the germline genome, by reducing the impact of germline variation in the regulatory sequences of the genes governing T and B cell homeostasis and responses. Indeed, the genes governing the development and function of each individual lymphocyte are partly regulated by the B or T cell antigen receptor signaling pathway, and are therefore influenced in each cell by the nature of each antigen receptor, possibly accounting for the rapid loss of heritability of most adaptive parameters. The adaptive immune system can be seen as "buffering" the influence of the germline in antigen-specific cells. Of course, adaptive immunity is also controlled by the germline, as illustrated by rare inborn errors of T cells and B cells, whether protective (e.g., CCR5 deficiency protecting T cells against HIV infection) or deleterious to the host (e.g., RAG deficiency preventing the development of T and B cells). However, the tremendous diversity of antigen receptors generated by somatic recombination may globally "loosen" the germline genetic chains of T and B cells, including common germline variations, providing us with some understanding of their negligible influence on immunological diseases. The equally tremendous spatial and temporal variety of antigenic challenges in the course of infections may leave countless somatic immunological imprints, each unique, on each individual, including identical twins. Like antigen-specific antibody titers, these various imprints may be more than a collection of scars; they may also ensure better-adapted and sustained host defense during recurrent or latent infections, at least at the population level.

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A Protein Restriction-Dependent Sulfur Code for Longevity

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The restriction of proteins has recently emerged as the most important factor for the beneficial effects of calorie restriction. Hine et al. now provide strong evidence for the role of the hydrogen sulfide (H_2S) gas in the protective effects of calorie and protein restriction against ischemia/reperfusion injury (IRI) but also implicate H_2S in longevity extension in model organisms.

A severe restriction of calories (CR) from all sources is among the most effective interventions to protect cells from toxins and extend both lifespan and healthspan, but it is the reduction in protein intake (PR) that has recently emerged as the most important factor responsible for lifespan- and healthspan-extending benefits (Bruce et al., 2013; Solon-Biet et al., 2014). In fact, the TOR-S6 kinase pathway, activated directly by certain amino acids, promotes aging in all major model organisms, and deficiency in insulin-IGF-1 signaling, which is induced by protein restriction, is implicated in lifespan extension in worms, flies, mice, and possibly humans (Fontana et al., 2010). In rodents, chronic cycles in which a normal diet is alternated with one lacking essential amino acids are sufficient to reduce IGF-1 levels and delay cognitive

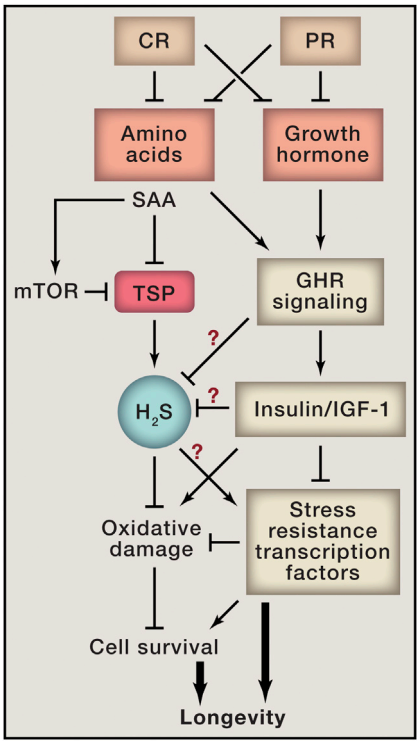

Figure 1. A Model for the Mechanisms of Calorie or Protein Restriction's Effects on Organismal Protection and Longevity

Specific sulfur amino acids activate nutrient-sensing pathways, including the insulin/IGF-1 and mTOR pathways, which accelerate the aging process in various organisms. Thus, CR, in part through protein/amino acid restriction, protects cells and organisms from stress by inhibiting insulin/IGF-1 and mTOR signaling and by switching cells to a stress-resistant mode characterized by entry into an alternative metabolic mode and H_2S generation. H_2S is important to prevent ischemia/reperfusion damage in the liver but has the potential to mediate many of the effects of CR/PR through yet poorly understood mechanisms.

decline in Alzheimer's disease models (Parrella et al., 2013), and in humans, a low protein intake is associated with reduced IGF-1 levels and a lower risk of mortality from all causes and cancer (Levine et al., 2014). Thus, the identification of the key nutrients regulating cellular protection and of the intracellular mediators of these effects is fundamental to the understanding of the mechanisms of aging. The ability of methionine deficiencies to lower IGF-1 and also protect against toxins (Miller et al., 2005) points to this essential sulfur amino acid as one of central importance in mediating the anti-aging effects of calorie restriction. In fact, methionine restriction extends lifespan in yeast, flies, and mice (Grandison et al., 2009; Miller et al., 2005; Ruckenstuhl et al., 2014). Thus, methionine can regulate the growth hormone-insulin-like growth factor 1 (GH-IGF-1) axis and probably the downstream mTOR-S6 kinase and PKA enzymes, as well as protective transcription factors (Figure 1). Although stress resistance transcription factors such as Msn2/4 in yeast and FOXOs in worms, flies, and mammals play essential roles in lifespan extension and can regulate both mitochondrial antioxidant enzymes and oxidative damage (Fontana et al., 2010), the underlying mechanisms connecting pro- and anti-aging pathways with stress resistance and longevity remain poorly understood.

In this issue of *Cell*, Hine and colleagues (2015) have now shed light on the mechanisms of CR-dependent protection by connecting calorie, protein, and methionine restriction to the activation of the transsulfuration pathway enzyme cystathionine γ-lyase (CGL), which is responsible for the generation of the gas hydrogen sulfide (H_2S). Restriction of all sources of calories or of only cysteine or methionine was sufficient to cause a major increase in H_2S and protection from hepatic ischemia reperfusion injury (IRI) in mice (Figure 1). This effect could be reversed by mTORC1 activation, indicating either that mTORC1 is functioning downstream of IGF-1 in this hepatic IR sensitization pathway or that cysteine and methionine can act directly to activate this kinase or upstream factors, as is well established for leucine (Sancak et al., 2008).

The demonstration that H_2S is not just a bystander but is, in fact, a mediator of the protective effects of CR came from the remarkable finding that injection of the H_2S precursor NaHS or H_2S itself in the drinking water conferred protection from the liver damage. Furthermore, CR did not protect mice lacking CGL from ischemia reperfusion injury, and CGL overexpression by adenoviral-mediated gene delivery improved the outcome after IRI. Evidence for a direct effect of H_2S on cellular stress resistance was generated by using an IRI in vitro hepatocyte model. H_2S protected a cultured hepatocyte cell line as well as primary hepatocytes during both the ischemic and reperfusion phases. To investigate further the molecular mechanisms of H_2S-dependent stress resistance, Hine and colleagues studied the role of target mitochondrial proteins, previously shown to be sulfhydrated and protect against stress-dependent damage/death. They show that the knockdown of the sulfide quinone oxidoreductase (SQR), which transfers electrons from H_2S to coenzyme Q, reduces the ability of H_2S to protect cells from ischemia- and reperfusion-dependent damage. The hypothesis is that CR-dependent protection against ischemia could be due, in part, to the ability of H_2S to facilitate electron transport in the wake of limited nutrient supply and possibly also to the generation of sulfites or sulfates, which in bacteria can act as terminal electron acceptors for ATP production under hypoxic conditions.

In an alternative mechanism, which was not investigated in this study, H_2S could also act as a signaling molecule that can itself activate transcription factors controlling stress resistance and longevity (Figure 1). Thus, it will be interesting to determine whether H_2S could function both at the mitochondrial level to prevent oxidative damage and in the cytoplasm and nucleus to regulate enzymes and transcription factors that control cell protection and aging. In fact, the authors provide evidence for the association between H_2S and CR-dependent lifespan extension of yeast, worms, and flies independently of an ischemia-reperfusion challenge. In yeast, they show that glucose restriction induces endogenous H_2S production via the TSP from sulfur amino acids (SAAs) and that the exogenous H_2S donors, NaHS and GYY4137, added early to the culture, extended chronological longevity. In worms, the *eat-2* mutant, a genetic model of lifespan extension by CR, produced more H_2S gas, and knockdown of CBS-1, another enzyme, which promotes H_2S generation, decreased *eat-2*-mediated lifespan extension, while its overexpression extended the lifespan of wild-type controls. In flies, maximum H_2S production capacity was associated with diets that maximize lifespan.

Thus, multiple dietary-restricted regimens that promote longevity and healthspan, including single amino acid restriction, increased H_2S production in

organisms ranging from yeast to mice, indicating that generation of this gas may play a conserved protective role in many organisms, at least when generated at a specific concentration. In fact, H_2S has been traditionally regarded as a toxic gas, but recent findings indicate that it functions as a signaling molecule with potential therapeutic applications in a variety of conditions, including its remarkable ability to induce suspended animation in mice. Hine and colleagues have identified specific dietary interventions that result in the generation of protective levels of H_2S but also provided data that advance our understanding of the anti-aging mechanisms induced by CR/PR. The identification of drugs that induce the generation of protective levels of H_2S while eliminating the risk of toxic side effects could have important clinical applications for the treatment of a variety of acute conditions, such as ischemia/reperfusion and other types of damage associated with surgery. The chronic use of drugs promoting H_2S for healthspan extension could instead be much more problematic, as it is difficult to predict what the side effects caused by long-term use could be. If the effects described in model organisms are conserved in humans, protein restriction without malnutrition or the consumption of plant-based proteins with low sulfur amino acid content may represent a safer strategy to induce H_2S generation and take advantage of its protective effects.

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Skeletal Stem Cells in Space and Time

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The nature, biological characteristics, and contribution to organ physiology of skeletal stem cells are not completely determined. Chan et al. and Worthley et al. demonstrate that a stem cell for skeletal tissues, and a system of more restricted, downstream progenitors, can be identified in mice and demonstrate its role in skeletal tissue maintenance and regeneration.

The groundbreaking concept that bone, cartilage, marrow adipocytes, and hematopoiesis-supporting stroma could originate from a common progenitor and putative stem cell was surprising at the time when it was formulated (Owen and Friedenstein, 1988). The putative stem cell, nonhematopoietic in nature, would be found in the postnatal bone marrow stroma, generate tissues previously thought of as foreign to each other, and support the turnover of tissues and organs that self-renew at a much slower rate compared to other tissues associated with stem cells (blood, epithelia). This concept also connected bone and bone marrow as parts of a single-organ system, implying their functional interplay. For many years, the evidence underpinning the concept has been incomplete. While multipotency of stromal progenitors has been demonstrated by in vivo transplantation experiments, self-renewal, the defining property of a stem cell, has not been easily demonstrated until recently in humans (Sacchetti et al., 2007) and mice (Méndez-Ferrer et al., 2010). Meanwhile, a confusing and plethoric terminology has been introduced into the literature, which diverted and confounded the search for a skeletal stem cell and its physiological significance (Bianco et al., 2013).