	27	29.3	70% at 10–30 min	Volpato et al. (2008)
80	17	6 h	Mouse female	No	No	21.2	Decrease of 5°C	n.d.	Hemelrijk et al. (2018)
0	5	6 h	Mouse female	No	No	21.2	Decrease of 7°C	n.d.	Hemelrijk et al. (2018)
60	21 (air)	30 min	Mouse	No	n.r.	23–24	36.7	50%	Haouzi et al. (2008)
60	21 (air)	30 min	Sheep	Yes	Yes	n.r.	No change	No change	Haouzi et al. (2008)
20–80	17.5	6 h (1.5 h steps)	Pig	Yes	n.r.	22	~33	Less decrease than control (no H_2S)	Li et al. (2008)

Values indicate the lowest T_b and the lowest metabolic rate (% of control) recorded at the end of H_2S gas exposure. Metabolic rate values refer to reported O_2 consumption rate. T_b values refer to reported core body temperature, except for data from Hemelrijk et al. (2018), where data refer to superficial body temperature measured by thermic camera. T_a, ambient temperature during H_2S exposure; n.r., not reported; n.d., not determined.

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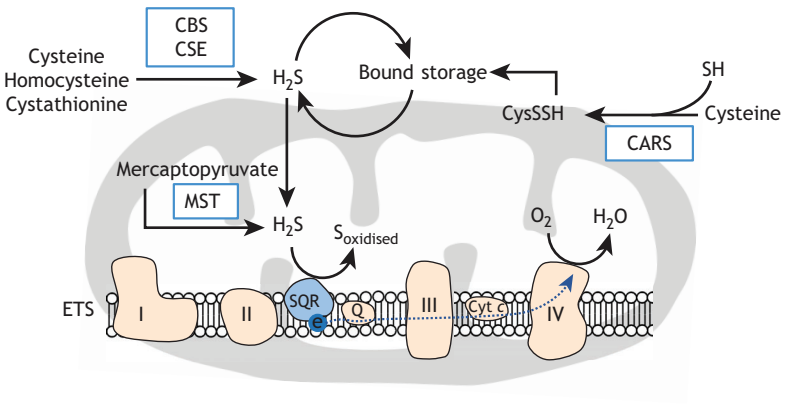

Fig. 2. Schematic overview of H_2S production and degradation. H_2S originates from various sources, including enzymatic production by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (MST), but also from the release of protein-bound sulfur, originating in part from cysteinyl–tRNA synthetase (CARS). The enzyme sulfide:quinone oxidoreductase (SQR), located in the inner mitochondrial membrane, tightly controls the levels of H_2S by continuous H_2S oxidation. In this process one electron (e) is donated to the ubiquinone (Q) of the electron transport system (ETS), thus stimulating respiration. CysSSH, cysteine persulfides; SH, thiol; I–IV, complexes I–IV.

brown bears is used not only for synthesis of H_2S but also for the synthesis of glutathione (Revsbech et al., 2014), a major antioxidant involved in the defense against reactive oxygen species (ROS).

In lung tissue from Syrian hamsters, H_2S synthesis has been found to increase during early and late torpor owing to increased expression of CBS, and both CBS expression and H_2S levels decreased to normal levels during IBE (Talaei et al., 2012). These changes are associated with the rapid lung remodeling taking place during torpor rather than with changes in lung metabolic rate (Talaei et al., 2012). Although this mechanism of H_2S regulation appears to be energetically costly, these results imply that CBS is partly degraded during the short period of IBE (typically <24 h) and then resynthesized during torpor in a cyclic manner, at least in the lung. In contrast, in thirteen-lined ground squirrels, a proteomic study reported lower CBS expression during torpor compared with IBE and summer in the liver, a major metabolic organ (Hindle et al., 2014). This finding aligns with the almost complete arrest of transcription and translation at the low body temperature of torpor in this tissue (Van Breukelen and Martin, 2001). Although regular changes in CBS expression may be a feasible strategy for regulating H_2S levels in the lung of hibernating Syrian hamsters, this does not seem to apply to other species or organs, particularly the liver, which undergoes significant metabolic depression during torpor (Muleme et al., 2006).

In addition to changes in the expression of H_2S-producing enzymes, changes in endogenous H_2S levels may also originate from existing bound sources of sulfur, including protein-bound persulfides and polysulfides (Kolluru et al., 2013). H_2S release from bound storage may be especially beneficial during hibernation, where free amino acids, such as cysteine, are a limited source and are largely preserved and directed away from the urea cycle to limit protein muscle breakdown (Rice et al., 2020). For instance, in the blood of winter-hibernating brown bears, levels of H_2S in red blood cells appear to derive in part from bound sulfane sulfur, allowing cysteine to be allocated to boost glutathione synthesis (Revsbech et al., 2014). The recently discovered cysteinyl–tRNA synthetase (CARS) pathway may contribute to the bound sulfur pool during protein translation. CARS can act as cysteine persulfide synthases that produce cysteine persulfides (CysSSH), which can be a source of protein persulfidation (R-SSH) (Akaike et al., 2017; Fukuto et al., 2018). Both CysSSH and protein persulfides can release free H_2S upon reduction, e.g. by thioredoxin (Dóka et al., 2016). Taken together, these data suggest that H_2S does not originate from a single source, but from a combination of enzymatic *de novo* synthesis, increased substrate availability and release from existing bound sulfur sources, depending on the species and the tissue. Specifically, the torpor-dependent upregulation of H_2S-producing enzymes remains to be investigated more broadly, to clarify in which hibernators and organs it may occur.

The effect of H_2S on mitochondrial respiration

Though changes in endogenous H_2S levels and in the expression of the enzymes involved in its production provide evidence that H_2S metabolism is associated with hibernation, they do not reveal the mechanisms by which H_2S is capable of suppressing metabolic rate in torpor. To elucidate this, we will now focus on the mitochondrial targets of H_2S signaling during torpor, an aspect that has been investigated recently.

The suppression of whole-animal metabolism during torpor is reflected by suppression of mitochondrial function – a key feature for understanding mechanisms of metabolic depression (Staples, 2014). This is exemplified by liver mitochondria isolated from thirteen-lined ground squirrels, showing ∼70% lower respiration rates in torpor than in IBE (Muleme et al., 2006; Staples, 2014). This suppression of mitochondrial metabolism originates from phosphorylation-dependent changes in the activities of ETS complexes I and II (Mathers and Staples, 2019), with ETS and mitochondrial content remaining unchanged (Mathers and Staples, 2015; Mathers et al., 2017). However, other inhibitory mechanisms can contribute to depress mitochondrial respiration further, specifically a direct inhibition of cytochrome *c* oxidase by H_2S.

The inhibitory effect of H_2S on respiration has been widely demonstrated in purified cytochrome *c* oxidase, isolated mitochondria, various cell lines and animal models (Collman et al., 2009; Cooper and Brown, 2008; Leschelle et al., 2005; Módis et al., 2014; Sun et al., 2012; Wu et al., 2011). The inhibitory interaction of H_2S with cytochrome *c* oxidase is complex and concentration-dependent. Inhibition is reversible at low H_2S concentrations, with a binding constant of ∼0.2 µmol l^{-1} (Cooper and Brown, 2008; Collman et al., 2009; Szabo et al., 2014), i.e. comparable to physiological levels of H_2S. However, the effect of H_2S on mitochondrial function is tightly regulated by the activity of the mitochondrial enzyme sulfide:quinone oxidoreductase (SQR), which is embedded in the inner membrane (Fig. 2). SQR is the first enzyme in the H_2S oxidation pathway that continuously oxidizes H_2S to thiosulfate, sulfite and sulfate through a series of enzymatic reactions, thereby regulating the *in vivo* levels of H_2S via its oxidative breakdown. Similar to cytochrome *c* oxidase, SQR binds H_2S tightly, with a reported binding constant of ∼0.1–2 µmol l^{-1} (Lagoutte et al., 2010; Abou-Hamdan et al., 2015). Once H_2S binds to SQR (Olson, 2011), electrons are donated to the ETS via ubiquinone to complex III. Thus, H_2S at low levels is capable of stimulating respiration by functioning as an inorganic substrate (Goubern et al., 2007; Módis et al., 2014; Szabo et al., 2014; Yong

and Searcy, 2001). However, sustaining ETS electron transport by sulfide oxidation is energetically inefficient as it consumes three times more oxygen than substrates such as NADH or succinate to generate the same proton gradient (Abou-Hamdan et al., 2015; Marutani et al., 2021). At high H_2S levels reaching SQR capacity, there is less H_2S oxidation and inhibition of ETS at complex IV increases. H_2S also inhibits its own oxidation, resulting in a positive feedback loop (Abou-Hamdan et al., 2015). Estimated values of ∼1–5 and ∼10–40 µmol l^{-1} H_2S are considered stimulatory and inhibitory, respectively, in several cell types (Lagoutte et al., 2010), but effective ranges of values would obviously depend on the SQR concentration and specific enzymatic activity present in a given organism or tissue. Thus, H_2S inhibits mitochondrial respiration at high concentrations but stimulates it at low concentrations.

Recently, we have investigated the effects of H_2S on mitochondrial respiration in the thirteen-lined ground squirrel (Jensen et al., 2021). In that study, we isolated liver mitochondria from squirrels during summer or hibernation during torpor and IBE and found a low SQR activity and a strong, dose-dependent inhibition of mitochondrial respiration by exogenous H_2S during torpor (Jensen et al., 2021). These findings imply that during torpor, inhibition of H_2S breakdown by a less active SQR allows levels of H_2S to increase and inhibit cytochrome *c* oxidase. Accordingly, endogenous *in vivo* levels of bioavailable H_2S increased to inhibitory levels (∼90 µmol l^{-1}) in the liver during torpor in hibernating thirteen-lined ground squirrels (Jensen et al., 2021). Thus, SQR appears as a key enzyme involved in the control of mitochondrial respiration by H_2S during torpor, at least in the liver, where metabolic suppression is particularly pronounced during torpor compared with other tissues (Brown and Staples, 2014; Brown et al., 2012; Muleme et al., 2006). This mechanism of SQR-mediated regulation of H_2S is highly tissue specific, as in mice SQR is absent in neural tissue, making the brain more sensitive to H_2S (Marutani et al., 2021), but highly expressed in the intestine (Lagoutte et al., 2010; Malagrinò et al., 2019). Interestingly, a recent study (Marutani et al., 2021) reports that the brain of the thirteen-lined ground squirrel contains 100-fold higher levels of SQR protein than that of mice and rats, correlating with a higher tolerance to sulfide inhibition of oxidative phosphorylation but also with a high tolerance to ambient hypoxia. These effects were reversed by SQR silencing (Marutani et al., 2021). It appears conceivable that species- and tissue-specific variations in the level of SQR expression, intrinsic activity and the presence of available sites for post-translational modifications are crucial in setting the threshold where H_2S has inhibitory or toxic effects, or no effect at all. Possibly, these variations explain in part the variable outcome of inhaled H_2S reported in the literature on various animal models (Table 1).

In addition to complex IV, H_2S may also interact with other mitochondrial complexes. For instance, H_2S has been shown to persulfidate two reactive cysteines at complex V, which increases its enzyme activity (Módis et al., 2016). Whether this occurs in hibernators remains to be investigated.

A mechanism for SQR inhibition

The inhibition of mitochondrial respiration by H_2S proposed in the thirteen-lined ground squirrel during torpor (Fig. 3) involves decreased SQR activity, which hampers the ability of mitochondria to metabolize H_2S (Jensen et al., 2021). In principle, these effects can be due in part to increased SQR protein degradation and resynthesis in torpor and IBE, respectively, but in the context of energy-saving hibernation, rapid and reversible post-translational modifications may provide a suitable and energetically inexpensive mechanism for the regulation of SQR activity. Protein acetylation is a prevalent post-translational protein modification during torpor in the thirteen-lined squirrel liver (Hindle et al., 2014), suggesting that cycles of acetylation and deacetylation may control SQR activity. In support of this possibility, an unidentified liver mitochondrial protein with the same molecular mass as SQR (∼50 kDa) has been found to be acetylated during torpor, but not during IBE in thirteen-lined ground squirrels (Mathers and Staples, 2019). Moreover, an acetylation site in mouse SQR has been reported (Rardin et al., 2013), suggesting that acetylation regulates SQR activity.

Sirtuin 3 is a major mitochondrial deacetylase, and deacetylation by sirtuin 3 has been correlated with increased activity of complex I, II and IV (Ahn et al., 2008; Cimen et al., 2010; Wu et al., 2013). It is unknown whether SQR is among sirtuin 3 targets, but in the liver of thirteen-lined ground squirrels, sirtuin 3 expression is significantly lower during winter compared with summer, with no difference between torpor and IBE (Hindle et al., 2014). Further complicating

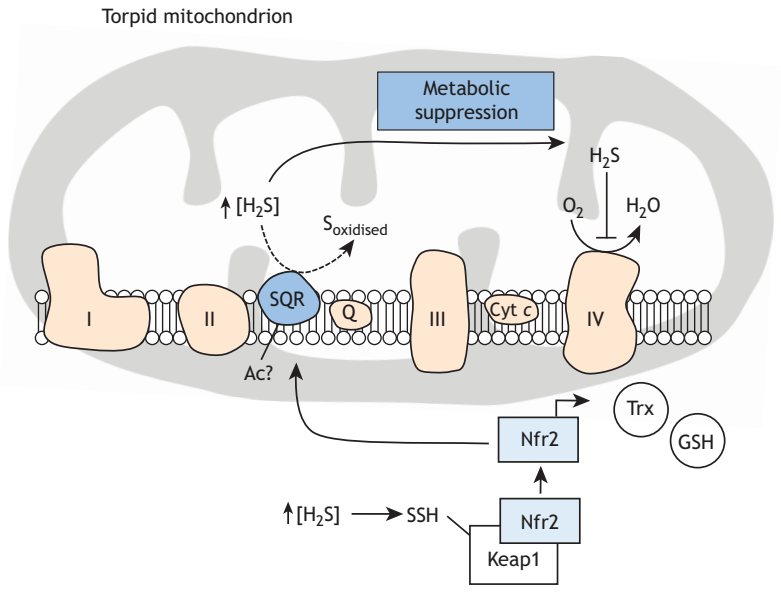

Fig. 3. Proposed mechanism of how H_2S controls metabolic suppression during torpor and promotes antioxidant properties. In the inner mitochondrial membrane, the sulfide: quinone oxidoreductase (SQR) enzyme catalyzing H_2S oxidation to an oxidized form ($S_{oxidized}$) is inhibited, likely by acetylation (Ac). This causes mitochondrial H_2S levels to increase and inhibit O_2 consumption at complex IV. Increased H_2S levels also promote persulfidation (SSH) of Kelch-like ECH-associated protein 1 (Keap1). This increases the stability of the transcription factor Nuclear factor-erythroid 2-related factor (Nfr2), which promotes synthesis of SQR and antioxidants, such as thioredoxin (Trx) and glutathione (GSH), both involved in reactive oxygen species scavenging.

the picture, sirtuin 3 may itself be activated by S-persulfidation (Yuan et al., 2019), suggesting an intricate mechanism where high H_2S may activate sirtuin 3 and trigger deacetylation of SQR, which would lead to accumulation of H_2S. Future work will clarify whether SQR is reversibly acetylated during the hibernation season and by which mechanism.

SQR expression is itself H_2S-dependent and regulated by the transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2), which also controls the expression of antioxidant genes such as thioredoxin and glutathione reductase (Harvey et al., 2009; Kensler et al., 2007). Nrf2 is tightly controlled by the repressor protein Keap1, which promotes Nrf2 degradation. Keap1 itself can be inactivated by H_2S, which persulfidates two cysteines on Keap1 (Hourihan et al., 2013; Yang et al., 2013). This alleviates repression of Nrf2, which subsequently translocates to the nucleus and facilitates gene transcription (Fig. 3). The H_2S-dependent stimulation of Nrf2 stability has been demonstrated in mice, where intravenous injection of H_2S increases Nrf2 levels in cardiac tissue after 30 min (Calvert et al., 2009). In thirteen-lined ground squirrels, levels of Nrf2 increase in the heart, liver and brown adipose tissue during torpor (Morin et al., 2008), and elevated H_2S levels during torpor could potentially contribute to Nrf2 stability and an increase in SQR expression. A study on Nrf2-deficient mouse embryonic fibroblasts found no change in the expression of SQR upon treatment with H_2S, whereas SQR expression increased in wild-type cells (Hourihan et al., 2013). Thus, increased levels of H_2S can increase Nrf2 stability, which may then contribute to enhanced SQR expression, leading to increased H_2S oxidation. This negative feedback loop acts as a safety valve that prevents H_2S levels from accumulating to toxic levels, but, as it involves protein synthesis, it would be an energetically expensive control mechanism in the context of torpor and arousal, but could be feasible in different seasons. During torpor, SQR activity is inhibited (Jensen et al., 2021), so its Nfr2-mediated expression is probably not substantial. Although SQR regulation by H_2S remains unclear in hibernators, potential mechanisms involve its post-translational acetylation and/or Nrf2-induced expression, both of which remain to be investigated further.

H_2S function goes beyond metabolic suppression

In addition to metabolic rate, H_2S controls a variety of physiological responses, such as vascular tone (Yang et al., 2008; Zhao et al., 2001), neuromodulation (Abe and Kimura, 1996; Kimura and Kimura, 2004) and cytoprotection (Calvert et al., 2010; Elrod et al., 2007). The redox potential and reactivity of H_2S are comparable to those of glutathione and cysteine (Carballal et al., 2011; Mishanina et al., 2015), but because *in vivo* levels of H_2S are much lower than those of glutathione and free cysteine (Mishanina et al., 2015), any direct ROS scavenging by H_2S is probably negligible. Instead, H_2S may be indirectly involved in protective mechanisms of ROS scavenging and enhancement of antioxidant defense through increasing activity and levels of antioxidants, such as glutathione and superoxide dismutase (Kimura and Kimura, 2004; Kimura et al., 2010; Sun et al., 2012).

Some mismatch between oxygen supply and consumption resembling ischemia/reperfusion may occur in the transitions between torpor and arousal and potentially cause a surge of mitochondrial ROS, but this does not seem to result in major oxidative stress in hibernators and effects appear to be tissue specific and transient (Staples, 2016). Hibernators appear in general more resistant to oxidative damage than mammals of similar size, suggesting some adaptive traits in these animals. Cardiac arrest does not damage hippocampal neurons (Dave et al., 2006) or the liver (Bogren et al., 2014) of Arctic ground squirrels compared with rats. Livers of thirteen-lined ground squirrels in the hibernation season tolerate ischemia/reperfusion better than those of rats or summer squirrels (Lindell et al., 2005). Arousal in Arctic ground squirrels is associated with some oxidative damage in brown adipose tissue but not in the liver (Orr et al., 2009).

In addition to metabolic suppression of torpor, H_2S could be involved in cytoprotection during the hibernation season, although more evidence is needed. Treatment with H_2S has been found to decrease ROS levels under ischemia/reperfusion of rat cardiomyocytes (Sun et al., 2012) and to prevent oxidative damage in porcine kidneys (Maassen et al., 2019). H_2S may also act by increasing antioxidant capacity through stimulation of the Nrf2 transcription pathway, as discussed above (Fig. 3). In general, H_2S has a broad range of functions, and thus H_2S signaling may be relevant in many physiological responses during hibernation, including changes in peripheral vasoconstriction, where H_2S may contribute through its ability to regulate vascular tone (Yang et al., 2008; Zhao et al., 2001).

Concluding remarks

Significant changes in the endogenous levels and composition of H_2S metabolites have been reported recently in several hibernating mammals. Although changes in the activity and expression of enzymes involved in H_2S production may be involved (Talaei et al., 2012), a key regulatory site appears to be the mitochondrial enzyme SQR, which controls H_2S levels *in vivo* via oxidation (Jensen et al., 2021; Marutani et al., 2021). The underlying mechanism involves decreased SQR activity during torpor, possibly mediated by acetylation, which causes a build-up *in vivo* of H_2S with consequent inhibition of complex IV and metabolic suppression. Several aspects need to be examined further, especially identifying the regulatory mechanism of SQR activity by post-translational modifications, possibly including reversible binding of acetyl group or H_2S. Other aspects that remain to be investigated are redox buffer capacity and the content of accessible cysteines in hibernator proteins and how these may help regulate *in vivo* levels of H_2S and determine thresholds of inhibitory levels and toxicity. Moreover, H_2S may be indirectly involved in other adaptive mechanisms during hibernation, such as cytoprotective antioxidant defense. Investigations on the nature of H_2S signaling and of mitochondrial regulation in comparative studies appear critical to improve our understanding on the mechanisms of torpor and hibernation.

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Competing interests

The authors declare no competing or financial interests.-

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