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Caenorhabditis elegans as an emerging model for studying the basic biology of obesity

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The health problem of obesity and its related disorders highlights the need for understanding the components and pathways that regulate lipid metabolism. Because energy balance is maintained by a complex regulatory network, the use of a powerful genetic model like *C. elegans* can complement studies on mammalian physiology by offering new opportunities to identify genes and dissect complicated regulatory circuits. Many of the components that are central to governing human metabolism are conserved in the worm. Although the study of lipid metabolism in *C. elegans* is still relatively young, much progress has already been made in tracing out genetic pathways that regulate fat storage and in developing assays to explore different aspects of metabolic regulation and food sensation. This model system holds great promise for helping tease apart the complicated network of genes that maintain a proper energy balance.

One great aspect of science is that interactions between researchers in different fields can introduce fresh perspectives and generate new ways to think about a problem. At our institution, several groups studying lipid metabolism in vastly different model systems have started a journal club to stimulate these types of discussions. In the first few meetings, exchanges like the following were quite common:

Mouse researcher: 'Do worms have a vagus nerve?'

Worm researcher: 'What's a vagus nerve?'

In many ways this interaction typifies the early conversations between researchers using mouse models of obesity and those using the nematode *C. elegans* to study lipid metabolism. Mouse researchers have questioned, with both curiosity and scepticism, how well worm metabolism and physiology match up with mammals. After all, decades of genetic and physiological studies of murine lipid metabolism have contributed enormously to our knowledge of human energy homeostasis. Worm researchers, however, are willing to overlook many of the vast physiological differences (the lack of a vagus nerve, for one) and instead highlight the conserved features that govern metabolism in diverse species. They can note, with pride, the numerous discoveries in worm genetics that have proven relevant for human disease and emphasize the need for powerful genetic systems to unravel complex processes like lipid metabolism. Given the spectacular success of 'simple' organisms, such as yeast, worms and fruit flies, in revealing universal biological principles, it should hardly be surprising that fundamental aspects of lipid homeostasis would also be highly conserved. The capacity to maintain an energy balance in a dynamic environment is crucial for survival. The wide range of metabolic, physiological and behavioral adaptations that allow animals to maintain an energy balance is, therefore, likely to have very ancient origins.

Energy homeostasis is maintained by an extremely complex network of signaling pathways that operate in many tissues in the body and that are employed to mediate different aspects of energy balance, including uptake of nutrients, storage of resources and modification of feeding behavior. For these processes to occur effectively, mechanisms have evolved to sense nutrients and coordinate various activities between tissues. Impairments in any of these functions may lead to a systemic imbalance, which can result in obesity. The health consequences of obesity are significant, as it is correlated with a vast array of pathological conditions, such as cardiovascular disease, type 2 diabetes and several types of cancer (Kopelman, 2000). The startling rise in the prevalence of obesity in many countries is a compelling reason to explore new models for studying energy balance. Because of the complexity of metabolic regulation, a genetically tractable system like *C. elegans* offers tremendous potential to unravel the connections between important components and tissues, and thereby complement mouse models of obesity. This Primer will outline the rapid progress made in understanding *C. elegans* lipid metabolism and demonstrate its potential to inform our knowledge of human obesity.

Conservation of key fat-regulatory genes and pathways in *C. elegans*

All organisms must balance energy input and output in order to live. Lipids are a vital component of cellular membranes and a form of stored energy throughout eukaryotes; as such, many of the proteins involved in making, burning and transporting fats are highly conserved between *C. elegans* and mammals. In mammals, fat metabolism is regulated at both peripheral sites of fat storage and in central pathways that coordinate energy balance and food-related behaviors; key genes from both categories play conserved roles in the worm (Fig. 1). *C. elegans* stores lipids in intestinal and skin-like hypodermal cells (Ashrafi, 2007; Hellerer et al., 2007). Although it is not known whether worms store lipids in organelles that are similar to mammalian lipid droplets, many of the key cellular regulators of fat storage and energy use are conserved (Mullaney and Ashrafi, 2009). These include fat, sugar and protein breakdown and synthesis pathways; for instance, fat mobilization in *C. elegans* is dependent on a conserved set of lipases whose mam-

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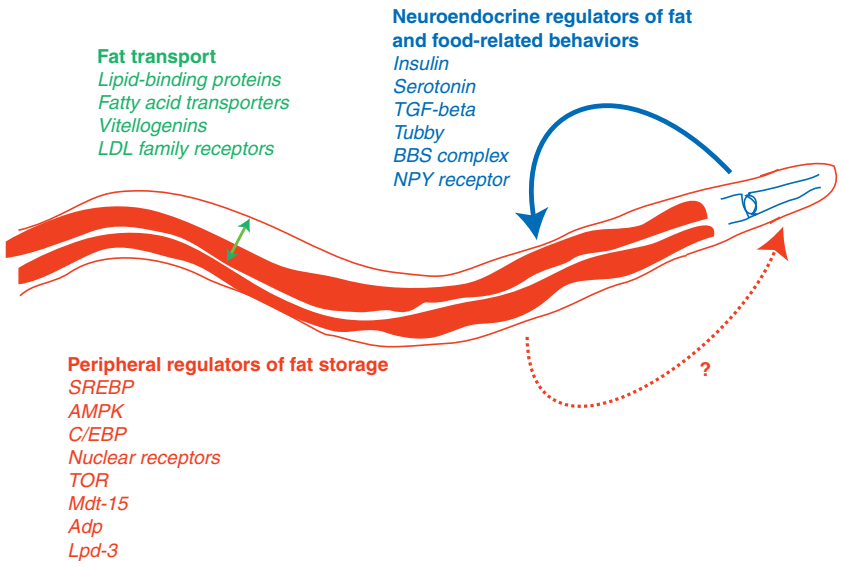

Fig. 1. Major fat-regulatory pathways are conserved between *C. elegans* and mammals. Many of the crucial regulators of lipid homeostasis in mammals serve similar functions in *C. elegans*. Regulators in the nervous system (blue) modulate fat metabolism, as well as feeding and food-related behaviors (they are able to regulate both aspects of fat balance by distinct mechanisms). Peripheral regulators of fat storage act directly in the tissues that contain the major fat depots for the organism (intestinal and hypodermal cells in *C. elegans*, shown in red). Some data suggest that fat regulators in the periphery can feed back and influence the nervous system, although the precise mechanism for this is currently unclear. Systems also exist to transport fats between cells and tissues (green). Prominent examples from each category are listed in the appropriate colors.

malian orthologs liberate the triglycerides stored in fat droplets (Wang et al., 2008; Narbonne and Roy, 2009). Fat storage and use in *C. elegans* are also dependent on conserved pathways such as the AMP-activated protein and target of rapamycin (TOR) kinases, and the sterol response element binding protein (SREBP) and CCAAT/enhancer binding protein (C/EBP) transcription factors (Long et al., 2002; McKay et al., 2003; Apfeld et al., 2004; Jia et al., 2004; Yang et al., 2006). Many nuclear receptors act as crucial regulators of metabolic gene transcription in mammals (Chawla et al., 2001; Sampath and Ntambi, 2005), and recent work has demonstrated that certain nematode nuclear receptors are likewise key regulators of metabolism (Van Gilst et al., 2005b; Van Gilst et al., 2005a). *C. elegans* also possesses machinery to transport fatty acids between tissues; the best characterized example of this is the shuttling of lipids and proteins (in the form of yolk particles) from intestinal cells to oocytes by a process involving a low-density lipoprotein (LDL) family receptor and receptor-mediated endocytosis (Grant and Hirsh, 1999). Other examples include fatty acid transporters and lipid-binding proteins (Hirsch et al., 1998; Plenefisch et al., 2000).

Although worm and mammalian physiologies differ greatly, many of the fat-regulatory components play conserved roles. The SREBP family exemplifies the remarkable conservation of metabolic regulators across eukaryotes. Although best characterized for the regulation of mammalian cholesterol and lipid synthesis in the liver (and as important proteins in adipocyte development), SREBP homologs are crucial for metabolism in yeast, worms and flies, all of which lack both a liver and adipocytes, and have vastly different requirements for cholesterol. The essential features, however, are conserved; these transcription factors induce the expression of specific genes in response to changes in membrane fluidity. This remarkable property has been exploited throughout evolution and adapted to the unique demands of each organism: fission yeast use it to monitor oxygen levels, flies use it to control phospholipid levels and mammals use it to control cholesterol synthesis (Brown and Goldstein, 1997; Dobrosotskaya et al., 2002; Hughes et al., 2005). The membrane component sensed by the *C. elegans* SREBP homolog is not yet known, but it is clear that this gene, as in mammals, is crucial for fat storage and the synthesis of certain fat species (McKay et al., 2003; Yang et al., 2006). Model organisms can, therefore, be useful in elucidating the underlying principles of fat regulation, despite their physiological divergence from humans.

Important neuronal and endocrine regulators of metabolism are also conserved in the worm. An insulin-like pathway in *C. elegans*, similar to the mammalian insulin pathway, regulates lipid metabolism and longevity (Kimura et al., 1997; Lin et al., 1997; Ogg et al., 1997; Ogg and Ruvkun, 1998). Serotonin signaling in the nervous system controls both fat metabolism and feeding behavior (Sze et al., 2000; Srinivasan et al., 2008), as it does in mammals (Tecott et al., 1995). The TUB-1 protein and Bardet-Biedl syndrome (BBS) complex act in ciliated sensory neurons to regulate peripheral lipid storage and various aspects of environmental sensation, just like their homologs in the mouse (Mak et al., 2006). Worms do lack several of the key features of human metabolism, such as leptin and other adipokines, a true adipose tissue (although regulators of adipogenesis can be discovered using *C. elegans*, as will be discussed later) and a hypothalamus. Homologs of some transcription factors that are essential for the development and function of the mammalian hypothalamus are, however, expressed in subsets of *C. elegans* neurons (Good et al., 1997) (K.A., unpublished).

Why use *C. elegans* as a model for fat regulation and food-related behaviors?

C. elegans offers several advantages for studying fat and feeding regulation that complement mouse studies in important ways. Because the energy balance is maintained by many regulatory pathways, redundant mechanisms and feedback loops, genetic analysis is essential for understanding the functional relevance of each gene and the regulatory connections between components. However, in most cases, fat and feeding regulatory mechanisms in mammals are analyzed in the context of single gene knockout or knock-in animals, yet the elucidation of homeostatic responses and feedback regulatory loops necessitates combinatorial analyses of mutants. Take, for example, the case of neuropeptide Y (NPY).

Although expression of NPY in the hypothalamus is leptin dependent, and administration of NPY causes hyperphagia and obesity, mice lacking NPY have no feeding or obesity phenotype (Erickson et al., 1996). However, when an NPY deficiency is crossed into the leptin-deficient *ob/ob* background, the importance of NPY in mediating leptin signaling is revealed: these mice display a lower body weight and less food intake than their *ob/ob* counterparts (Erickson et al., 1996). In mice, spectacular genetic successes like this are often only possible when examining known fat regulators. The short generation time (around three days at room temperature) and the ease of genetic manipulation (both for forward screening and reverse genetics) make *C. elegans* an ideal model for unbiased screening, as well as simultaneous manipulation of multiple genes, such that crosstalk between behavioral and metabolic components can be deciphered and the contributions of each tested rigorously. The amenability of *C. elegans* to screens for lipid storage genes has already led to the discovery of genes required for proper energy balance in mice, including a gene required for adipogenesis of cultured 3T3-L1 cells despite worms lacking a dedicated adipose tissue (McKay et al., 2003; Suh et al., 2007). Elegant, cross-species studies like these provide hints of the enormous benefits that worm genetics can yield for studies of human obesity.

Although the identities and organization of the mammalian neural circuits that respond to internal cues of energy storage are just beginning to be formulated, the enormous complexity of the mammalian nervous system makes this a very daunting task. In addition to internal signals, visual, olfactory, gustatory and other sensory cues modulate feeding behavior. Little is known about the molecular and neural circuits that underlie perception of environmental conditions, or about their interplay with internal cues and how these inputs elicit behavioral, physiological and metabolic outputs. The ability to gauge the energy status of cells and organisms is essential for lipid homeostasis. Decades of rigorous genetic studies on dauer formation (a long-lived, alternate stage of development in *C. elegans*) have made it clear that the worm has numerous mechanisms to alter its metabolism, developmental progression and life span in response to changes in food intake and other signals (Hu, 2007). Many of the components used by the worm to regulate dauer entry and survival are homologous to mammalian regulators of lipid storage, including insulin signaling and an AMP-activated protein kinase (Hu, 2007; Narbonne and Roy, 2009). More recently, another developmental decision in the life cycle of the worm has been demonstrated to be dependent upon nutrition; after hatching, worms progress through larval development in the presence of food and arrest in its absence. Like the dauer decision, this larval arrest is an organismal response mediated by insulin signaling (Baugh and Sternberg, 2006; Fukuyama et al., 2006), and genetic examination of larval arrest has already yielded a regulator of insulin release that acts in pancreatic β-cells (Kao et al., 2007). Once in this arrested state, survival is a tightly regulated process that also involves sensory perception of the environment and insulin release in a distinct subset of neurons (Lee and Ashrafi, 2008). The simple nervous system of the worm makes it an attractive system for studying how organisms sense nutrients and adjust their behaviors, and even morphologies, to adapt.

Human feeding behavior reflects a complex mix of social, economic, psychological and physiological factors. Nevertheless, *C. elegans* is well suited for investigating the molecular pathways, as well as the neural circuits, that allow animals to assess nutrient availability and govern behavioral strategies. Feeding can be examined in *C. elegans* by measuring the rate of pharyngeal pumping (the muscular contraction of the pharynx that forces bacteria through the grinder and into the gut of the animal), a behavior that is modulated by food (Avery and Horvitz, 1990). In response to food, worms alter their rates of feeding, egg laying and locomotion, and many of the components required for these behaviors also regulate human feeding behavior, such as serotonin (Tecott et al., 1995; Sawin et al., 2000). As in mammals, there is evidence that signals from peripheral sites of fat metabolism also govern feeding behavior in *C. elegans* (Srinivasan et al., 2008). Additionally, worms engage in more sophisticated food-related behaviors, like social feeding. The decision to dine alone or with friends is made by a worm homolog of the NPY receptor (de Bono and Bargmann, 1998), which, as discussed earlier, is a major regulator of mammalian feeding behavior. The feeding behavior of *C. elegans* is also influenced by food quality and prior experiences (Shtonda and Avery, 2006). Furthermore, based on a unique quiescence behavior, worms have even been proposed as a model for studying satiety signaling (You et al., 2008).

The relatively simple nervous system of *C. elegans* makes it suitable for functionally dissecting neural circuits that govern energy balance; studies on the neuronal regulation of fat storage and feeding have already yielded important findings that deepen our understanding of how these processes are regulated. Serotonin, for example, has been shown to govern feeding rate and fat metabolism by independent mechanisms, suggesting that neural pathways can govern fat storage by determining food intake and concomitantly modulating energy use, allowing another level of regulation for the overall energy balance (Srinivasan et al., 2008). As such, *C. elegans* studies have broadened the understanding of serotonergic mechanisms of fat loss. In another example, neural transforming growth factor-beta (TGF-β) signaling has been shown to regulate fat storage and feeding through a peptidergic circuit that uses non-synaptic communication (Greer et al., 2008). Moreover, as in the case of serotonin signaling, analyses of this TGF-β signaling pathway have revealed that neural regulation of fat can be molecularly dissociated from regulation of feeding behavior (Greer et al., 2008), suggesting that the major peptidergic signaling pathways that regulate mammalian energy balance, including leptin, NPY and the agouti-related protein, may also regulate feeding behavior and metabolic rate through distinct mechanisms.

Methods for examining fat storage and metabolism in *C. elegans*

A historical introduction to the techniques used to examine fat in the worm will serve to illustrate how rapidly the field has evolved over the last decade and its potential to contribute to the study of metabolism. An early method for assessing fat content was the use of Sudan Black, a classic lipophilic dye, to stain fixed animals (Kimura et al., 1997). For the first time, this allowed a visual assessment of stored lipids in the transparent bodies of *C. elegans* (see Fig. 2 for a comparison of the various lipid-staining methods). Since this method uses fixed animals, the staining is not dependent on any endogenous pathways for uptake and transport. However, because of the fix-

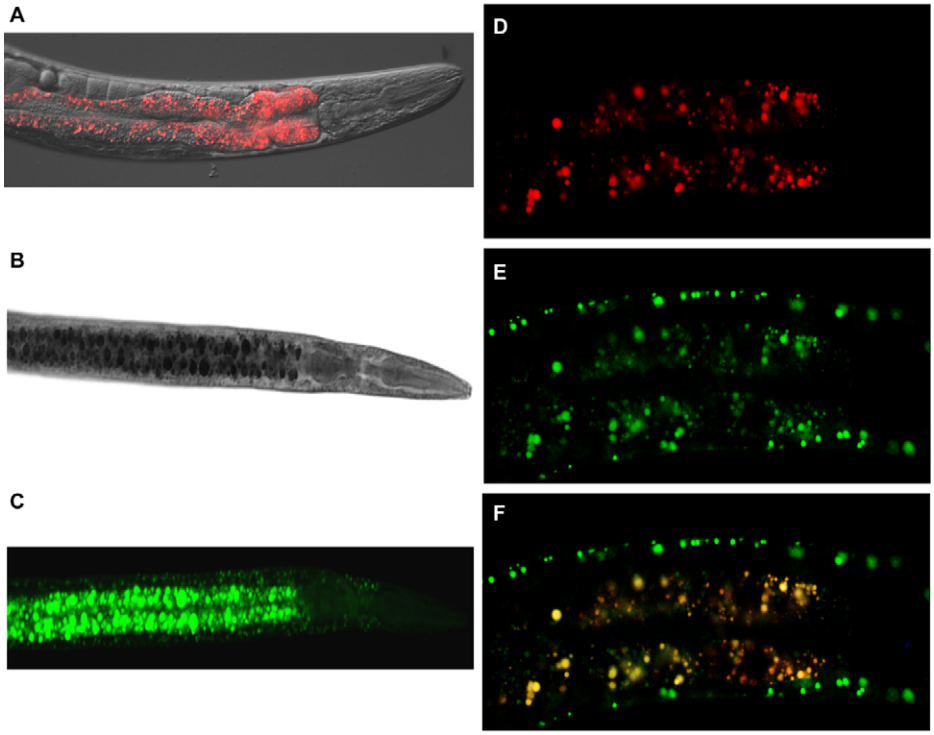

Fig. 2. Methods to stain lipids in *C. elegans*. (A) Nile Red staining of a live animal. This composite image was made by overlaying fluorescence and DIC channels. Note that the animals shown in A-C are not matched for developmental stage or genotype; images are simply provided as examples for each staining methodology. (B) Sudan Black B staining of a fixed animal. (C) LipidTOX neutral lipid staining of a fixed animal. (D-E) Nile Red (D) and fatty acid-conjugated BODIPY staining (E) of a live animal. This higher magnification image, centered on the anterior intestinal cells, was taken with a spectral confocal microscope. The images in D and E were derived from spectral unmixing of the image in F to separate Nile Red from BODIPY fluorescence. Note that both intestinal and hypodermal lipids are visible in E. (F) Image of a live animal stained with both Nile Red and fatty acid-conjugated BODIPY, taken on a spectral confocal microscope. Note the extensive overlap of Nile Red-stained and fatty acid-conjugated BODIPY-stained lipid particles in the intestinal cells.

ation process, this method is not amenable to high-throughput screening; therefore, its use has been restricted to the examination of a limited number of genotypes or conditions, usually to confirm genetic interactions that have already been established by other phenotypes, most notably dauer formation (Kimura et al., 1997; Ogg et al., 1997; Jia et al., 2004). Staining is also quite variable within samples, making it difficult to study subtle phenotypes. In our experience, very rigorous staining methods are required to minimize experimental variations and obtain accurate and reproducible data, making this method most applicable to examining dramatic fat phenotypes (Greer et al., 2008). Fluorescent dyes, such as LipidTOX neutral lipid stains, may be used in place of Sudan Black and use a similar fixation protocol for easier visualization of fats (Fig. 2).

The next major advance was the use of vital dye staining to examine fat content in intact living animals. The most commonly used dyes have been Nile Red (Greenspan et al., 1985) and fatty acid-conjugated BODIPY. This methodology made it possible to apply the strengths of worm genetics to the problem of lipid metabolism by enabling forward and whole-genome RNAi screening, leading to the identification of hundreds of fat-regulatory genes in *C. elegans* (Ashrafi et al., 2003). It also makes large-scale epistasis analyses very easy to conduct. The fluorescent properties of these dyes make them generally quite sensitive, allowing the detection of subtle differences in fat levels, and the ease of staining living worms with a vital dye has made this method very popular in elucidating the pathways affecting fat metabolism (Mukhopadhyay et al., 2005; Van Gilst et al., 2005b; Mak et al., 2006; Srinivasan et al., 2008). As living worms are used, visualization of fat depots by these dyes is dependent upon endogenous uptake and transport pathways, as well as the physical properties of the dyes themselves. For example, BODIPY-conjugated fatty acids stain hypodermal stores of fat readily, whereas Nile Red does not (both dyes stain the same compartments in the intestinal cells, see Fig. 2). In certain cases, even intestinal fat stores may not be amenable to visualization by these dyes (Schroeder et al., 2007). Thus, although in most cases the fluorescence intensity of these dyes accurately reflects endogenous fat levels, this may not always be the case. To get around these particular limitations, coherent anti-Stokes Raman scattering (CARS) microscopy has been used to visualize stored fats in intact animals without exogenous dyes (Hellerer et al., 2007). Although promising, this method has not yet been widely used owing to the need for specialized equipment.

Rigorous dissection of metabolic pathways in the worm has given rise to the need for assays that can resolve individual parameters of lipid metabolism, such as fatty acid synthesis, energy consumption and fat breakdown. In recent years, these challenges have begun to be met and invaluable secondary assays that look at specific aspects of lipid metabolism are now emerging. Perhaps the first was the use of gas chromatography to measure individual lipid species; this was combined with the power of worm genetics in an elegant screen that delineated the pathway for polyunsaturated fatty acid synthesis (Watts and Browse, 2002). Since this development, numerous genes have been shown to regulate the balance of polyunsaturated fat species, which appears to be under strict control (Van Gilst et al., 2005b; Brock et al., 2006; Taubert et al., 2006; Yang et al., 2006; Brock et al., 2007). A metabolic labeling strategy has been developed to resolve the individual contributions of de novo fat synthesis and dietary absorption of fats to overall fat metabolism (Perez and Van Gilst, 2008). To examine the rates of energy use, oxygen consumption has been examined as a proxy for overall metabolic flux. Combined with the genetic and pharmacological perturbation of specific genes involved in fat breakdown, this approach can be used to probe the role of lipid oxidation in fat regulation (Srinivasan et al., 2008). Additionally, based on sequence homology, researchers have

Advantages of *C. elegans* as a model for understanding obesity and related disorders

- Highly tractable genetics can be used to identify genes and order complex regulatory pathways
- The simple nervous system is a powerful system for elucidating neural circuits that govern metabolism, food-related behaviors and nutrient perception
- Emerging assays allow the examination of specific processes underlying energy balance and fat storage
- Many of the key components that regulate human metabolism play conserved roles in the worm

identified a number of genes that are known to be involved in lipid metabolism, including components of β-oxidation and fatty acid synthesis, and acyl-CoA synthetases. This makes it possible, through expression profiling and/or epistasis analysis using candidate metabolic genes, to identify the specific components and pathways that are acting downstream of fat regulators (Van Gilst et al., 2005b; Van Gilst et al., 2005a; Taubert et al., 2006; Srinivasan et al., 2008; Wang et al., 2008; Narbonne and Roy, 2009). It is now possible to move beyond overall fat content and examine the specific metabolic pathways that contribute to lipid homeostasis, allowing the power of worm genetics to be applied to these processes as well.

Animals such as *C. elegans* are often referred to as model organisms. At the risk of sounding pedantic, it is important to remember that they, like humans, live successfully in complicated and dynamic environments. Therefore, it is a safe bet that many of the molecular mechanisms that allow these animals to maintain energy homeostasis will be conserved in more complex models, such as mice, and in humans. Although worms cannot serve as a model for all of the factors regulating human obesity, in the conserved areas where they can contribute, they will continue to do so powerfully.

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COMPETING INTERESTS

The authors declare no competing financial interests.

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