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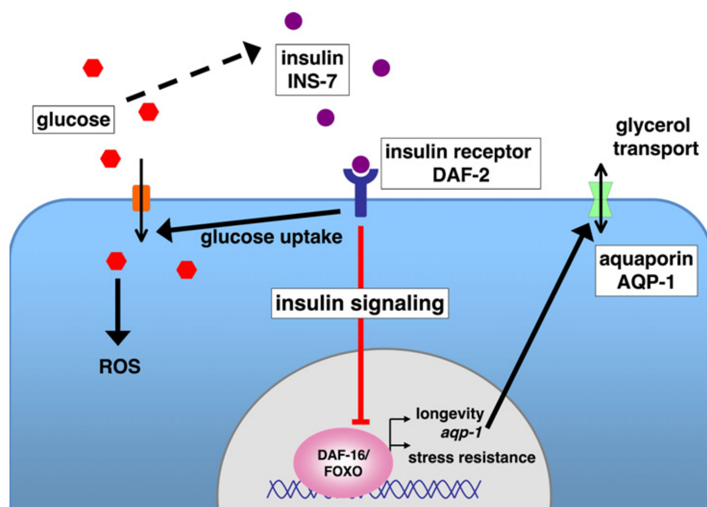

Figure 1. Effects of Glucose on Insulin-like Signaling and Longevity
Glucose stimulates release of insulin, which induces glucose uptake. Insulin-like signaling also inhibits FOXO/DAF-16, which positively regulates *aqp-1* and other stress resistance and longevity genes. High glucose levels may increase cellular ROS. In *C. elegans*, these events all appear to occur in the intestine, although insulin-like signaling responses vary among tissues.

hyperglycemia and resultant tissue damage, it seems that one might want to get by with needing as little insulin as possible, in order to minimize its inhibition of the life span-extending effects identified in animal models. The present study may also have implications for understanding effects of calorie restriction, a condition that prolongs life in essentially every organism examined (Bishop and Guarente, 2007). By identifying a specific life span-inhibitory effect of glucose, its results raise the question of whether the effects of limiting calories and glucose might be distinguishable. Evidently, we can't have our cake and eat it too just yet, but hope remains that a better understanding of how low insulin activity increases life span could allow these prolongevity mechanisms to be harnessed without impairing the essential activities of insulin. In the meantime, this work provides additional motivation to skip dessert.

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A Tale of Two Carboxypeptidases

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Proopiomelanocortin (Pomc) neurons play a central role in energy homeostasis. Despite the complexity of Pomc posttranslational processing, regulation of *Pomc* gene expression often takes center stage. Complementary papers that zero in on distinct carboxypeptidases (Plum et al., 2009; Wallingford et al., 2009) now refocus the spotlight on regulated peptide cleavage.

Hypothalamic proopiomelanocortin (Pomc) neuronal circuits play a critical role in tightly matching body weight to a fixed set point, primarily by inhibiting appetite and feeding behavior (Cone, 2005). Multiple regulatory steps in Pomc biosynthesis ultimately determine Pomc-derived peptide tone in the CNS in response to hormonal, metabolic, and *trans*-synaptic inputs to Pomc neurons (Figure 1). Indeed, Pomc has served as a paradigm for investigating posttranslational processing of prohormones into multiple bioactive peptides (Pritchard and White, 2007). Among the well-characterized enzymes are the prohormone convertases subtilisin/kexin type PC1 and PC2, carboxypeptidase E (Cpe), and peptidylglycine α-amidating monooxygenase (PAM). Spontaneous loss-of-function mutations in *PCSK1* or *Cpe* cause obesity in humans

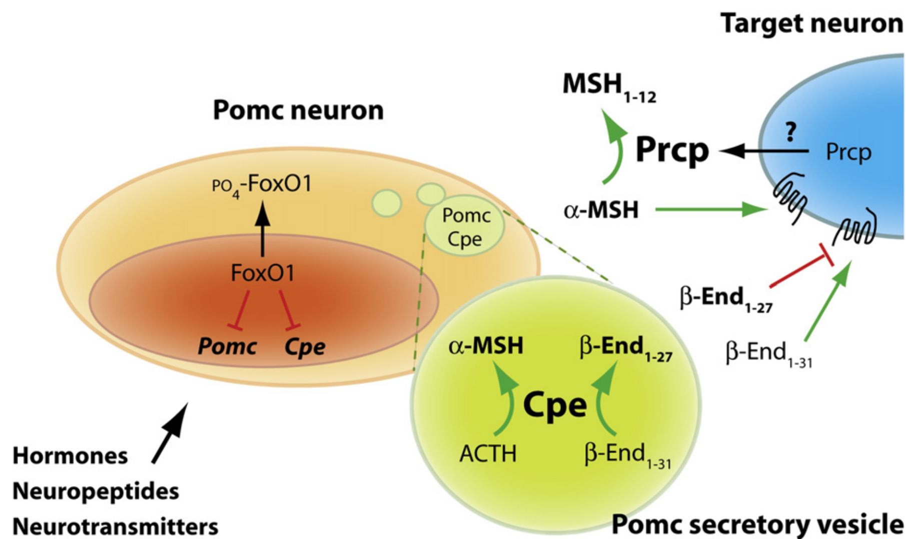

Figure 1. Integrated Signaling from Multiple Humoral and Neural Inputs Regulates Pomc Expression, Posttranslational Processing, and Peptide Release from Axon Terminals

Constitutively nuclear FoxO1 has been implicated previously as a transcriptional repressor of *Pomc*. Evidence linking FoxO1 to inhibition of *Cpe* expression in Pomc neurons illuminates a second pathway for this protean transcription factor to coordinate metabolic signaling by inducing changes in the mix of melanocortin and β-Endorphin peptide isoforms cleaved from Pomc and released from secretory vesicles. The fate of neuropeptides in the extracellular space has received relatively little attention compared to the earlier steps in their biosynthesis. A new study implicates Prcp in the degradation of biologically active α-MSH to inactive α-MSH_{1-12}, thereby limiting the stimulation of melanocortin receptors on target neurons and illustrating another regulated step in Pomc peptide processing that mediates anorexigenic tone in Pomc neural circuits.

and mice (*fa/fa*), respectively, but their pleiotropic range of substrates complicates the assignment of causation to any single neuropeptide precursor. Additionally, Pomc processing raises an intriguing biological paradox due to the stoichiometric production of melanocortins, which in the most simplistic terms activate G_s-coupled receptors on target neurons to inhibit feeding, and the opioid peptide β-$endorphin_{1-31}$, which activates G_i-coupled receptors to stimulate feeding.

It is in this context that Plum and colleagues writing in *Nature Medicine* explored a molecular pathway linking forkhead box-containing transcription factor of the O subfamily type 1 (FoxO1) to Cpe-mediated Pomc peptide processing in hypothalamic Pomc neurons (Plum et al., 2009). FoxO1 plays a coordinating role in several well-explored developmental and metabolic intracellular signaling programs in response to insulin (Gross et al., 2008). Constitutively nuclear FoxO1 is phosphorylated via an insulin receptor-Irs1-Akt pathway and then translocated to the cytoplasmic compartment, thereby abrogating its function as a regulator of gene expression (Fukuda et al., 2008). FoxO1's transcriptional valence depends on the particular promoter context and recruitment of coactivator or corepressor complexes. *Pomc* itself has been implicated as a target of nuclear FoxO1, although the precise mechanism for its inhibitory effect is controversial (Kitamura et al., 2006; Yang et al., 2009) and studies performed in transfected, heterologous cell lines may not truly represent the endogenous milieu present in Pomc neurons. Moreover, the full set of physiologically relevant transcriptional targets for FoxO1 apart from *Pomc* and the genes encoding neuropeptide Y (*NPY*) and Agouti-related protein (*Agrp*) (Kim et al., 2006) in neurons of the feeding control circuits remains unknown.

Therefore, Plum and colleagues created a new mouse model of FoxO1 deficiency restricted to POMC neurons and pituitary cells (Plum et al., 2009). Major findings in the mice were a phenotype of leanness, decreased food intake under basal and postfasting/refeeding conditions, but unaltered energy expenditure, locomotor activity, and corticosterone secretion. *Pomc-Foxo1*$^{-/-}$ mice were also partially protected from weight gain induced by a high-fat diet. Notably, despite the aforementioned reports of *Pomc* serving as an inhibitory target for FoxO1, there was no evidence for increased *Pomc* mRNA expression in the adult mutant mice under basal conditions or upon refeeding after an 18 hr fast. However, the altered physiological parameters were associated with increased mediobasal hypothalamic content of α-MSH compared to stable Pomc precursor and ACTH levels and an increased ratio of β-$endorphin_{1-26}$ (β-End_{1-26}) and β-End_{1-27} relative to the more potent full-length β-End_{1-31} upon refeeding. Consistent with this anorexigenic Pomc peptide profile, *Cpe* mRNA levels were increased specifically in the arcuate nucleus. Furthermore, wild-type but not mutant mice fed a high-fat diet exhibited a substantial decrease in *Cpe* mRNA levels, while generalized overexpression of *Cpe* in the arcuate nucleus from an adenoviral vector mimicked the attenuated refeeding response after a fast that was observed in *Pomc-Foxo1*$^{-/-}$ mice. In vitro studies using transfected Neuro2A cells suggested that FoxO1 suppresses *Cpe* promoter activity by acting as a corepressor independent of direct DNA binding.

On the face of it, the paper lays out a compelling argument for *Cpe* as a physiologically meaningful nuclear target of FoxO1 signaling. However, the absolute magnitude of change in body weight, body composition, and food intake in *Pomc-Foxo1*$^{-/-}$ mice was relatively small and the alterations in mediobasal

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hypothalamic peptide ratios were only measureable after a fasting, refeeding paradigm and not under basal conditions. Cell counts revealed a trend toward decreased numbers of arcuate Pomc neurons based on their identification by in situ hybridization for *Pomc* mRNA. The cause of this decrease is unknown, but in any event, it would be expected to tip the balance in favor of weight gain, not the weight loss observed in *Pomc-Foxo1*$^{-/-}$ mice. The final proof of altered Cpe expression playing the proximate causative role in the phenotype may come from a conditionally activated/inactivated *Cpe* allele specifically in Pomc neurons. If confirmed, drug development targeted at Cpe to decrease appetite without a compensatory drop in metabolic rate may indeed be a rationale new approach to the management of obesity and insulin resistance, as suggested by the paper's authors.

A complementary study published in the *Journal of Clinical Investigation* by Wallingford and colleagues (Wallingford et al., 2009) further highlights the potential for targeted pharmacotherapy aimed at modulation of Pomc posttranslational processing to tweak Pomc circuits in favor of an anorectic and catabolic mode. The authors are the first to identify a selective degradation pathway for extracellular α-MSH involving the enzyme prolylcarboxypeptidase (Prcp). *Prcp* was identified as a candidate obesity gene after positional cloning of the introgressed locus from a lean, subcongenic mouse strain. Recombinant enzyme efficiently cleaved the amidated valine at residue 13 of α-MSH, converting it into the biologically dead peptide α-MSH_{1-12}. Furthermore, an inactivating gene-trap mutation in murine *Prcp* resulted in a lean phenotype with increased hypothalamic content of α-MSH and an elevated α-MSH/ACTH ratio. Pharmacologic inhibition of endogenous Prcp in wild-type mice acutely reduced food intake, consistent with the chronic phenotype associated with genetic deficiencies in *Prcp*. Based on its localization in the hypothalamus, the authors speculate that regulated release of the enzyme from postsynaptic sites on second order neurons may modulate the strength of melanocortin signaling by accelerated degradation of α-MSH.

In several aspects, the tale of these two carboxypeptidases is analogous except that Cpe cleaves Pomc-derived intermediates into a more potent anorexigenic mix of peptides while Prcp terminates α-MSH bioactivity by slicing just one amino acid too deep. Both papers clearly underscore the importance of quantifying levels of all processed forms of Pomc peptides, in addition to total peptide levels and *Pomc* mRNA expression, to fully understand the molecular basis of associated phenotypes. The ultimate step in linking altered Pomc peptide profiles with biological function must include development of more sophisticated and sensitive technologies to simultaneously measure multiple peptides, not just at the anatomic level of whole hypothalamic areas under a static time frame as performed in the two studies, but in more discretely defined projection areas of Pomc neurons and under dynamic conditions.

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