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Major Urinary Protein Regulation of Chemical Communication and Nutrient Metabolism

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Summary

The major urinary protein (MUP) family members contain a conserved β-barrel structure with a characteristic central hydrophobic pocket. They are secreted by the liver and excreted into the urine. MUPs bind via their central pockets to volatile pheromones or other lipophilic molecules, and regulate pheromone transportation in the circulation, excretion in the kidney, and release into the air from urine marks. MUPs are highly polymorphic, and the MUP profiles in urine function as individual identity signatures of the owners. The MUP signatures are detected by the main and accessory olfactory systems and trigger adaptive behavioral responses and/or developmental processes. Circulating MUPs serve as a metabolic signal to regulate glucose and lipid metabolism. Recombinant MUP1 markedly ameliorates hyperglycemia and glucose intolerance in mice with type 2 diabetes. MUP1 suppresses hepatic gluconeogenesis and promotes energy expenditure in skeletal muscle by stimulating mitochondrial biogenesis and function. MUPs are unique members of the lipocalin super-family that mediate both chemical and metabolic signaling.

Keywords

Major urinary protein; lipocalin; pheromone; chemical signaling; obesity; type 2 diabetes

I. Introduction

Animals as well as human beings have evolved a variety of communication mechanisms to exchange information. Chemical communication plays a key role in regulating both behavioral and physiological responses in the animal kingdom. Individuals generate scent substances which are excreted into the environment via sweat, urine and feces. These scent substances serve as chemical signals and are perceived by conspecifcs to trigger adaptive behavioral and physiological responses in the receivers (Brennan and Kendrick, 2006; Tirindelli et al., 2009). Most scent substances are unstable, volatile small molecules and bind to their cognate protein carries. These carries not only extend the lifetime but also regulate the release of the scent substances (Hurst, 2009; Tirindelli et al., 2009). The major urinary protein (MUP) family proteins bind to, concentrate, and stabilize many volatile scent substances (e.g. pheromones), thereby controlling both pheromone transport in circulation

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and pheromone release into the air from urine scent marks (Brennan and Kendrick, 2006; Hurst, 2009; Tirindelli et al., 2009). Additionally, MUPs themselves may serve as chemical signals to convey their owners' identity information to conspecifics (Chamero et al., 2007). Recent studies reveal that the MUP family members also regulate nutrient metabolism independently of chemical signaling (Hui et al., 2009; Zhou et al., 2009). Nutrient metabolism provides energy supply to power behavioral and physiological activities. Therefore, the MUP family members appear to coordinate behavioral response and energy metabolism by serving as both chemical and metabolic signals.

II. MUP protein structure and polymorphism

MUPs belong to the lipocalin super-family (Cavaggioni and Mucignat-Caretta, 2000; Finlayson et al., 1965). The lipocalin family members have relatively low similarity in their amino acid sequences; however, their tertiary structures are extremely conserved with a characteristic β–barrel consisting of eight β–strands and an α–helix (Bocskei et al., 1992). Most lipocalin family members bind, via their central hydrophobic pockets formed by these eight β–strands, to small lipophilic molecules, including fatty acids, steroids, retinol and pheromones (Schlehuber and Skerra, 2005).

MUPs bind to pheromones via their central β-barrel cavities

The MUP family members were initially discovered in urine as a group of small proteins with molecular weights around 18 kD (Finlayson et al., 1965; Lane and Neuhaus, 1972). MUPs are mainly synthesized in the liver and secreted into the bloodstream (Shaw et al., 1983). Due to their small sizes, MUPs are efficiently filtered through the glomeruli and excreted into the urine (Kimura et al., 1991). The isoelectric points of MUPs in urine are varying from 4.6 to 5.3 (Bocskei et al., 1992; Clissold and Bishop, 1982). Each individual adult male mouse excretes approximately 8–14 different MUP isoforms in urine (Hurst, 2009).

MUP tertiary structures have been extensively studied by both X-ray crystallography and NMR spectroscopy (Bocskei et al., 1992; Darwish Marie et al., 2001; Lucke et al., 1999; Timm et al., 2001; Zidek et al., 1999). The structures of both endogenous and recombinant MUP1 protein have been characterized (Bocskei et al., 1991; Timm et al., 2001). The MUP family members contain a characteristic eight antiparallel β-sheets that are linked by seven loops to form a β-barrel (Bocskei et al., 1992; Darwish Marie et al., 2001; Lucke et al., 1999; Timm et al., 2001; Zidek et al., 1999). The first loop is a large Ω-loop that functions as a dynamic lid of the β-barrel, and the other six are typical short β-hairpin (Timm et al., 2001). The interior of the β-barrel forms a hydrophobic pocket that binds directly to hydrophobic pheromones.

The affinity of different MUP isoforms for pheromones varies. For instance, MUP4 has a 23-fold higher affinity for (±)-2-sec-butyl-4,5-dihydrothiazole than MUP1, but has a 4-fold lower affinity for 6-hydroxy-6-methyl-3-heptanone than MUP1 (Darwish Marie et al., 2001; Sharrow et al., 2002). The affinity for pheromones is determined by the amino acids of the binding pockets (Darwish Marie et al., 2001; Sharrow et al., 2002). A single-amino-acid substitution in the binding pocket can result in a dramatic change in the affinity of a MUP

family member (Darwish Marie et al., 2001). The binding of pheromones to MUPs not only protects against pheromone decomposition in blood and urine but also sustains pheromone action by slowly releasing MUP-bound volatile pheromones into the air from urine marks. Therefore, MUP expression levels control pheromone levels in blood and urine (Sharrow et al., 2002).

MUPs are highly polymorphic

MUP expression is sexually dimorphic in rodents, and the levels of MUPs are much higher in males than in females (Geertzen et al., 1973; Lane and Neuhaus, 1972). Androgen potently stimulates MUP expression, resulting in male-dominant expression and excretion of MUPs (Johnson et al., 1995; Kurtz and Feigelson, 1977). MUPs are synthesized mainly in the liver and excreted into the urine (Finlayson et al., 1965; Shaw et al., 1983). MUP synthesis accounts for 3.5–4% of total hepatic protein synthesis in adult male mice (Berger and Szoka, 1981). Urinary MUPs mediate chemical signaling in conspecifics (Tirindelli et al., 2009). The MUP family members are also expressed in the submaxillary, lachrymal, nasal, parotid, mammary glands, and hypothalami; however, the function of extrahepatic MUPs is unknown (Cavaggioni and Mucignat-Caretta, 2000; De Giorgio et al., 2009; Shaw et al., 1983).

The MUP family proteins are encoded by multiple paralogous genes clustered on chromosome 4 in mice and chromosome 5 in rats (Hastie et al., 1979). Rat MUPs are also called α_{2U}-globulins (Lane and Neuhaus, 1972). The amino acid sequences of MUPs are 65% identical between mice and rats (Cavaggioni and Mucignat-Caretta, 2000). The mouse genome contains 21 *MUP* genes and additional 21 *MUP* pseudogenes (Logan et al., 2008). The *MUP* genes and pseudogenes have been independently evolved from a single ancestral gene (Logan et al., 2008). The *MUP* genes contain 6 coding exons, and the pseudogenes contain premature stop codons due to an insertion or deletion. The *MUP* genes and pseudogenes are classified into two groups. The first group consists of 6 *MUP* genes (e.g. *MUP1*, *MUP2*, *MUP18*, *MUP24*, *MUP25* and *MUP26*) and 5 pseudogenes (Logan et al., 2008). The cDNA sequences of these six *MUP* genes are 82–94% identical. The second group consists of the remaining 15 *MUP* genes and 16 pseudogenes (Logan et al., 2008; Mudge et al., 2008). The cDNA sequences of these 15 *MUP* genes are >97% identical.

The *MUP* genes are extremely polymorphic in wild or outbred mice (Cheetham et al., 2009; Finlayson et al., 1965; Robertson et al., 2007). Each individual adult male mouse normally expresses 8–14 different MUP isoforms; therefore, the number of MUP expression patterns is extremely expanded due to MUP polymorphism (Beynon et al., 2002; Evershed et al., 1993; Hurst, 2009). Polymorphic *MUP* genes serve as a specific genetic marker of individual identity, and the MUP profiles in urine are recognized as an individual identity signature of the owners by conspecific receivers (Cheetham et al., 2007; Hurst et al., 2001; Sherborne et al., 2007).

III. MUP regulation of chemical communication

Pheromones are diverse, biologically active substances that are excreted to the outside by individuals. Pheromones are detected by conspecifics and trigger specific behavioral,

physiological, and/or developmental responses in the receivers, including aggression, mating, territory marking, estrous cycles and pregnancy (Hurst, 2009; Tirindelli et al., 2009).

MUPs function as volatile pheromone carriers

Many pheromones are small volatile organic molecules which are unstable in aqueous environments (e.g. blood and urine) (Hurst and Beynon, 2004; Stowers and Marton, 2005). The MUP family members bound via their center hydrophobic cavities to a variety of pheromones (Bocskei et al., 1992; Peele et al., 2003; Sharrow et al., 2002). The MUP-pheromone physical interactions protect against pheromone destruction during both transportation in the bloodstream and excretion into the urine. Additionally, free volatile pheromone molecules are quickly evaporated into the air from scent urine marks. MUPs not only facilitate pheromone transportation as pheromone carries but also prolong pheromone lifetime by slowly releasing their bound pheromones into the air from scent marks (Humphries et al., 1999; Hurst et al., 1998).

MUPs act as pheromones to directly regulate behavioral and physiological responses

Interestingly, the MUP1 protein moiety is sufficient to activate sensory neurons in the vomeronasal organ (VNO) and to trigger ovulation (More, 2006). Recombinant MUP1 promotes inter-male aggression in the absence of pheromones (Chamero et al., 2007). Moreover, purified MUP1 directly stimulates Gαo-coupled V2R receptors in VNO neuron cultures (Chamero et al., 2007). Therefore, MUP proteins also act as involatile pheromones in addition to as pheromone carriers.

The MUP profiles serve as an individual identity signature

MUPs and their bound pheromones profoundly modulate the behaviors and development of conspecifics. Urine from intact but not castrated males promotes male aggression (Mugford and Nowell, 1970). Males advertize their social status to attract females via urinary pheromones (Bronson and Caroom, 1971; Jemiolo et al., 1991). MUPs accelerate female puberty and promote ovulation (More, 2006; Mucignat-Caretta et al., 1995). The urine scents of unfamiliar males block pregnancy in recently mated females (Bruce, 1959), and MUPs bind to the volatile pheromones involved in pregnancy block (Peele et al., 2003). The polymorphic patterns of MUPs serve as an individual identity signature in urine marks (Hurst and Beynon, 2004; Hurst et al., 2001). Females use the MUP signatures to recognize individual scent owners, preferentially associated with heterozygous males, and avoid inbreeding (Cheetham et al., 2007; Thom et al., 2008).

Pheromones are believed to stimulate sensory neurons in VNO when animals make nasal contact with scent marks (Breer et al., 2006; Halpern and Martinez-Marcos, 2003; Meredith, 1994). Each pheromone activates a specific subset of sensory neurons that convey unique signals to the brain (Dulac and Torello, 2003). VNO neurons project to the accessory olfactory bulb, and the second-order neurons in the accessory olfactory bulb project to amygdala that innervates hypothalamic neurons either directly or indirectly (Tirindelli et al., 2009). In contrast, the airborne volatile odorants are believed to stimulate sensory neurons in the main olfactory epithelium (MOE) which project to the main olfactory bulb (Tirindelli et

al., 2009). The second-order neurons in the main olfactory bulb project to higher centers in the brain, including the piriform cortex and the cortical amygdale (Tirindelli et al., 2009). However, recent studies suggest that both the vomeronasal and the main olfactory systems are involved in pheromone detection (Hurst, 2009).

IV. MUP regulation of nutrient metabolism

Behavioral and developmental responses are powered by energy derived from nutrient metabolism. It is not surprising that many factors simultaneously regulate both behaviors and metabolism. Glucose and fatty acids are the primary fuel substrates to power cellular activity that underlies behavioral and developmental responses. Animals have evolved a sophisticated neuroendocrine system that maintains glucose and lipid homeostasis. For instance, a rise in blood glucose derived from ingested food stimulates pancreatic β cells to secrete insulin. Insulin in return reduces blood glucose levels by stimulating glucose uptake into skeletal muscle and adipose tissue as well as by suppressing glucose production from the liver (Saltiel and Kahn, 2001). In contrast, a fall in blood glucose during fasting stimulates the secretion of counterregulatory hormones (e.g. glucagon and catecholamines) which increase blood glucose levels by stimulating liver glucose production (Jiang and Zhang, 2003). Therefore, blood glucose homeostasis is maintained mainly by a balance between insulin and counterregulatory hormones. Impaired ability of insulin to decrease blood glucose (insulin resistance) is the primary risk factor for the development of type 2 diabetes. Insulin sensitivity is regulated by multiple humoral factors, including MUP1.

MUP1 is involved in nutrient sensing

Recent studies show that the expression and secretion of MUP1 are regulated by nutrient signals. Fasting markedly reduced MUP1 expression in the liver, which is reversed by refeeding (Hui et al., 2009). The liver plays a key role in nutrient sensing and metabolism. In agreement with this observation, caloric restriction (chronic malnutrition) also dramatically reduces MUP1 expression in mouse livers (Dhahbi et al., 2004; Miller et al., 2002). The expression of other MUP family members, including MUP4 and MUP5, is also suppressed in calorie-restricted mice (Dhahbi et al., 2004). Interestingly, MUP1 deficiency is associated with obesity and type 2 diabetes. Two groups reported independently that hepatic MUP1 expression and circulating MUP1 levels are markedly reduced in mice with either genetic (leptin receptor-deficient *db/db*) or dietary fat-induced obesity (Hui et al., 2009; Zhou et al., 2009). Interestingly, MUP1 is also expressed in several extrahepatic tissues, and MUP1 expression is similarly reduced in both adipose tissues and the hypothalamus in response to nutrient deprivation (De Giorgio et al., 2009; van Schothorst et al., 2006). Adipocytes and hypothalamic neurons are also key players in nutrient sensing. These observations suggest that MUP1 and/or the other MUP family members are likely involved in the nutrient sensing process, and defects in MUP-mediated nutrient sensing might contribute to the development of metabolic diseases, including type 2 diabetes.

MUP1 regulates nutrient metabolism in multiple tissues

There are multiple lines of evidence supporting an important role of MUP1 in glucose metabolism. In mice with either genetic (*db/db*) or dietary-induced type 2 diabetes, liver-

specific overexperssion of MUP1 markedly reduces hyperglycemia and glucose intolerance (Zhou et al., 2009). Similarly, chronic administration of purified recombinant MUP1 proteins also ameliorates hyperglycemia and improves glucose intolerance in *db/db* mice (Hui et al., 2009). The MUP1 therapy also improves systemic insulin sensitivity in diabetic mice as expected (Hui et al., 2009; Zhou et al., 2009). Interestingly, rosiglitazone (a potent PPARβ agonist) and resveratrol (a natural product abundant in grape skins), two chemically distinct compounds that decrease hyperglycemia and glucose intolerance in diabetic mice, also stimulate MUP1 expression in the liver (Baur et al., 2006; Hui et al., 2009).

MUP1 treatment enhances insulin signaling in the skeletal muscle but not livers of diabetic mice, suggesting that skeletal muscle is a physiological target of MUP1 (Hui et al., 2009). Moreover, recombinant MUP1 directly suppresses glucose production in primary hepatocyte cultures independently of insulin (Zhou et al., 2009). Additionally, liver-specific overexpresion of MUP1 markedly decreases triglyceride levels in the livers of *db/db* mice (Zhou et al., 2009). Therefore, MUP1 may also regulate hepatic glucose and lipid metabolism in an autocrine and/or paracrine fashion. Interestingly, MUP1 expression is also regulated by nutrients in adipose tissue and the hypothalamus, suggesting that MUP1 may regulate the metabolic activity of these two tissues in a similar autocrine and/or paracrine manner (De Giorgio et al., 2009; van Schothorst et al., 2006).

MUP1 regulates metabolism by multiple mechanisms

MUP1 reduces blood glucose levels at least in part by suppressing the hepatic gluconeogenic program (Zhou et al., 2009). In both animals and primary hepatocyte cultures, recombinant MUP1 markedly inhibits the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), two rate-limiting enzymes for gluconeogenesis (Zhou et al., 2009). Insulin is well known to suppress hepatic gluconeogenesis; however, MUP1 suppresses hepatic glucose production independently of insulin, suggesting that MUP1 regulates the hepatic gluconeogenic program by a novel mechanism (Zhou et al., 2009). Hepatic gluconeogenesis is abnormally elevated in type 2 diabetes, thus significantly contributing to hyperglycemia and glucose intolerance (Ali and Drucker, 2009; Jiang and Zhang, 2003). Interestingly, type 2 diabetes is associated with a marked reduction in MUP1expression, suggesting that reduced expression of hepatic MUP1 contributes to abnormally-elevated hepatic gluconeogenesis (Hui et al., 2009; Zhou et al., 2009).

Chronic MUP1 treatment also decreases the levels of plasma lipids in *db/db* mice (Hui et al., 2009; Zhou et al., 2009). Moreover, liver-specific overexpression of MUP1 results in a marked reduction in hepatic lipid levels, presumably due to suppression of lipogenic genes in the liver, including the *stearoyl-CoA desaturase-1*, *fatty acid synthase*, *carbohydrate response element binding protein*, and *peroxisome proliferator-activated receptor-β* (*PPARβ*) genes (Zhou et al., 2009). Chronic administration of purified recombinant MUP1 also decreases lipid levels in the skeletal muscles of *db/db* mice (Hui et al., 2009). Together, these observations suggest that MUP1 regulates both glucose and lipid metabolism in multiple tissues.

MUP1 improves insulin sensitivity in skeletal muscle at least in part by increasing energy expenditure (Hui et al., 2009). Chronic administration of purified MUP1 proteins increases

energy expenditure, body temperature and ambulatory locomotion in *db/db* mice (Hui et al., 2009). MUP1 increases not only mitochondrial biogenesis but also the capacity of mitochondrial oxidative phosphorylation (Hui et al., 2009). Interestingly, MUP1 promotes mitochondrial biogenesis and function specifically in the skeletal muscle but not other tissues (e.g. adipose tissues and livers) of *db/db* mice (Hui et al., 2009). An increase in mitochondrial content and function is likely to result in an increase in fatty acid β-oxidation and a decrease in lipid levels in skeletal muscles, thereby ameliorating lipotoxicity and insulin resistance in MUP1-treated mice with type 2 diabetes.

Recombinant MUP1 inhibits the hepatic gluconeogenic program directly in primary hepatocyte cultures, suggesting that MUP1 regulates metabolic function in the liver by activating its own cognate receptors (Zhou et al., 2009). Additionally, in animals, circulatory MUP1 binds to, concentrates, and slowly releases various lipophilic molecules (Cavaggioni and Mucignat-Caretta, 2000; Sharrow et al., 2002). These lipophilic molecules may be bioactive and regulate nutrient metabolism; therefore, MUP1 may also regulate metabolism indirectly by controlling the stability, concentrations, and/or activity of these bioactive lipophilic molecules.

V. Conclusions and future directions

MUPs belong to the lipocalin super-family whose tertiary structure contains a conserved β-barrel with a characteristic central hydrophobic pocket. The MUP family members are expressed mainly by the liver and secreted into the bloodstream (Fig. 1). Various pheromones and other small lipophilic molecules bind to the central pockets of MUPs and are transported through the circulation. MUPs are excreted into the urine in the kidney, and urinary MUPs prolong pheromone lifetime by slowing the release of MUP-bound pheromones into the air from urine scent marks. MUPs are highly polymorphic, and the urinary MUP profiles are recognized as an individual identity signature of the scent owners by conspecifics. MUPs and MUP-bound pheromones are detected by both the main and the accessory olfactory systems. These two systems act coordinately to convey the information about the individual identity of signalers to the brain of conspecific receivers and to trigger behavioral responses and/or developmental processes. However, it remains completely unclear how the MUP detection system and the central nervous system extract the individual identify information encoded in the MUP profiles. Interestingly, circulating MUPs may play an important role in regulating nutrient metabolism. MUPs, particularly MUP1, suppress the hepatic gluconeogenic and lipogenic programs. MUP1 also promotes mitochondrial biogenesis and oxidative phosphorylation in skeletal muscles, thus increasing energy expenditure and insulin sensitivity. However, it is unclear whether MUP1 regulates metabolism directly through its own cognate receptors or indirectly by controlling the stability, the release, and/or the activity of MUP-bound small molecules. It also remains unclear whether hypothalamic and adipose MUP1, whose expression is regulated by nutrients, regulates metabolism. Additionally, the therapeutic potential of MUP1 in treating type 2 diabetes and metabolic disorders remains to be determined.

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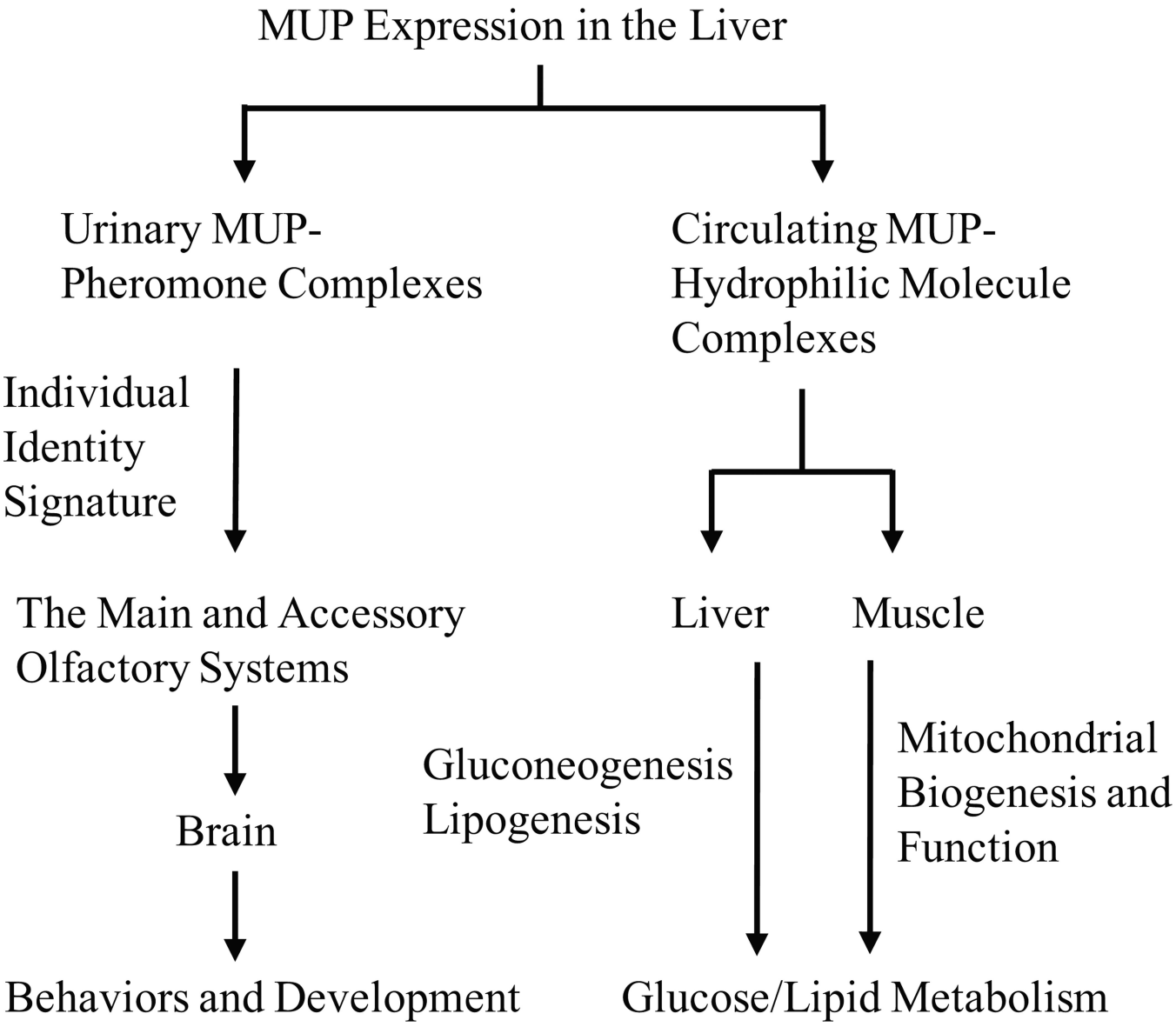

Figure 1. A model of MUP action

The MUP family members are expressed mainly by the liver and secreted into the bloodstream. MUPs bind to various volatile pheromones or other lipophilic small molecules, and regulate the transportation and bioactivity of these small molecules. MUPs and MUP-bound pheromones are excreted into the urine and detected by the main and accessory olfactory systems of conspecifics. MUPs are highly polymorphic, and the MUP profiles in urine are recognized as an identity signature of the owners by receivers. Additionally, circulating MUPs and MUP-bound bioactive molecules also regulate metabolism by suppressing the hepatic gluconeogenic and/or lipogenic programs as well as by promoting mitochondrial biogenesis and function and insulin sensitivity in skeletal muscles.