The Mechanism Enabling Hibernation in Mammals

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Abstract

Some rodents including squirrels and hamsters undergo hibernation. During hibernation, body temperature drops to only a few degrees above ambient temperature. The suppression of whole-body energy expenditure is associated with regulated, but not passive, reduction of cellular metabolism. The heart retains the ability to beat constantly, although body temperature drops to less than 10 °C during hibernation. Cardiac myocytes of hibernating mammals are characterized by reduced Ca^{2+} entry into the cell membrane and a concomitant enhancement of Ca^{2+} release from and reuptake by the sarcoplasmic reticulum. These adaptive changes would help in preventing excessive Ca^{2+} entry and its overload and in maintaining the resting levels of intracellular Ca^{2+}. Adaptive changes in gene expression in the heart prior to hibernation may be indispensable for acquiring cold resistance. In addition, protective effects of cold-shock proteins are thought to have an important role. We recently reported the unique expression pattern of cold-inducible RNA-binding protein (CIRP) in the hearts of hibernating hamsters. The CIRP mRNA is constitutively expressed in the heart of a nonhibernating euthermic hamster with several different forms probably due to alternative splicing. The short product contained the complete open reading frame for full-length CIRP, while the long product had inserted sequences containing a stop codon, suggesting production of a C-terminal deletion isoform of CIRP. In contrast to nonhibernating hamsters, only the short product was found in hibernating animals. Thus, these results indicate that CIRP expression in the hamster heart is regulated at the level of alternative splicing, which would permit a rapid increment of functional CIRP when entering hibernation. We will summarize the current understanding of the cold-resistant property of the heart in hibernating animals.

Keywords

Hibernation · Cold-shock protein · Hypothermia

Abbreviations

CIRP	Cold-inducing RNA-binding protein
CNS	The central nervous system
ECG	Electrocardiograms
HNF	Hepatocyte nuclear factor

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HP	Hibernation-specific protein
ICV	Intracerebroventricular
RBM3	RNA-binding motif 3
SERCA2	asarco(endo)plasmic reticulum Ca^{2+}-ATPase 2a

3.1 Hibernation of Mammals

Most mammals have the ability to maintain their body temperature within a narrow range even in a cold environment. In a cold environment, thermoregulatory responses to minimize heat loss (e.g., peripheral vasoconstriction and piloerection) are evoked (Tansey and Johnson 2015). In addition, heat-producing responses in skeletal muscles (shivering thermogenesis) and in brown adipose tissue (non-shivering or metabolic thermogenesis) are activated, and thereby a drop in body temperature is prevented (Tansey and Johnson 2015). If the body temperature of homotherms drops extremely, they cannot survive because the heart cannot keep beating, and organs fall into ischemia (Ivanov 2000).

On the other hand, several mammalian species undergo hibernation (Carey et al. 2003; Ruf and Geiser 2015) (see Table 3.1). During hibernation, body temperature drops to only a few degrees above ambient temperature (Carey et al. 2003; Ruf and Geiser 2015). Hibernating animals stay unmoving and usually show a curly shape to minimize heat dissipation from the body surface (Fig. 3.1). The hypothermic condition of mammalian hibernators is fundamentally different from that of poikilotherms (amphibians and reptiles). The body temperature of poikilothermic animals directly correlates with changes in ambient temperature due to a lack of efficacious mechanisms for maintaining body temperature (Jackson and Ultsch 2010; Malan 2014). As a result, body temperature drops passively in response to a decrease in ambient temperature. In contrast, mammalian hibernators possess a thermoregulatory mechanism similar to that of nonhibernators, and they can control their body temperature in a nonhibernating state despite exposure to a wide range of surrounding temperatures (Carey et al. 2003; Ruf and Geiser 2015). Even in an extremely cold environment, they do not necessarily undergo hibernation if enough food is available. Furthermore, mammalian hibernators do not always continue in a hibernating condition throughout winter; they sometimes interrupt hibernation and spontaneously recover their body temperature even though they are consistently exposed to a cold environment (Carey et al. 2003). This behavior provides further evidence for the notion that hypothermia during mammalian hibernation is actively induced, since passively induced hypothermia may not recover unless ambient temperature is increased. Thus, hibernation of mammals is considered to be an adaptive strategy to survive in a severe environment during winter.

Table 3.1 Hibernating mammals

Order	Species	Body temperature in hibernation (°C)
Monotremata	Echidna	4
Marsupialia	Pygmy possum, feathertail glider, Chiloe opossum	1.3–7.1
Eulipotyphla	Hedgehog	1–9.7
Afrosoricida	Tenrec	8.6–15
Chiroptera	Bat	−2 to 13.9
Primates	Lemur	6.5–9.3
Carnivora	Badger, bear	28–32.5
Rodentia	Prairie dog, marmot, woodchuck, ground squirrel, chipmunk, pocket mouse, kangaroo mouse, hamster, jerboa, dormouse	−2.9 to 15

Ruf and Geiser (2015)

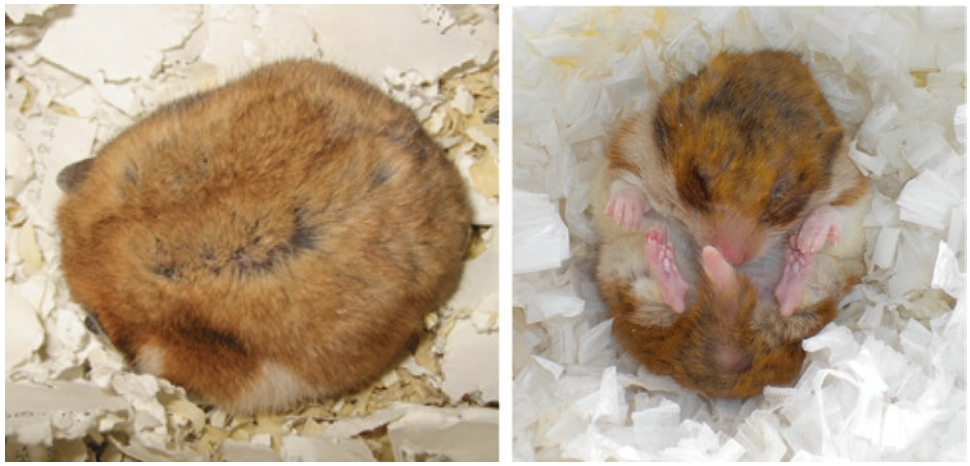

Fig. 3.1 Hibernating hamster. Pictures show curly shape that is usually observed during hibernation in Syrian hamsters

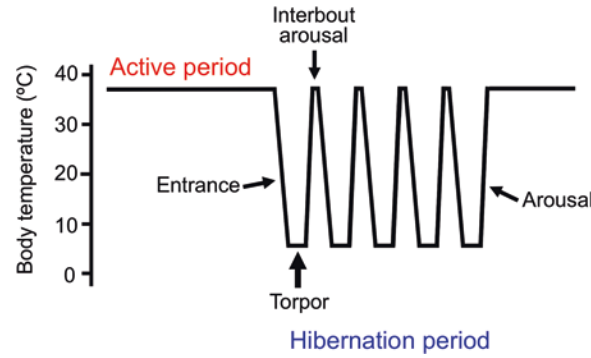

Fig. 3.2 A schema of body temperature during hibernation

3.2 Variation of Hibernation

During hibernation, the degree of body temperature reduction and duration of the hypothermic state vary widely among animal species (Carey et al. 2003; Ruf and Geiser 2015). In black bears, the body temperature during hibernation is around 33 °C, which is much higher than that in small hibernators (Carey et al. 2003; Ruf and Geiser 2015). In contrast, the body temperature of arctic ground squirrels drops to as low as −3 °C during hibernation (Barnes 1989). Several mammalian species undergo daily torpor, in which duration of the hypothermic state is less than 24 h (Breukelen and Martin 2015). In daily torpor, reduction of body temperature is relatively moderate compared with that in deep hibernation. Exceptionally, it has been reported that body temperature of the rock elephant shrew (*Elephantulus myurus*) reached 5–10 °C during daily torpor (Mzilikazi et al. 2002). Some species including tenrec and mouse lemurs adopt either daily torpor or hibernation depending on the ambient temperature (Lovegrove and Génin 2008; Kobbe and Dausmann 2009; Kobbe et al. 2011). A typical deep hibernation is characterized by extended duration of torpor bouts. As shown in Fig. 3.2, the hypothermic state during deep hibernation is interrupted by periods of arousals to euthermia, so-called interbout arousals. The duration of torpor bouts is from a few days to up to 5 weeks. The interbout arousals are maintained for 12–24 h before reentry into torpor (Carey et al. 2003). The periodic hibernation-arousal cycles suggest that the central nervous system (CNS) is continuously operated even at a low temperature during hibernation.

Table 3.2 Physiological parameters in active and hibernating hamsters ($n = 6$)

	Active control	Hibernation in summer	Hibernation in winter
Body temperature (°C)	35.2 ± 0.6	5.0 ± 0.9	5.5 ± 0.3
Heart rate (beats/min)	369 ± 13	15.8 ± 3.1	15.0 ± 2.7
Respiratory rate (breaths/min)	92.2 ± 8.5	2.3 ± 1.7	3.0 ± 1.4

It is known that seasonal hibernators, e.g., Richardson's ground squirrel (*Spermophilus richardsonii*) and Siberian chipmunk (*Tamias sibiricus asiatics*), rarely hibernate in summer even if they are placed in a cold condition (Kondo 1987). This suggests that the endogenous circannual rhythm plays a critical role in the induction of hibernation in seasonal hibernators. In contrast, hamsters hibernated even in summer when they were placed in a condition suitable for induction of hibernation (Miyazawa et al. 2008). No significant differences in parameters including body temperature, heart rate, respiratory rate (Table 3.2), and incidence of ECG abnormalities were found between hibernation in summer and that in winter (Miyazawa et al. 2008). Therefore, the endogenous circannual rhythm might only have a minor contribution, if any, to the induction of hibernation in this species. Of course, this does not necessarily rule out the possibility of involvement of the circannual rhythm in the induction of hibernation in hamsters. It may be appropriate to consider that the relative importance of endogenous and environmental factors varies among species and that this variation is a determinant for seasonal or nonseasonal hibernators.

3.3 Metabolic Regulation During Hibernation

It is generally accepted that the primary purpose of hibernation is to decrease metabolic activity, allowing energy expenditure to be balanced with reduced energy supply due to limited food availability during the winter season. For instance, the metabolic rate of the hibernating ground squirrel is reduced to less than 5% of that observed in the nonhibernating euthermic counterpart (Wang and Lee 1996). The suppression of whole-body energy expenditure is associated with regulated, but not passive, reduction of cellular metabolism. It has been demonstrated that a serine/threonine protein kinase, Akt (also known as protein kinase B), is inactivated by dephosphorylation in hibernating animal organs, typically in skeletal muscles and the liver (Abnous et al. 2008). Considering that Akt activation plays an important role in anabolic and catabolic responses in various cells, the dephosphorylation of Akt in hibernating animal cells would be suitable for a decrease in metabolic activity. Interestingly, the dephosphorylation is promoted immediately prior to entering hibernation (Hoehn et al. 2004). Accordingly, the reduction of cellular metabolism during hibernation does not arise as a consequence of lowered temperature (i.e., general suppression of enzyme activity). Rather, cellular metabolism is suppressed actively before entering hibernation, and this can therefore be a cause of decrease in body temperature. Consistent with this, Akt activity is increased during arousal from hibernation (Lee et al. 2002; Fleck and Carey 2005).

Some species do not feed during hibernation, whereas other species store food and feed during interbout arousals (Humphries et al. 2003; Geiser 2004). Regardless of these differences, hibernation in both groups of species can be considered as a fasting condition (Humphries et al. 2003). To tolerate the long-term fasting condition, major metabolic substrate switches from glucose to lipid occur during the hibernation period in ground squirrels and black bears (Serkova et al. 2007; Andrews et al. 2009) as evidenced by the fact that respiratory quotient values are about 0.7 in hibernating animals (Fedorov et al. 2009).

Global analysis of gene expression by using DNA microarrays would allow speculation regarding differences in metabolic conditions between hibernating hypothermic animals and active euthermic animals (Williams et al. 2005). In the liver of hibernating bears, expression levels of key glucogenic enzymes are increased,

whereas expression levels of glycolytic enzymes are decreased (Fedorov et al. 2009). A similar shift from glycolysis to gluconeogenesis was observed at the mRNA and protein levels in the liver of hibernating ground squirrels (Yan et al. 2008). These changes would contribute to the provision of glucose as an energy source for the brain and other tissues in fasting conditions during hibernation. Also, genes involved in cellular respiration are downregulated during hibernation (Williams et al. 2005; Yan et al. 2008; Fedorov et al. 2009). This is consistent with the reduced metabolic rate in hibernating animals (Carey et al. 2003). Reduction of gene expression for anabolic enzymes with concomitant induction of gene expression for catabolic enzymes is also the case in lipid metabolism. A coordinated induction of genes involved in fatty acid β-oxidation and downregulation of genes involved in lipid biosynthesis at transcriptional (Williams et al. 2005; Yan et al. 2008) and proteomic levels (Shao et al. 2010) have been shown in the livers of hibernating bears and ground squirrels. In contrast, genes involved in amino acid catabolism are downregulated during hibernation (Fedorov et al. 2009). Reduction of amino acid breakdown would be reasonable, since genes involved in protein biosynthesis in the liver and skeletal muscles are increased in this state (Fedorov et al. 2009). The enhanced protein biosynthesis is considered to be a molecular adaptation that contributes to the ability to reduce muscle atrophy over prolonged periods of immobility during hibernation.

3.4 Endogenous Regulators of Hibernation

3.4.1 Factors Related to Hibernation

Although the precise mechanism responsible for regulating hibernation remains unknown, a number of studies have revealed important factors controlling hibernation behavior. It has been demonstrated that adenosine acting through the adenosine A1-receptor in the CNS plays a key role in the entrance phase of hibernation in hamsters (Tamura et al. 2005). The importance of central adenosine is suggested by the fact that intracerebroventricular (ICV) injection of an adenosine A1-receptor antagonist to hamsters in the process of dropping body temperature inhibits entrance to hibernation (Tamura et al. 2005). The effect of adenosine would be related to hibernation onset but not to maintenance of a hypothermic condition, because decreased body temperature cannot be reversed in animals in which deep hypothermia has already been established (Tamura et al. 2005). Vice versa, ICV injection of an A1-receptor agonist to euthermic hamsters decreases body temperature (Miyazawa et al. 2008). In the CNS, adenosine acts as a neuromodulator, and the A1-receptor mediates the presynaptic inhibition of neurotransmission. Thus, activation of the A1-receptor would act as a suppressor of the thermoregulatory mechanism in the CNS. In accordance with this, it has been reported that activation of the A1-receptor promotes sedation and depression of locomotor activity (Radulovacki et al. 1982; Wauquier et al. 1987; Nikodijevic et al. 1991; Ticho and Radulovacki 1991; Malhotra and Gupta 1997).

Similar approaches to identify possible regulators of hibernation have revealed that opioid peptides such as β-endorphin and endomorphine in the hypothalamus are related to the maintenance phase via the μ1-opioid receptor in hamsters (Tamura et al. 2005) and via the δ-opioid receptor in ground squirrels (Yu and Cai 1993a, b). The contribution of opioid peptides to maintenance of the hypothermic state leads an interesting hypothesis that continuous release of opioid peptides during hypothermia may induce tolerance, and therefore hamsters cannot maintain hypothermia for a long time (Tamura et al. 2005). Although further study is needed to verify this hypothesis, it provides a rational explanation for the presence of energy-demanding interbout arousals.

Several lines of evidence suggest that histamine in the hippocampus is involved in the maintenance of hibernation. In general, histamine decreases sleep and promotes wakefulness (Nishino et al. 2001). However, infusion of hista-

mine into the dorsal hippocampus brings about prolonged duration of the torpor bout. This finding is also interesting since it supports the idea that hibernation is an arousal state distinct from any known euthermic arousal state, rather than being homologous to sleep (Kilduff et al. 1993).

The preferential use of lipids during hibernation seems to suggest that excessive fat accumulation is appropriate for entering hibernation. However, it has been demonstrated that a high body mass inhibits the induction of hibernation (Bieber et al. 2014; Zervanos et al. 2014). Conversely, a reduction of body mass triggers the entrance to hibernation in order to reduce the consumption of limited amounts of stored fat. Thus, the decision of whether or not to enter hibernation depends on the body mass and amount of fat deposits (Humphries et al. 2003; Chayama et al. 2016). One possible hormonal mediator that reflects the amount of fat deposits is leptin (Houseknecht et al. 1998). In line with this, a high circulating level of leptin negatively impacts the induction of hibernation. In little brown bats, dissociation of leptin secretion and adiposity is found during the pre-hibernation period, and the decreased leptin level in the absence of a decrease in body mass permits the entrance to hibernation (Kronfeld-Schor et al. 2000). Accordingly, leptin can be considered to be an important regulator of hibernation.

3.4.2 Hibernation-Specific Protein

Many studies have been conducted to identify factors responsible for hibernation (Wang et al. 1988; Shintani et al. 2005; Tamura et al. 2005, 2006, 2012; Kondo 2007; Chayama et al. 2016). The most typical factors that may play a role in physiological adaptation prior to the onset of hibernation are hibernation-specific proteins (HP), originally discovered in the chipmunk (*Tamias asiaticus*) in 1992 (Kondo and Kondo 1992). The protein identified in the plasma of the chipmunk is a 140-kDa protein complex that consists of four components: three highly homologous proteins (HP-20, HP-25 and HP-27) and a proteinase inhibitor (HP-55) (Kondo and Kondo 1992; Takamatsu et al. 1993). HP-20, HP-25, and HP-27 contain an N-terminal collagen-like domain and a C-terminal globular domain homologous to the complement C1q (Takamatsu et al. 1993). The proteins can be detected in the plasma of hibernators, but not in nonhibernators, including tree squirrels and rats (Kondo and Kondo 1992; Takamatsu et al. 1993). The lack of HP in tree squirrels (*Callosciurus caniceps*) is interesting because tree squirrels are a species closely related to chipmunks but do not undergo hibernation (Kojima et al. 2001). This provides support for the pivotal role of HP in hibernation.

The plasma level of the HP complex decreases markedly in hibernating chipmunks (Kondo and Kondo 1992; Takamatsu et al. 1993). Concomitantly, HP gene expression in the liver, in which HP is exclusively produced, is down-regulated (Takamatsu et al. 1993). However, reduction of the circulating HP complex level would not be totally dependent on reduced production in the liver. In contrast to the plasma level of the HP complex, the level of a heterotrimer composed of HP-20, HP-25, and HP-27 (called HP20c) is increased in the brain (Kondo et al. 2006). Therefore, transport to the brain is attributable to the reduced circulating level of the HP complex. The currently accepted mechanism for activation of HP involves dissociation of the HP complex to HP20c and HP-55. HP20c, being free from HP-55, can be actively transported to the brain, where it regulates brain functions for hibernation. In support of this model, a neutralizing antibody against HP20c decreases the duration of hibernation (Kondo et al. 2006). Furthermore, hibernation is never induced in animals lacking an increase in HP20c even in a cold environment (Kondo et al. 2006). The precise action of HP20c in regulation of hibernation remains to be elucidated.

Interestingly, HP gene expression levels in the liver, as well as plasma HP levels, show seasonal oscillations even when chipmunks are kept under a warm condition with a 12-h photoperiod (Kondo et al. 2006). This indicates that gene expression of HP is regulated by endogenous circannual rhythms, rather than environmental factors (Kondo et al. 2006). It has been demon-

strated that hepatocyte nuclear factor 4 (HNF-4) activates *HP-25* transcription (Kojima et al. 2000). In nonhibernating chipmunks, HNF-4 binds to the *HP-25* promoter, leading to *HP-25* transcriptional activation. On the other hand, small heterodimer partner (SHP), which is a negative regulator of HNF-4, is upregulated in the liver of hibernating chipmunks, resulting in the dissociation of HNF-4 from the *HP-25* promoter and the repression of *HP-25* gene transcription (Tsukamoto et al. 2017). Accordingly, SHP, which controls HNF-4 binding to the *HP-25* gene promoter, would be one of the key regulators of HP gene expression.

3.5 Regulation of Cardiac Function During Hibernation

3.5.1 Innate Characteristics of the Heart of Hibernators

Although heart rate in hibernating animals is dramatically lowered compared with that in euthermic counterparts, normal sinus rhythm is fundamentally maintained (Harris and Milsom 1995; Milsom et al. 1999; Mertens et al. 2008). This is in contrast to nonhibernating mammals, in which ventricular dysfunction and arrhythmias such as atrioventricular block and ventricular fibrillation develop when their body temperature drops to less than 20 °C (Johansson 1996; Fedorov et al. 2008). Contraction of cardiac muscle, analogous to that of skeletal muscle, is induced by intracellular Ca^{2+} transients (Kurihara 1994). Hence, a rise in intracellular Ca^{2+} concentration sufficient for inducing contraction is needed to maintain heart function at a cold temperature. In the rat myocardia, basal intracellular Ca^{2+} concentration, which is usually about 140 nM at 30–35 °C, is increased to 200–300 nM in response to a cold temperature around 10 °C (Liu et al. 1991; Wang and Zhou 1999). Such a rise in the basal Ca^{2+} concentration would negatively impact cardiac function, since it enhances basal tone, resulting in insufficient dilation during the diastolic filling period. Furthermore, a rise in the basal Ca^{2+} concentration also lowers the degree of Ca^{2+} increment even if the amplitude of the cytosolic Ca^{2+} transient remains similar. It is therefore considered that a rise in the basal Ca^{2+} concentration is an underlying basis for cardiac dysfunction at a cold temperature.

Interestingly, in the ground squirrel, a typical hibernator, it has been reported that the basal intracellular Ca^{2+} concentration of myocardia is not increased at a cold temperature (Wang et al. 2002). In addition, amplitude of the Ca^{2+} transient is increased at a cold temperature (Wang et al. 2002). In agreement with this, myocardial contractile force at a low temperature is greater than that at a temperature comparable to body temperature of the active state. The greater myocardial contractile force at a low temperature would be reasonable as a compensatory mechanism for the marked reduction of heart rate. The remarkable differences in Ca^{2+} dynamics between hibernators and nonhibernators suggest that an ability to maintain cardiac contractility under an extremely hypothermic condition can be recognized as an inherent feature of hibernators.

The cold-resistant nature of the heart of a hibernator has also become apparent from experiments in which attempts were made to induce artificial hypothermia in both hibernators and nonhibernators. When extreme hypothermia was forcibly induced by pentobarbital anesthesia combined with cooling of the whole body, cardiac contractility was maintained in hamsters (Miyazawa et al. 2008). This is in sharp contrast to nonhibernators, in which cardiac arrest is usually induced at a low temperature (Duker et al. 1983; Caprette and Senturia 1984; Johansson 1996). In fact, the same procedure for inducing artificial hypothermia in hamsters was lethal in rats (Miyazawa et al. 2008). In addition to the cold resistance, the heart of a hibernator is known to be resistant to various harmful stimuli. For instance, the heart of one of the hibernators, hedgehog dog, is hardly affected by manipulations that elicit atrial fibrillation (e.g., aconitine administration, high concentration of $CaCl_2$ administration, or ligation of the hepatic artery) (Johansson 1985, 1996).

3.5.2 Adaptive Changes in the Heart Prior to Hibernation

As mentioned above, the specific innate characteristics of the heart of hibernators would be important for enabling hibernation. It should be noted, however, that maintenance of cardiac function during hibernation does not totally depend on the innate ability of the heart. It is believed that adaptive changes that occur in response to a short photoperiod and cold ambient temperature are also essential for entering deep hibernation, as well as for keeping a hypothermic state and for recovery to a euthermic state. Therefore, numerous studies have been carried out to reveal remarkable differences between hibernating animals and their euthermic counterparts.

The most striking adaptive changes in the heart of hibernating animals are alterations in intracellular Ca^{2+} mobilization involving cardiac excitation-contraction coupling (Kondo and Shibata 1984; Lakatta and Guarnieri 1993). In general, intracellular Ca^{2+} for contraction of cardiac muscle is supplied by its entry into the cell through the L-type Ca^{2+} channel followed by Ca^{2+} release from the sarcoplasmic reticulum, a Ca^{2+} storage organelle (Kurihara 1994). In hibernating chipmunks, it has been demonstrated that activity of the L-type Ca^{2+} channel is suppressed and thereby entry of extracellular Ca^{2+} is limited (Kondo and Shibata 1984). Since excessive Ca^{2+} entry and its overload would damage cardiomyocytes through induction of apoptosis and/or necrosis, maintenance of Ca^{2+} homeostasis is essential for preventing profound arrhythmia and ventricular fibrillation (Lakatta and Guarnieri 1993). Thus, it can be considered that suppression of the L-type Ca^{2+} channel activity is an appropriate adaptive event for hibernating animals. Meanwhile, suppression of the channel activity may have a negative impact on cardiac contractility. To compensate for the reduced Ca^{2+} entry, release of Ca^{2+} from intracellular stores is enhanced during hibernation (Kondo and Shibata 1984). It is also important, in addition to the increased release, that reuptake of Ca^{2+} by the sarcoplasmic reticulum is enhanced (Belke et al. 1991). Collectively, suppression of channel activity in the plasma membrane with concomitant activation of store function enables efficacious Ca^{2+} cycling at a cold temperature.

3.5.3 Molecular Basis for the Adaptive Changes in the Heart of Hibernating Animals

As mentioned above, cardiac myocytes of hibernating mammals are characterized by reduced Ca^{2+} entry into the cell membrane (Alekseev et al. 1996; Yatani et al. 2004; Dibb et al. 2005) and a concomitant enhancement of Ca^{2+} release from and reuptake by the sarcoplasmic reticulum (Kondo and Shibata 1984; Belke et al. 1991; Wang et al. 2002). These adaptive changes would help in preventing excessive Ca^{2+} entry and its overload and in maintaining the resting levels of intracellular Ca^{2+} (Wang et al. 2002). The molecular basis of reduced Ca^{2+} entry into the cell membrane would not be due to reduced expression of the L-type Ca^{2+} channel protein but rather due to a decrease in channel activity by phosphorylation of the molecule (Kokoz et al. 2000) (Fig. 3.3).

As for the increased release of Ca^{2+} from intracellular stores, there has been a study demonstrating that the density of ryanodine receptors is increased in the sarcoplasmic reticulum, although the expression level of the receptors remains unchanged (Milner et al. 1991). The ryanodine receptor is the major Ca^{2+} release channel on the sarcoplasmic reticulum required for excitation-contraction coupling in cardiac muscle (Kurihara 1994). In addition, expression of sarco(endo) plasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a) is upregulated, and a negative regulator of SERCA2a, phospholamban (PLB), is downregulated during hibernation (Brauch et al. 2005). These changes enable a prompt removal of cytosolic Ca^{2+}, thereby ensuring diastolic filling (Fig. 3.3).

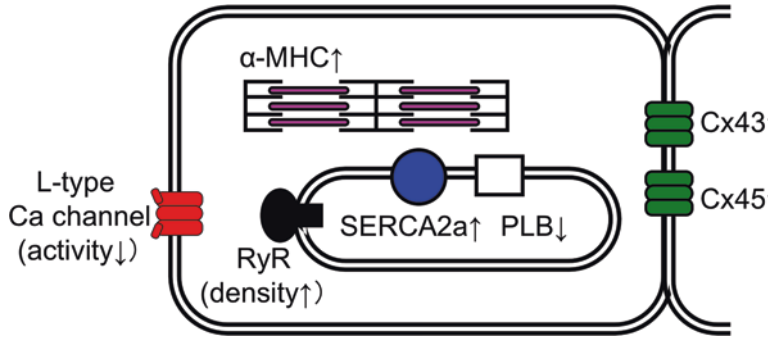

Fig. 3.3 Adaptive changes in molecules related to maintenance of intracellular Ca^{2+}, contractility or synchronous contraction in cardiac myocytes of hibernating animals. *RyR* ryanodine receptors, *SERCA2a* sarco(endo)plasmic reticulum Ca^{2+}-ATPase 2a, *PLB* phospholamban, *α-MHC* myosin heavy chain-α, *Cx* connexin

Moreover, the expression of functional proteins related to contractility (e.g., myosin heavy chain-α, ventricular myosin light chain, and the troponin C) and the expression of proteins involved in synchronous contraction (e.g., connexin43) have been shown to be upregulated or downregulated appropriately in hibernating animals (Saitongdee et al. 2000; Brauch et al. 2005; Fedorov et al. 2005). Importantly, the onset of these changes precedes the onset of hibernation (Kondo 1987; Saitongdee et al. 2000), indicating that these changes in gene expression and subsequent functional remodeling are preparatory processes for entering hibernation and are therefore indispensable for acquiring cold resistance (Fig. 3.3).

3.5.4 Autonomic Regulation of the Heart During Hibernation

The adaptive changes prior to hibernation would alone be insufficient to maintain cardiac pulsatility under an extremely hypothermic condition during hibernation, although these changes are undoubtedly indispensable. The operation of autonomic regulation for maintaining proper cardiac pulsatility during hibernation has been suggested by experiments focusing on artificially induced hypothermia in hamsters. By combining pentobarbital anesthesia with cooling of the animal, forced hypothermia that is comparable to that in hibernating animals can be successfully produced (Miyazawa et al. 2008). This procedure may reproduce a hypothermic condition without promoting possible autonomic functions that would usually be triggered in natural hibernation.

Even after sufficient exposure to an environment that is appropriate for induction of adaptive changes, hamsters show abnormal electrocardiograms (ECG) such as J wave and/or signs related to atrioventricular block when the hypothermic condition is forcibly induced (Miyazawa et al. 2008). The J wave, which is typically described in hypothermia in nonhibernating mammals (Brunson et al. 2005), is a wave located at the point of the end of the QRS complex and occupying the initial part of the ST segment (Gussak et al. 1995). The origin of the J wave during hypothermia has been attributed to injury current, delayed ventricular depolarization and early repolarization, tissue anoxia, and acidosis (Brunson et al. 2005). If the adaptive changes exclusively contribute to cold tolerance of the heart, heart pulsatility of well-adapted hamsters can be maintained appropriately not only during natural hibernation but also during a forcibly induced hypothermic condition. Therefore, the fact that the J wave as well as abnormal ECG signs related to conduction block are not observed in natural hibernation (Mertens et al. 2008; Miyazawa et al. 2008) can be rationally explained by the operation of regulatory mechanisms during natural hibernation to coordinate the cardiac

conducting system properly and to prevent cardiac impairment caused by hypothermia. The precise regulatory mechanisms have so far remained elusive.

3.6 Mechanism of Protection Against Cold Temperature

3.6.1 Cold-Shock Proteins-Associated Protection During Hibernation

Generally, the heart of mammalians cannot keep beating in a deep hypothermic condition (Ivanov 2000), suggesting that a cold temperature is harmful to the heart. In contrast, the heart of hibernating animals is capable of maintaining constant beating despite a decrease in body temperature to less than 10 °C during hibernation (Carey et al. 2003). Therefore, in addition to the adaptive changes prior to entering hibernation and the operation of autonomic regulatory mechanisms during hibernation, protection of cardiomyocytes against harmful effects of a cold temperature would be essential to maintain heart function under a condition of extreme hypothermia. In relation to the protective mechanism, recent studies have been focused on functional roles of cold-shock proteins, including cold-inducing RNA-binding protein (CIRP) and RNA-binding motif 3 (RBM3) (Zhu et al. 2016). It has been demonstrated that CIRP and RBM3 are induced by cold stress in cultured cells (Nishiyama et al. 1997; Gotic et al. 2016; Zhu et al. 2016). These proteins regulate gene expression at the level of translation (i.e., mRNA splicing, stability, and transport) and thus allow cells to respond rapidly to cold stress (Lleonart 2010; Zhu et al. 2016). Accumulating evidence indicates that CIRP and RBM3 play important roles in the protection of various types of cells against harmful effects of a cold temperature (Gualerzi et al. 2003; Saito et al. 2010).

The prominent action of cold-shock proteins, which was originally revealed in cells of nonhibernators, has been shown to function during hibernation. For example, it has been reported that RBM3 is increased in hibernating mammals such as black bears (Fedorov et al. 2009, 2011) and ground squirrels (Epperson et al. 2004; Williams et al. 2005) and plays an important role in neuroprotection (Tong et al. 2013; Peretti et al. 2015). Also, CIRP is expressed in response to a cold stress in the treefrog (Sugimoto and Jiang 2008). Accordingly, cold-shock proteins might help to protect organs including the heart against a harmful low temperature during hibernation.

3.6.2 Hibernation-Specific Alternative Splicing of the *CIRP* Gene

We recently reported the unique expression pattern of *CIRP* in the hearts of hibernating hamsters (Sano et al. 2015). In our study, RT-PCR analysis revealed that CIRP mRNA is constitutively expressed in the heart of a nonhibernating euthermic hamster with several different forms probably due to alternative splicing. The short product contained the complete open reading frame for full-length CIRP. On the other hand, the long product had inserted sequences containing a stop codon, suggesting production of a C-terminal deletion isoform of CIRP. The RNA-binding domain in the N-terminal region (Lleonart 2010) is conserved in the long isoform, indicating that the isoform possesses RNA-binding activity equal to that of full-length CIRP. However, the isoform lacks critical phosphorylation and methylation sites located in the C-terminal region, the phosphorylation and/or methylation of which is related to activation of CIRP (De Leeuw et al. 2007; Lleonart 2010). It is thus probable that the C-terminally truncated isoform plays a dominant-negative role over the full-length CIRP. In contrast to nonhibernating hamsters, only the short product is expressed in hibernating animals. It is therefore speculated that the dominant-negative regulation is important to mask the function of CIRP under a nonhibernating condition. The dominant-negative regulation combined with constitutively active transcription may permit rapid expression of CIRP function by switching the splicing pattern, leading to avoidance of hypothermic damage in the heart (Fig. 3.4).

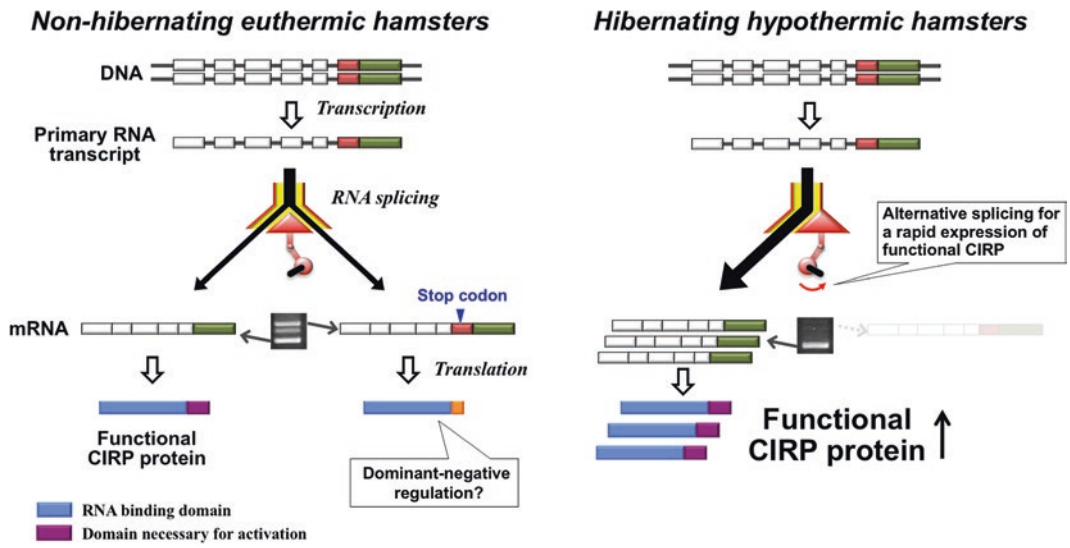

Fig. 3.4 Alternative splicing of cold-inducible RNA-binding protein (CIRP) gene in nonhibernating euthermic and hibernating hypothermic hamsters. (The figure was modified from our published article Sano et al. 2015)

It would be of interest to uncover the factors causing the shift in alternative splicing of CIRP. Recent evidence from cultured cells suggests that a mild cold temperature (32 °C) is a possible trigger for splicing regulation of the CIRP gene, since mild cold exposure increases the expression of CIRP mRNA without affecting its pre-mRNA levels (Gotic et al. 2016). This assumption can be applicable to the shift in alternative splicing of CIRP under the condition of natural hibernation, since animals go through a gradual decrease in body temperature, and they would maintain mild hypothermia, about 25–30 °C, for several hours (Horwitz et al. 2013). Taken together, it is reasonable to consider that mild hypothermia during the induction period of hibernation might induce hibernation-specific alternative splicing of CIRP in the hamster heart.

3.7 Induction of Artificial Hypothermia in Nonhibernating Animals

3.7.1 Significance of Therapeutic Hypothermia

Hypothermia results in a reduction of cellular metabolic rate and oxygen consumption, indicating that it may have therapeutic efficacy (Hale and Kloner 2011; Tissier et al. 2012). Mild hypothermia, 32–35 °C, is very potent for reducing myocardial infarct size in some experimental animal models such as rabbits, dogs, sheep, pigs, and rats (Tissier et al. 2012). In addition, induced hypothermia has been shown to reduce the risk of cerebral ischemic damage both in animal studies and in humans, who have been resuscitated following cardiac arrest (Galvin et al. 2015). Thus, it is important to devise a method by which hypothermia can be induced safely and simply in nonhibernating mammals including humans.

Therapeutic hypothermia is generally induced by a combination of anesthesia with cooling in the patient (Galvin et al. 2015). In addition, safe and simple pharmacological approaches to achieve therapeutic hypothermia have been investigated. For instance, hydrogen sulfide can induce a state of hypothermia in mice by inhibiting cytochrome oxidase, which decreases their metabolic rate and core body temperature (Guo et al. 2012). Administration of capsaicin also reduces body temperature by about 2–3 °C (Jakab et al. 2005; Swanson et al. 2005; Jones et al. 2009; Dow et al. 2014) since capsaicin is an agonist of TRPV1, which can detect a painful hot temperature (>42 °C) (Montell and Caterina 2007) and would be recognized as heat exposure, leading to reduction of body temperature mediated by the thermoregulatory center. It should be

noted, however, that the target temperature is generally about 30 °C, which is categorized as mild hypothermia, and that it is difficult to induce hibernation-like extreme hypothermia even by these methods.

3.7.2 Induction of Hypothermia by Activation of Central Adenosine A1-Receptor

One of the profound problems that occur during induction of artificial hypothermia is heart dysfunction such as ventricular fibrillation and cardiac arrest. Even in hibernators such as hamsters, abnormal ECG is recorded during nonhibernation artificial hypothermia induced by pentobarbital anesthesia and cooling (Miyazawa et al. 2008). To devise a safe method for induction of hypothermia, elucidation of the mechanisms for tolerance to cold stress during hibernation would provide valuable information. Central adenosine A1-receptor-mediated signals play a role in the induction and maintenance of hibernation (Tamura et al. 2005; Jinka et al. 2011; Iliff and Swoap 2012). The predominant role of adenosine A1-receptor-mediated signals leads to the idea that activation of adenosine A1-receptors would induce hypothermia in both hibernating and nonhibernating mammals. In accordance with this, central administration of an adenosine A1-receptor agonist and subsequent cooling induces extreme hypothermia in hamsters (Miyazawa et al. 2008) and rats (Tupone et al. 2013; Shimaoka et al. 2018) without accompanying atrioventricular block or abnormal ECG. These findings suggest that central adenosine A1-receptor-mediated signals would provide an appropriate condition for maintaining normal sinus rhythm during extreme hypothermia.

3.8 Conclusion

Entering hibernation in mammals confers resistance to various harmful events such as low body temperature, severe ischemia, bacterial infection, irradiation, and muscle disuse. Therefore, hibernation mechanisms are considered to be a potential therapeutic target for the treatment of several diseases. Although the application of this unique phenomenon to medical fields has been strongly desired, a poor understanding of the mechanisms limits the progress toward developing novel therapeutic strategies. A large number of previous experiments focused on adaptive changes in the heart prior to hibernation. It is clear that adaptive changes are involved in the beneficial properties of hibernating animals. However, it remains unclear whether these changes are solely responsible for the establishment of a hibernating condition. For instance, it is uncertain whether the changes at the molecular level (see Fig. 3.3) are sufficient for maintaining cardiac pulsatility under an extremely hypothermic condition. On the other hand, artificially induced hypothermia may provide a valuable tool to answer the question. The method for inducing hypothermia forcibly in hamsters allows reproduction of a hypothermic condition in the absence of possible hibernation-specific reactions. Unlike hypothermia in natural hibernation, the forced induction of hypothermia causes irreversible injury of the myocardium (Miyazawa et al. 2008). Comparison of the heart in forced hypothermia with that during hibernation would be valuable for identifying critical factors related to cold resistance of the heart. Thus, it is expected that further studies using artificial hypothermia may provide a breakthrough in understanding the hibernation mechanisms.

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