around the nuclear periphery, but also displaced from the nucleoplasm to the periphery (Figure 1). Will mammalian centrosome-associated SUN domain proteins make forays to collect telomeres as does Sad1?

Perhaps the most mysterious aspect of the bouquet is its function. Although it is clear that bouquet mutants suffer reduced homolog pairing and recombination, the effects on recombination are not necessarily consonant with the severity of their effects on ascus morphology and spore viability. For example, the frequency of normal ascus formation in *bqt1*Δ and *bqt2*Δ cells was similar to that of cells lacking Rec12, the Spo11 homolog required for meiotic double-strand break formation and recombination. However, recombination is only mildly reduced in *bqt*Δ cells, whereas it is nearly abolished in *rec12*Δ cells (De Veaux et al., 1992). Likewise, loss of the dynein heavy chain abolishes horsetail movement and confers reduced recombination but has little effect on spore viability. Thus, it remains possible that the extraordinarily conserved meiotic bouquet serves additional unanticipated functions.

REFERENCES

Chikashige, Y., Ding, D.Q., Funabiki, H., Haraguchi, T., Mashiko, S., Yanagida, M., and Hiraoka, Y. (1994). Science *264*, 270–273.

Chikashige, Y., Tsutsumi, C., Yamane, M., Okamasa, K., Haraguchi, T., and Hiraoka, Y. (2006). Cell, this issue.

De Veaux, L.C., Hoagland, N.A., and Smith, G.R. (1992). Genetics *130*, 251–262.

Goto, B., Okazaki, K., and Niwa, O. (2001). J. Cell Sci. *114*, 2427–2435.

Malone, C.J., Fixsen, W.D., Horvitz, H.R., and Han, M. (1999). Development *126*, 3171–3181.

Martin-Castellanos, C., Blanco, M., Rozalen, A.E., Perez-Hidalgo, L., Garcia, A.I., Conde, F., Mata, J., Ellermeier, C., Davis, L., San-Segundo, P., et al. (2005). Curr. Biol. *15*, 2056–2062.

Niwa, O., Shimanuki, M., and Miki, F. (2000). EMBO J. *19*, 3831–3840.

Scherthan, H. (2006). In Telomeres, 2nd Edition, T. de Lange, V. Lundblad, and E. Blackburn, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 225–259.

Yamamoto, A., West, R.R., McIntosh, J.R., and Hiraoka, Y. (1999). J. Cell Biol. *145*, 1233–1249.

Hibernation Proteins: Preparing for Life in the Freezer

Michael H. Hastings[1,*] and Francis J.P. Ebling[2]
[1]MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, United Kingdom
[2]School of Biomedical Sciences, University of Nottingham Medical School, Nottingham, NG7 2UH, United Kingdom
*Contact: mha@mrc-lmb.cam.ac.uk
DOI 10.1016/j.cell.2006.03.019

Hibernation is an extreme response to a seasonal environment, yet we know almost nothing about how it is timed or how vital cellular functions are sustained in the face of plummeting body temperature. In this issue of *Cell*, Kondo et al. (2006) identify a liver-derived protein complex as an essential coordinator of this adaptation to the depredations of winter.

All environments are seasonal, and thus animals have evolved strategies to schedule their behavior and physiology accordingly. At higher latitudes, the suspension of homeothermic physiology and brain function enables an animal to withdraw from a hostile world and sustain life for months, eking out precious energy reserves by reducing metabolic rate for part of each day (torpor) or for more prolonged intervals (hibernation). In this way, species from bats to bears to rodents extend their geographical range into the harshest of habitats. The trick to their success is twofold. First, an endogenous timer enables them to anticipate and prepare for the onset of winter. Second, they are able to protect vital cellular activities from the ravages of prolonged hypothermia and hypoxia. This has more than passing biological interest, because understanding how tissues cope with the cardiovascular and oxidative stresses associated with hibernation or torpor (Osborne and Hashimoto, 2006) may have direct clinical relevance.

In short-lived species, seasonal timing relies upon an internal photoperiodic calendar based on melatonin, whereas longer-lived animals employ a true annual clock, synchronized by day length. Consequently, when isolated in continuous cold and darkness, hibernators such as the Siberian chipmunks used by Kondo et al. (2006) maintain precise circannual rhythms of core body temperature for up to a decade, each animal cycling through its personal year.

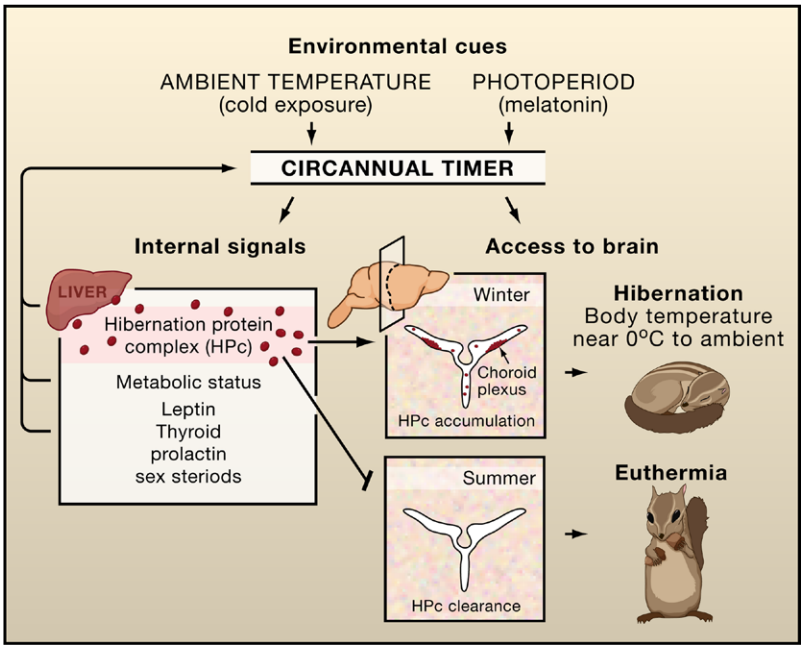

Figure 1. Hibernation Cycles Are Driven by a Circannual Clock

Hibernation and torpor are a common strategy for conservation of energy stores. External cues including the annual change in photoperiod and ambient temperature influence their timing. The current study (Kondo et al., 2006) demonstrates a key role for an endogenous circannual clock in timing hibernation bouts in chipmunks and indicates that it influences both the production of a systemic signal from the liver, the hibernation protein complex (HPc), and also the entry of this signal into the brain via the choroid plexus. Studies in other seasonal species indicate that several other endocrine signals (for example leptin and thyroxine) are also transported into the brain to regulate hivernal physiology. The transport and metabolism of such signals by epithelial tissues (such as the choroid plexus and ependymal tanycytes) may be a major regulatory node. For example, ependymal cells in the Siberian hamster exhibit a clear seasonal change in the expression of the gene encoding the retinol binding protein, CRBP1 (Ross et al., 2004). Expression is high in summer conditions but low in winter when hamsters display bouts of torpor, which is characterized by a periodic drop in body temperature, whereas in chipmunks, HPc accumulates in the brain to initiate and maintain hibernation. The concentration of HPc in the brain decreases at the termination of hibernation.

What we know about circannual clocks, however, revolves around what they are not. Lesion studies indicate that the well-characterized daily (circadian) timer of the suprachiasmatic nuclei of the hypothalamus is not essential for the expression of circannual rhythms (Ruby et al., 1998)—a biological year is not defined as 360-odd circadian days.

Therefore, an alternative way to identify the circannual clock is to characterize its likely effector pathways and then work backward. This has the added advantage that it could simultaneously reveal how peripheral organs are able to function during hypothermia. Kondo et al. (2006) highlight the hibernation-specific protein complex (HPc) as one such entry point. HPc consists of HP55 (a homolog to α 1-antitrypsin) combined with the HP20c complex (which is formed from three structurally homologous proteins: HP20, 25 and 27, bound together by helices in their N-terminal collagen-like domains). Synthesized in the liver, HPc titers in the blood decline in advance of hibernation and rise with its termination. In animals that fail to hibernate, HPc titers remain high. These circannual cycles are not, however, a passive consequence of periodic hypothermia, because when chipmunks are housed at 23°C to prevent entry into hypothermia, they still show marked circannual rhythms in blood HPc titers. HPc levels therefore mark circannual time, not body temperature. Moreover, acute cold exposure triggers hypothermia in animals with low HPc titers but not in individuals with elevated HPc, thereby implicating HPc in thermoregulation.

So, is HPc the gate-keeper, barring entry to cold-induced hypothermia? If so, it would be expected to act within thermoregulatory circuits of the brain. Kondo et al. (2006) therefore assayed HPc titers in the cerebrospinal fluid (CSF), and this is where the story takes an unanticipated twist. Remarkably, they found a dramatic increase in HPc in the CSF as blood levels declined (Figure 1). Furthermore, termination of hibernation, which is associated with rising levels of HPc in the blood, coincided with a fall in HPc concentration in the CSF. Given the absence of brain expression of HPc components, Kondo et al. (2006) looked for evidence of transfer from blood to CSF and observed HPc-like immunoreactivity in choroid plexus, consistent with an active transcellular transport from serum to CSF. Intriguingly, size-exclusion chromatography and coimmunoprecipitation indicated that, within CSF, the HPc complex dissociates into HP20c and HP55 components. Rather than barring entry into hypothermia, it now looked as though central HP20c proteins actually facilitate it. To test this, Kondo et al. (2006) blocked the neural activity of HP20c by central infusion of anti-HP20 serum. Immunoneutralization terminated bouts of hibernation and caused a return to euthermia: an impressive outcome for a heroic, long-term physiological experiment.

These findings are intriguing at several levels. First, why should peripheral levels of HPc be the inverse of those found in the CNS—that is, why go to the trouble of synthesizing all of that HP in the liver at times when it is not needed in the brain? Does HP20c have functions in the periphery different from its central role? One might expect that all tissues would require comparable protection against hypothermia, thus if HP components serve this general function in the brain, why not also

for peripheral tissues? Perhaps vital tissues require custom-made protective programs, for example, the heart exhibits highly specific cellular modifications to allow it to function at low temperature and in the face of increased blood viscosity (Dibb et al., 2005). Does the fall in peripheral HPc levels contribute to this, or is there an active uptake into cardiac and other tissues, mimicking that seen in the brain, such that these systemic adaptations are promoted by elevated intracellular HP20c even as serum levels decline? Knowing more about the cellular actions of HP20c is an essential step in decoding these protective programs and perhaps exploiting them in transplant and vascular surgery. What signaling cascades does it affect, and is dissociation from HP55 the key to switching on or off the functions of HP20c? Given its putative antiproteinase activity, HP55 may simply be a protective shield responsible for delivering the active agents of HP20c safely to their targets.

The question of selective transport of HP complexes also looms large. First, how is it carried across the choroid plexus: by dedicated transporter mechanisms/ binding proteins or does the HP20c piggyback on more generalized pathways? Given the inverse relationship between serum and CSF levels, the former is more likely than a simple mass action effect. Second, how is transport across endothelial cells facilitated at some phases of the annual cycle but retarded at others? Conveyance across the choroid is not unprecedented. The adipocyte-derived hormone leptin, which provides a key signal of fat reserves to the brain, is actively transported across the choroid by the short form of the leptin receptor (Tartaglia et al., 1995). Moreover, entry into winter physiology is gated by leptin levels: in the case of true hibernators, high levels of leptin signal that fat reserves are adequate to embark on this process, whereas in species displaying torpor, it is the low levels of leptin that signal that fat reserves are depleted, and thus short-term metabolic savings must be made. In both cases, leptin and HPc, it seems that the choroid plexus is a key tissue, integrating systemic endocrine signals and selectively transporting such signals into the brain to regulate long-term adaptive cycles.

Although it is unclear whether leptin uptake by the choroid is seasonally regulated, in the context of HPc, the circannual clock drives these specialized transport cells. Indeed, they may even be circannual oscillators themselves. A precedent for such long-term, cell-based programs comes from the pars tuberalis of the pituitary, a target for melatonin, which after prolonged exposure to winter-like melatonin signals, reverts spontaneously to a spring-like cellular phenotype (Lincoln et al., 2005). Perhaps the circannual timer may not be a true oscillator after all. Rather it may be based on two interval timing mechanisms, akin to egg-timers, that govern alternating cellular states of the choroid, interlocked in some form of reciprocal switch.

Whatever the nature of the timer, its impact upon the choroid plexus across the hibernation cycle of chipmunks also resonates with studies in Siberian hamsters and Japanese quail, which exhibit marked photoperiod-regulated changes in gene expression within the tanycyte cells of the ependyma of the hypothalamic third ventricle (Ross et al., 2004) (Watanabe et al., 2004). Tanycytes are thought to regulate transport and processing of many hormones and energy metabolites between the hypothalamic portal capillaries and the cerebrospinal fluid (Rodriguez et al., 2005) and thus constitute the selectively permeable blood-brain barrier of the arcuate nucleus, the preeminent neural regulator of long-term metabolic cycles. For example, seasonal metabolic and reproductive cycles are particularly dependent on changes in the transport and processing of thyroid hormones within the hypothalamus, where conversion of thyroxine into the active metabolite tri-iodothyronine is regulated by type II deiodinase expressed in tanycytes (Watanabe et al., 2004) (Yoshimura et al., 2003). The circannual changes in HP immunoreactivity in the choroid plexus in the chipmunks may therefore be part of a more general mechanism in which seasonally specific changes in the transporter cells of the brain (choroid plexus epithelium and tanycytes) are a common underlying substrate for winter adaptations. Indeed, given the decrease in activity in the nervous system and the long time course of events, it may be that endocrine and paracrine signaling are better suited to initiate, maintain, and terminate hibernation.

REFERENCES

Dibb, K.M., Hagarty, C.L., Loudon, A.S., and Trafford, A.W. (2005). Am. J. Physiol. Regul. Integr. Comp. Physiol. *288*, R607–R614.

Kondo, N., Sekilima, T., Kondo, J., Takamatsu, N., Tohya, K., and Ohtsu, T. (2006). Cell, this issue.

Lincoln, G.A., Johnston, J.D., Andersson, H., Wagner, G., and Hazlerigg, D.G. (2005). Endocrinology *146*, 3782–3790.

Osborne, P.G., and Hashimoto, M. (2006). Behav. Brain Res. *168*, 208–214.

Rodriguez, E.M., Blazquez, J.L., Pastor, F.E., Pelaez, B., Pena, P., Peruzzo, B., and Amat, P. (2005). Int. Rev. Cytol. *247*, 89–164.

Ross, A.W., Webster, C.A., Mercer, J.G., Moar, K.M., Ebling, F.J., Schuhler, S., Barrett, P., and Morgan, P.J. (2004). Endocrinology *145*, 13–20.

Ruby, N.F., Dark, J., Heller, H.C., and Zucker, I. (1998). Brain Res. *782*, 63–72.

Tartaglia, L.A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T., Deeds, J., et al. (1995). Cell *83*, 1263–1271.

Watanabe, M., Yasuo, S., Watanabe, T., Yamamura, T., Nakao, N., Ebihara, S., and Yoshimura, T. (2004). Endocrinology *145*, 1546–1549.

Yoshimura, T., Yasuo, S., Watanabe, M., Iigo, M., Yamamura, T., Hirunagi, K., and Ebihara, S. (2003). Nature *426*, 178–181.