Regulation of body size in Caenorhabditis elegans: effects of environmental factors and the nervous system

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ABSTRACT Body size is one of the basic traits of animals and is regulated to adapt to the environment. Animals perceive environmental stimuli with sensory neurons, and signals from the nervous system alter the size of organs, thus regulating body size. The model animal Caenorhabditis elegans is particularly suited for genetic analysis of body size regulation, and has already contributed to the elucidation of various genetic pathways that regulate body size. In this review, we summarize the available literature regarding environmental factors that regulate body size and the role of the nervous system in such regulation. We discuss in detail a recent report on body size regulation by the neurotransmitter, dopamine.

KEY WORDS: body size, environmental factors, dopamine, Caenorhabditis elegans

Environmental factors control animal body size

Body size is one of the most important features of an organism. Several environmental factors are known to affect animal body size, and food is one of the most influential ones. Food-rich conditions promote cell division and allow animals such as Drosophila to grow larger (de Moed et al., 1997). Size differences could also arise due to the quality of food. Intake of high-fat diets during early life results in obesity in mice (Lin et al., 2000). High-fat diets could also epigenetically regulate the metabolism via modification of adiponectin and leptin genes (Masuyama and Hiramatsu, 2012). Apart from nutritional factors, an association between body size and ambient temperature has also been reported. Tail length and surface area of the ear of mice have been positively correlated with ambient temperature (Serrat et al., 2008). Lower temperatures can cause animals to grow larger. For example, lower temperatures have been reported to increase the body size of female Drosophila by increasing the cell size; in males, not only the cell size, but the cell number also increases, indicating that the mechanism of body size regulation differs between females and males (French et al., 1998; de Moed et al., 1997; Robertson, 1959). In addition, Drosophila also responds to changes in humidity; surface area of the wings was found to be larger in low-humidity conditions (Kennington et al., 2003). Although several such environmental factors affect the body size, the mechanism behind this regulation is not fully understood. The nematode Caenorhabditis elegans is particularly suited for the study of body size regulation because it exhibits rapid development and is amenable to extensive genetic analyses. Various signaling pathways that regulate body size have been identified through studies using C. elegans.

TGF-β pathway regulates body size

Transforming growth factor-β (TGF-β) signaling regulates the animal body size (Patterson and Padgett, 2000; Tuck, 2014). TGF-β superfamily ligands are important in the regulation of cell identity, function, and survival, and play important roles in many diseases (Massagué, 2012; Wu and Hill, 2009). TGF-β ligands are secreted by various cell types, and activate TGF-β receptors, which are heterodimers of types 1 and 2 receptor subunits. The TGF-β receptors activate SMAD (Sma- and Mad-related) proteins, which enter the nucleus upon activation and function as transcription factors to induce the expression of various genes (Fig. 1). The TGF-β ligand, DBL-1, has been identified in C. elegans (Morita et al., 1999; Suzuki et al., 1999). DBL-1 is homologous to Drosophila Decapentaplegic (Dpp) and mammalian bone morphogenetic proteins. dbl-1-deficient mutants are smaller than the wild-type animals. DBL-1 does not affect cell division; instead, it

Abbreviations used in this paper: MAP kinase, mitogen-activated protein kinase; SMAD, Smad and Mad-related; TGF-β, transforming growth factor-β; TOR, target of rapamycin.

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regulates body size by stimulating the growth of hypodermal cells, which surround the animal body. In mammals, changes in cell size and number could result in variations in body size. However, the number of cells in large and small C. elegans mutants has been found to be unchanged in all analyses performed so far. This is presumably because of the small number of cells and the rigid cell lineage (Morita et al., 2002). DAF-4 and SMA-6 form a complex that functions as the DBL-1 receptor, which activates the SMAD transcription factors, SMA-2, SMA-3, and SMA-4 (Estevez et al., 1993; Krishna et al., 1999). SMA-3 interacts with LIN-31, a winged helix transcription factor (Wang et al., 2005). RNAI knockdown of lin-31 results in slightly reduced body size (Rual et al., 2004). LIN-31 is also involved in the TGF-β signaling for tail development in male C. elegans (Baird and Ellazar, 1999). In addition, the SMAD-interacting transcription cofactor SMA-9, which is homologous to the Drosophila Schnurri (Shn), functions as a downstream factor of DBL-1 in body size regulation (Liang et al., 2003).

DBL-1 is expressed in the neurons, whereas the receptor SMA-6 and the SMADs function in the hypodermal cells to regulate body size (Yoshida et al., 2001). Therefore, it has been proposed that DBL-1, secreted from neurons, transmits the signals to the hypodermal cells, thus regulating body size. LON-1 is homologous to the cysteine-rich secretory protein family and acts downstream of the TGF-β pathway in body size regulation. Expression of lon-1 is suppressed by DBL-1 via the SMAD proteins (Maduizia et al., 2002; Morita et al., 2002). LON-1 regulates the polyplidization of hypodermal cells, which in turn regulates hypodermal cell size, thereby affecting body size.

**Other genetic factors**

Insulin/IGF-1 signaling is involved in many metabolic and neural regulations in animals, including energy metabolism, development, longevity (Dyer et al., 2016; Nässel et al., 2015), as well as body size. In mammals, the insulin/IGF signal acts downstream of the growth hormone (GH) to regulate body size (Blutke et al., 2014; Lundberg et al., 2015). Insulin/IGF also regulates body size in invertebrates, which do not possess GH. Drosophila with mutations in the insulin/IGF-1 signaling pathway (chico and divir) are smaller than wild-type animals. The insulin/IGF-1 signal has been reported to regulate the body size in a cell-autonomous manner (Böhn et al., 1999; Brogiolo et al., 2001). In addition to the insulin-like peptides, the steroid molting hormone (ecdysone) and the sesquiterpenoid juvenile hormone are involved in body size regulation in Drosophila (Truman and Riddiford, 2007). These two hormones regulate growth and body size through signals related to the nutritional condition (Layalle et al., 2008; Shiao et al., 2008; Shimada-Niwa and Niwa, 2014). In C. elegans, forty insulin-like peptides have been identified. Each peptide is differentially expressed in different organs, including many neurons, and their expression levels change under different environmental conditions or during the different developmental stages (Ritter et al., 2013). Despite these different insulin-like peptides, C. elegans has only a single receptor, DAF-2. As with mammals, phosphoinositide 3-kinase (PI3K) and serine/threonine kinase (Akt) have been reported to function downstream of DAF-2 in C. elegans as well (Paradis et al., 1999; Paradis and Ruvkun, 1998). Akt kinases phosphorylate the DAF-16/FOXO transcription factor and negatively regulate the transcriptional activity of DAF-16. Insulin/IGF signaling is important for the regulation of development and behaviors in response to environmental factors (Gems et al., 1998; Tomioka et al., 2006). In addition, the presence of certain strains of bacteria in the diet has been reported to increase the body size of C. elegans. Animals fed E. coli HB101 were reported to grow larger than those fed a standard E. coli OP50 diet (So et al., 2011). The effect of the HB101 diet was reduced in daf-2 mutants, suggesting that DAF-2 insulin/IGF receptors are involved in the regulation of body size through food quality.

Signals from the germ line have also been reported to regulate lifespan and body size (Patel et al., 2002). Germ line ablation leads to longer lifespans and this lifespan extension is suppressed by daf-16 mutations. Ablation of germ line cells also causes animals to grow larger. Unlike the regulation of longevity, the effect of germ line signaling on body size is independent of DAF-16. As body size regulation by germ line signaling has also been shown to be...
independent of the TGF-β ligand, DBL-1, the molecular mechanism of this regulation is currently unknown.

The target of rapamycin (TOR) pathway affects developmental regulation and acts downstream of insulin signaling (Scott et al., 1998; Sekulic et al., 2000). The serine-threonine kinase TOR functions in two distinct complexes, TORC1 and TORC2. The TORC1 pathway regulates cell growth through the initiation factor 4E-BP, which is associated with translation factors and ribosomal S6 kinase (S6K) in animals (Gingras et al., 2001). Mutations in the Drosophila TOR homolog, dTOR, cause a reduction in body size because of a reduction in cell size and number (Zhang et al., 2000). The dS6K mutants are also smaller than wild-type Drosophila (Montague et al., 1999). TORC2 also regulates body size in Drosophila and C. elegans (Hietakangas and Cohen, 2007; Lee and Chung, 2007).

Rictor, which encodes one of the TORC2 components, positively regulates body size in C. elegans, and Rictor mutants (rick-1) are smaller than wild-type animals (Jones et al., 2009). Furthermore, Rictor functions independent of the TGF-β pathway or akt-1 and daf-16, which are components of the insulin-like signaling pathway, while sgk-1, the homolog of the serum- and glucocorticoid-induced kinase, acts downstream of TORC2.

Mutants for sma-5, which encodes a MAP kinase BMK1/ERK5, are smaller than wild-type C. elegans (Watanabe et al., 2005). The MAPK pathway also affects body size independent of the TGF-β pathway. Double mutants, with defects in both MAPK and TGF-β pathways, are smaller than single mutants (Watanabe et al., 2007).

The Hippo pathway was first identified in Drosophila, and its role in the regulation of development is well established (Justice et al., 1995; Xu et al., 1995; Yu et al., 2015). The components of this pathway are evolutionarily conserved and involved in cell proliferation, thus controlling organ and body sizes. Some genes encoding homologs of the Hippo pathway components have been identified in C. elegans. RNAi knockdown of the warts homolog, wts-1, results in small body size in C. elegans (Cai et al., 2009). However, the function of wts-1 in C. elegans may be different from that in Drosophila, which negatively regulates overgrowth. Analyses of epistasis showed that wts-1 regulates body size independent of the TGF-β pathway.

Body size is regulated by the quality and quantity of food

Body size is also affected by the quality of diet. For example, animals fed Comamonas DA1877 developed faster and grew larger than those fed E. coli OP50 (MacNeil et al., 2013). This was because of B12, which is produced by Comamonas DA1877, but not by E. coli OP50 (Watson et al., 2014). As mentioned in the previous section, the E. coli HB101 diet has also been reported to cause an increase in body size (So et al., 2011), and this regulation requires insulin/IGF signaling. In addition, quantity of diet also affects body size. C. elegans swallow bacteria by rhythmically moving their pharynx (pharyngeal pumping). The rate of pharyngeal pumping determines the amount of food taken in. Mutants with defective pharyngeal pumping have been shown to take in food less efficiently, and to be smaller, than wild-type animals (Mohr and Pilon, 2006; So et al., 2011). For example, eat-2 mutants, which have defects in the mechanism for receiving signals from the pharyngeal neurons, are smaller than wild-type animals. The mutants for pha-2 and pha-3, which are required for normal pharyngeal development, also exhibit smaller body size than wild-type animals. Similarly, animals grown in conditions with food scarcity are smaller than those grown with sufficient food (Lenaerts et al., 2008). These results indicate that reduced food intake results in smaller body size. Moreover, decreased food intake has a transgenerational effect. Animals that experience starvation during the L1 larval stage produce progeny with shorter body lengths (Jobson et al., 2015), suggesting that severe starvation causes decreased body length in progeny through epigenetic regulation.

Environmental factors regulate body size

Environmental stimuli can also regulate body size. In general, ectotherms grown at lower temperatures achieve larger sizes, and this phenomenon is called the temperature-size rule. Standard N2 wild-type animals used in most laboratory experiments seems to comply with this rule and grow larger when cultured at 12 °C than at 24 °C (Karmenga et al., 2007). However, body size alteration based on temperature is not observed in a wild isolate, CB4856. This is due to a single nucleotide polymorphism in tra-3, which encodes a calpain-like protease. Furthermore, CB4856 animals are smaller than N2 animals, even when grown at standard temperatures, due to a variation in npr-1, which encodes a G protein-coupled receptor related to neuropeptide Y receptors (Andersen et al., 2014). C. elegans, when raised in isolation, exhibit decreased developmental rates and smaller body sizes compared to animals raised in groups (Rose et al., 2005). Isolated animals also exhibit decreased responses to mechanical stimulation. However, mechanosensory stimulation during development reversed the effect of isolation on body size and mechanosensory response. Furthermore, isolation had no effect on the body size of mutants for mec-4, which is essential for the functioning of touch-sensing neurons. These results demonstrate that mechanosensory neurons regulate the body size based on the level of sensory stimulation received during development.

Calcineurin, a calcium- and calmodulin-dependent serine/threonine protein phosphatase, is also involved in body size regulation. Mutants for tax-6 and cnb-1, which encode the calcineurin subunits A and B, respectively, are smaller than wild-type animals (Bandypadhyay et al., 2002; Kuhara et al., 2002). Although the effect of tax-6 mutants on body size could be partially suppressed by the expression of TAX-6 in body wall muscles, it is fully suppressed only by pan-neuronal expression of the tax-6 cDNA. These results suggest that calcium signaling is induced in neurons by environmental stimuli, and that it regulates body size via the calcineurin pathway.

Ciliated sensory neurons can perceive environmental stimuli such as odor, salt, temperature, and pH. che-2 gene, which encodes a WD protein, is required for cilia formation and for perception of various stimuli (Fujiwara et al., 1999). che-2 mutants are smaller than wild-type animals, suggesting that perception of environmental stimuli is important for the regulation of body size (Fujiwara et al., 2002). However, mutants for egl-4, which encodes a GMP-dependent protein kinase and is expressed in most neurons, are larger than wild-type animals. The egl-4 mutation suppresses the reduction in body size of che-2 mutants; the effect of egl-4 is mediated by its function in the ciliated sensory neurons. The egl-4 gene regulates body size by negatively regu-
Dopamine regulates body size

In mammals, amine neurotransmitters such as dopamine and noradrenaline play critical roles in the regulation of behavior and metabolism. In *C. elegans*, dopamine is involved in sensing food and in food-dependent behavioral changes (Hills et al., 2004; Sawin et al., 2000). There are eight dopaminergic neurons in hermaphrodite *C. elegans* and they are all morphologically mechanosensory neurons (Sulston et al., 1975). It has been suggested that these neurons directly sense bacteria by touch and release dopamine in the presence of suitable food. The released dopamine causes multiple behavioral changes, including reduced locomotion and increased backward movement (Kindt et al., 2007; Sanyal et al., 2004). Five dopamine receptors have been identified in *C. elegans*: a D1-like receptor, DOP-1 (Sanyal et al., 2004; Suo et al., 2002); D2-like receptors, DOP-2 and DOP-3 (Chase et al., 2004; Sugiura et al., 2005; Suo et al., 2003); an invertebrate-specific receptor, DOP-4 (Sugiura et al., 2005); and a dopamine-gated chloride channel, LGC-53 (Ringstad et al., 2009). Octopamine is another monoamine neurotransmitter, considered the biological equivalent of noradrenaline (Roeder, 1999). In *C. elegans*, octopamine signaling is activated in the absence of food (Su et al., 2006), and dopamine suppresses this octopamine signaling (Kimura et al., 2010; Suo et al., 2009). This regulation prevents the activation of octopamine signaling in the presence of food, as dopamine signaling is active. There are three octopamine receptors identified to date: Gq-coupled receptors, SER-3 and SER-6, and a Gi-coupled receptor, OCTR-1 (Suo et al., 2006; Wragg et al., 2007; Yoshida et al., 2014). Although many of the roles that these neurotransmitters and their receptors play in the regulation of behavior and metabolism of *C. elegans* have been elucidated, the effects of dopamine on development are still unclear.

It was recently found that dopamine negatively regulates body size in *C. elegans* (Nagashima et al., 2016). Mutants for cat-2 gene, which encodes tyrosine hydroxylase, a rate-limiting enzyme for dopamine synthesis, have a decreased level of dopamine and exhibits greater body length and width, compared to wild-type animals (Fig. 2). This increased body length of cat-2 mutants was suppressed by the introduction of the wild-type cat-2 gene or exogenous dopamine, suggesting that endogenous dopamine suppresses body size. Interestingly, the regulation of body size by dopamine is limited to hermaphrodites. The body length of male cat-2 mutants was not significantly different from that of wild-type males. The D2-like dopamine receptor, DOP-3, is likely the receptor through which dopamine functions for body size regulation. Among the five dopamine receptor mutants tested, dop-3 mutants were longest, with similar length as cat-2 mutants. In contrast, dop-4 mutants were shorter than wild-type animals. The body length of dop-3;dop-4 double mutants was not different from that of dop-3 single mutants, suggesting that dop-3 plays a major role in body size regulation by dopamine. These results demonstrate that dopamine negatively regulates body size and that Dop-3 receptors are critical for body size regulation by dopamine.

Factors downstream of dopamine

Genetic analyses have revealed that octopamine signaling acts downstream of dopamine for body size regulation (Nagashima et al., 2016). *tbh-1* encodes tyramine β-hydroxylase, which is required for octopamine synthesis, and therefore, *tbh-1* mutants lack octopamine (Alkema et al., 2005). The body length of cat-2;tbh-1 double mutants was not different from that of wild-type animals (or *tbh-1* single mutants), indicating that the increased body length of cat-2 mutants was suppressed by *tbh-1*. This result suggests that octopamine signaling is activated in cat-2 mutants, and that the activated octopamine signaling leads to increased body size; however, this effect is absent in cat-2;tbh-1 double mutants, in which octopamine signaling is inactivated. Furthermore, the body length enlargement of cat-2 mutants was at least partially suppressed in the background of octopamine receptor mutants, ser-3 and ser-6. These results suggest that the octopamine-SER-3/6 signaling pathway functions downstream of dopamine to regulate the body size in *C. elegans*.

As reduced food intake results in reduced body size, it is possible that dopamine affects food intake to regulate the body size. The pharyngeal pumping rate of *cat-2* mutants was not significantly different from that of wild-type animals, suggesting that the quantity of food is not related to body size regulation by dopamine. Food quality also does not appear to play a role in the dopamine-induced regulation of body size, because even when *cat-2* mutants were raised on the DA1877 or HB101 diets, they were larger than wild-type animals. However, body size regulation by dopamine did depend on the insulin/IGF receptor, DAF-2, which is also essential for the diet-dependent increase in body size. Body length of *cat-2;daf-2* double mutants was not significantly different from...
that of the \textit{dat-2} single mutants; increased body length of \textit{cat-2} mutants was suppressed in the background of the \textit{dat-2} mutation. This result suggests that the insulin/IGF signal acts downstream of dopamine for body size regulation.

The relationship between dopamine and the TGF-\(\beta\) pathway

The relationship between body size regulation by dopamine and the TGF-\(\beta\) pathway has been studied (Nagashima \textit{et al.}, 2016). Both \textit{che-2} (sensory functions) and \textit{egl-4} (cGMP-dependent protein kinase) act upstream of the TGF-\(\beta\) signals (Fujiwara \textit{et al.}, 2002). \textit{cat-2;che-2} double mutants were longer than \textit{che-2} single mutants, suggesting that dopamine regulates body size independent of \textit{che-2}. However, the body length of \textit{cat-2;egl-4} double mutants was not significantly different from that of the \textit{egl-4} single mutants, suggesting that dopamine depends on \textit{egl-4} for the regulation of body size. The \textit{egl-4} gene is expressed in many neurons and supports various biological functions (Daniels \textit{et al.}, 2000; Hao \textit{et al.}, 2011). Therefore, it is possible that dopamine requires \textit{egl-4} in neurons (other than the sensory neurons), in which \textit{egl-4} functions downstream of \textit{che-2} for body size regulation.

The relationship between dopamine and the TGF-\(\beta\) ligand, DBL-1, was examined (Nagashima \textit{et al.}, 2016). Three different alleles of the \textit{dbl-1} mutants and two different alleles of the \textit{cat-2} mutants were investigated. The increase in body length of \textit{cat-2} mutants was not suppressed in any of the allele combinations, except one. This result supports the idea that dopamine regulates body size independent of the TGF-\(\beta\) ligand. \textit{sma-2} and \textit{sma-3} encode SMAD transcription factors that act downstream of \textit{sma-6}, which encodes a subunit of the DBL-1 receptor. Dopamine appears to act independent of the SMAD transcription factors, because double mutants for \textit{cat-2} and \textit{sma-2} or \textit{sma-3} were longer than the \textit{sma-2} or \textit{sma-3} single mutants, respectively, although the difference was very small. These results also support the possibility that dopamine regulates body size independent of the TGF-\(\beta\) pathway. However, \textit{cat-2;smal-6} double mutants were extremely sick, and the relationship between \textit{cat-2} and \textit{sma-6} is still unknown. Thus, the apparent suppression of \textit{cat-2} by \textit{dbl-1} in one allele combination suggests a possibility that the regulation of body size by dopamine partially depends on the TGF-\(\beta\) pathway.

Roles of dopamine in body size regulation

Regulation of body size by dopamine has been reported in several organisms. In mice, D2 dopamine receptors regulate body size by regulating GH secretion (Noain \textit{et al.}, 2013). In humans, hypersecretion of GH and elevated levels of IGF-1 cause acromegaly, a disease associated with increased body size. Patients with acromegaly are treated with dopamine agonists such as cabergoline, which suppresses GH secretion (Abs \textit{et al.}, 1998). Moreover, an association between stature and polymorphism in the promoter region of the human D2 dopamine receptor gene has been reported; lower transcriptional activity of the receptor results in greater stature (Arinami \textit{et al.}, 1999). In mammals, dopamine signaling is also affected by food in the context of body size regulation. It has been reported that offspring of mice fed high-fat diets gain weight through epigenetic regulation. In the offspring, the expression of dopamine reuptake transporters was increased, whereas that of D1 and D2 dopamine receptors was reduced (Vucetic \textit{et al.}, 2010). Taken together, these results suggest that dopamine-induced regulation of body size may be conserved across species.

The TGF-\(\beta\) pathway regulates polyplidization of hypodermal nuclei and this could affect body size by regulating the size of the hypodermal cells (Morita \textit{et al.}, 2002). The TGF-\(\beta\) ligand, DBL-1, is produced in the neurons and transmits the signal to

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Fig. 3. A model for the regulation of body size by environmental factors in \textit{Caenorhabditis elegans}. Several stimuli from the ambient environment affect body size. These stimuli are perceived by sensory neurons and the resultant signals from the nervous system influence body size (blue arrows). Dopamine, which is thought to be released upon tactile perception of food, negatively regulates body size through the DOP-3 dopamine receptor. Dopamine signaling negatively regulates octopamine signaling, which signals through SER-3 and SER-6 octopamine receptors. The observation that body size is regulated by dopamine, which has a role in the perception of food, suggests that not just ingestion, but the perception of food also regulates body size via the nervous system. The perception of mechanical and other stimuli by the amphid sensory neurons affects body size through MEC-4 and CHE-2, respectively. Low temperatures cause larger body sizes in animals, and this regulation requires TRA-3, a calpain-like protease. Both quality and quantity of food intake affect body size (red arrows). Animals fed the bacterial strain HB101 grow larger than those fed OP50, and this regulation is mediated by the insulin/IGF receptor, DAF-2. Bacterial strains that contain vitamin B12, such as Comamonas DA1877, also accelerate the animal development. PHA-2/3 and EAT-2 are required for the normal development and function, respectively, of the pharynx. Food intake is reduced in \textit{pha-2/3} and \textit{eat-2} mutants, which reduces the size of these animals.
the hypodermal cells. The SMA-6 receptor, which is expressed in the hypodermal cells, regulates gene expression through the transcription factors, SMA-2 and SMA-3, which in turn regulate the polypliodization. If dopamine does indeed act independent of DBL-1, it may be using different molecules to transmit the signal from neurons to other tissues. It is unlikely that dopamine itself, or octopamine, signals the hypodermis because there have been no reports on the expression of dopamine receptors or octopamine receptors in the hypodermis. As dopamine requires the insulin/IGF receptor, DAF-2, for the regulation of body size, it is possible that insulin/IGF ligands transmit the signal from the neurons to the non-neuronal cells.

Food is an important factor in the regulation of body size (Fig. 3). Both quantity and quality of food have been shown to exert a significant effect on the body size of C. elegans. Dopamine does not appear to play a role in regulation of food intake since cat-2 mutants exhibited normal pharyngeal pumping. Dopamine is also not involved in the alteration of body size due to different bacterial strains in the diet. However, dopamine has been reported to be involved in food sensing in C. elegans (Hills et al., 2004; Sawin et al., 2000). The dopaminergic neurons in C. elegans are mechanosensory (Sulston et al., 1975). The sensory endings of these neurons are embedded in the cuticle and can directly sense food bacteria by touch (Sawin et al., 2000). It has been proposed that the tactile perception of food bacteria in the environment induces dopamine release from these neurons, even without ingestion of the bacteria. The observation that dopamine negatively regulates body size suggests that perception of food regulates body size and so, food affects body size not only as a nutrient, but also through its sensory perception.

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