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Endocrine Regulation of Energy Balance by *Drosophila* TGF-β/Activins

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The Transforming growth factor beta (TGF-β) family of secreted proteins regulates a variety of key events in normal development and physiology. In mammals, this family, represented by 33 ligands, including TGF-β, activins, nodal, bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs), regulate biological processes as diverse as cell proliferation, differentiation, apoptosis, metabolism, homeostasis, immune response, wound repair, and endocrine functions. In *Drosophila*, only 7 members of this family are present, with 4 TGF-β/BMP and 3 TGF-β/activin ligands. Studies in the fly have illustrated the role of TGF-β/BMP ligands during embryogenesis and organ patterning, while the TGF-β/activin ligands have been implicated in the control of wing growth and neuronal functions. In this review, we focus on the emerging roles of *Drosophila* TGF-β/activins in inter-organ communication via long-distance regulation, especially in systemic lipid and carbohydrate homeostasis, and discuss findings relevant to metabolic diseases in humans.

1. Introduction

Similar to TGF-β/activin signaling transduction in mammals, the three *Drosophila* TGF-β/activin ligands, Dawdle (Daw), Activin-β (Actβ), and Myostatin (Myo), signal through two shared type-II receptors, Punt (Put), and Wishful thinking (Wit), and a single type-I receptor, Baboon (Babo), to regulate the transcription factor complex Smox/Med[1] (**Figure 1**). These ligands were initially thought to act as paracrine/autocrine signals, however, recent findings have revealed that they also play roles as hormones involved in inter-organ communication.

Actβ, the first fly activin homolog characterized,[2] was initially believed to act principally as a neuropeptide since it is mainly expressed in the central nervous system (CNS) and the peripheral nervous system (PNS).[3–5] CNS-derived Actβ plays critical autocrine/paracrine roles in neuronal development, including neuroblast proliferation, remodeling of mushroom bodies, optic lobe development and photoreceptor axon targeting, and metamorphosis.[3,6,7] In addition, PNS-derived Actβ is involved in regulation of postsynaptic activity and hemocyte maintenance.[4,5] Interestingly, Myo, another TGF-β/activin ligand with similar expression in both CNS and PNS,[8] also plays essential roles in neuronal remodeling and synaptic function.[8]

Daw, a divergent Actβ paralog most closely related to vertebrate TGFβ1, is expressed in multiple somatic tissues including the embryonic mesoderm, oenocytes, fat body, glial cells and hindgut; the larval musculature, imaginal discs and gut; and adult muscles and fat body.[9–12] Early studies showed that *daw* is necessary for migration of axonal growth cones during embryogenesis and that it acts redundantly with Actβ in the larval brain to regulate neuroblast proliferation.[6,12] *daw* null mutants show multiphasic lethality during development with 15–20% of the mutants dying as embryos and the rest at various phases of larval and pupal development.[11]

Here, we focus on recent studies describing the remarkable roles of Activins as metabolic hormones acting in inter-organ communication. Note that in addition to Actβ, Myo, and Daw, Maverick (Mav) has been proposed to also encode an Activin ligand, but, as it fails to activate dSmad2 in cultured cells and appears to signal through TGF-β/BMP type-I receptor Thickvein (Tkv) in synaptic growth regulation [13,14], we will not discuss it further in this review.

2. *Drosophila* Activins and Sugar Metabolism

Recent studies have elucidated the nature of Actβ beyond a neuropeptide and demonstrated its endocrine role in systemic carbohydrate homeostasis. *Actβ* null mutants exhibit hypoglycemic phenotypes.[15] In addition to its nervous system expression, Actβ has recently been reported to be produced by enteroendocrine cells (EEs) located in the larval midgut.[15] Midgut-derived Actβ is further found to cause physiological glycemic

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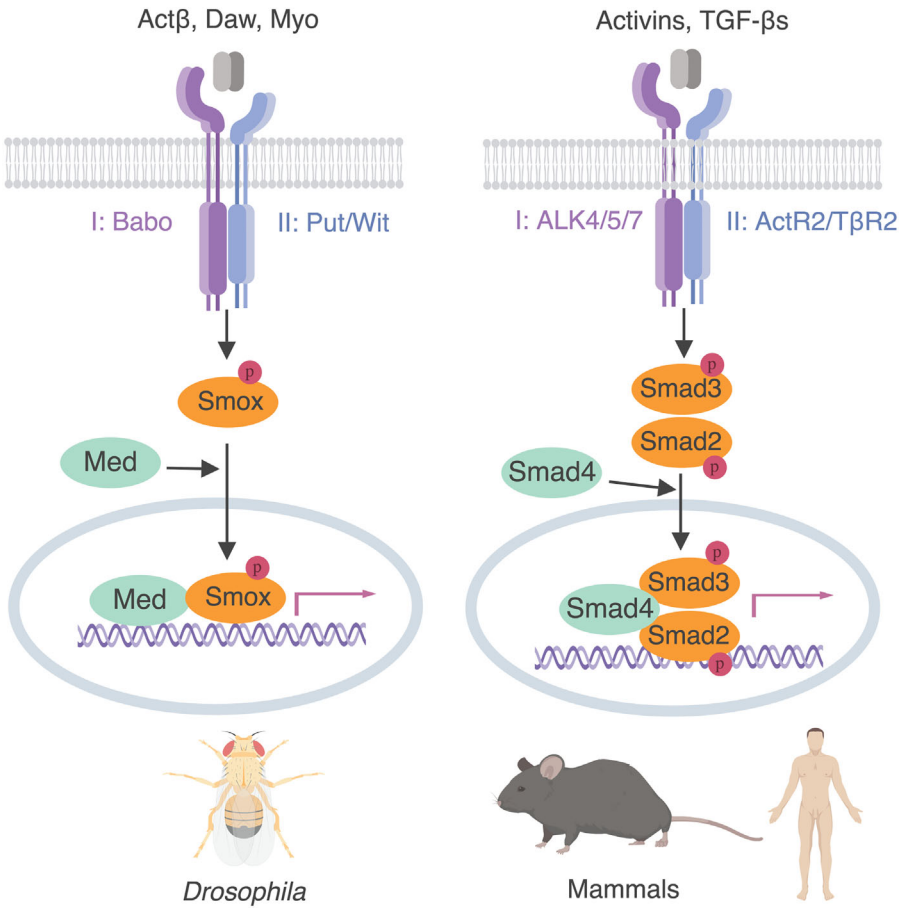

Figure 1. Core components of activin signaling in *Drosophila* and mammals. *Drosophila* TGF-β/activin ligands, Actβ and Daw, bind to the type I receptor Babo and type II receptors Put/Wit, resulting in activation of R-Smad Smox and co-Smad Med that regulate transcription. In mammals, TGF-βs and Activins signal through Activin type I receptor-like kinases 4/5/7 (Alk4/5/7) and Activin type II (ActR2) or TGF-β type II (TβR2) receptors to activate R-Smad Smad2/3 and co-Smad Smad4. Ligands, receptors, and R-Smad and co-Smad transcription factors between *Drosophila* and mammals are highly homologous in both protein sequences and structures.

imbalance by remotely signaling to the fat body and enhancing the action of Adipokinetic hormone (Akh) to promote carbohydrate release. In fat cells Actβ triggers Babo/Smox signaling via Babo-A, one of the Babo isoforms, to increase expression of the Akh receptor (*AkhR*) and enhance Akh sensitivity.[15] Similar to energy overload in mammals, flies also develop severe lipid accumulation, insulin resistance, and elevated glycemic level (circulating glucose + trehalose levels) under a chronic high-sugar diet (HSD) during the larval stage.[16] Interestingly, Actβ derived in the midgut, but not in the brain, is largely induced by HSD and is essential for hyperglycemia development associated with HSD.[15] Modulation of either Babo or Akh signaling in the fat body barely affects insulin signaling, indicating that Actβ-associated glycemic control through this midgut-to-fat-body axis is insulin-independent.[15]

Note that, compared to *Actβ* mutants, deficiency of midgut-derived Actβ is not able to fully restore hyperglycemia under HSD,[15] suggesting that functional Actβ can be produced from other tissues as well. In support to this model, Actβ expression is also induced by HSD in the body wall containing the PNS.[15] PNS-derived Actβ was recently shown to regulate proliferation of hemocytes, their location in resident clusters attached to PNS, as well as their circulation in the hemolymph.[5] As circulating fly hemocytes behave as macrophages secreting inflammatory cytokines, such as Upd3, cause systemic insulin resistance and hyperglycemia under a high-caloric diet,[17] it is possible that PNS-derived Actβ also participates in glycemic control by modulating hemocyte distribution.

The role of Daw in regulating sugar homeostasis was first suggested by the observation that lethality associated with *daw* loss of function mutations can be rescued by rearing the animals in low sugar and non-acidic pH conditions.[18] Subsequent studies on the metabolic roles of Daw revealed that it regulates multiple aspects of sugar homeostasis. First, *daw* mutant larvae show a hyperglycemic phenotype that is partially caused by loss of insulin release from the larval insulin producing cells (IPCs).[18] Second, canonical TGF-β signaling mediated by Daw can regulate mitochondrial activity in peripheral tissues as evidenced by increased expression of multiple mitochondrial genes and increased accumulation of TCA cycle intermediates in the fat body of mutant larvae. The effects of Daw on mitochondrial gene expression most likely extend to other

peripheral tissues like the gut since rearing flies on HSD, that causes increased expression of *daw*, leads to suppression of multiple nuclear encoded mitochondrial enzymes in the fly gut. Interestingly, the effects of Daw on mitochondrial activity and insulin release seems to be mutually independent events since the mitochondrial phenotypes can be completely rescued by activating TGF-β signaling in peripheral tissues without rescuing the hyperglycemic phenotype of *daw* mutant larvae. Third, Daw signaling in the fly gut is required for initiating glucose repression: a phenomenon whereby increased glucose consumption causes strong suppression of digestive enzymes like amylase that presumably protects the animals against sugar toxicity.[10] The extent to which the effects of Daw on glucose repression influence the hyperglycemic and mitochondrial phenotypes in *daw* mutant larvae warrants further investigation. Loss of the canonical TGF-β signaling components in the gut does lead to a higher spike in post-prandial circulating sugar levels indicating a potential link between glucose repression and circulating sugar levels. Nevertheless, this effect is not observed during sustained feeding on HSD, possibly due to secondary effects that either limit food intake by the experimental flies or due to HSD induced insulin resistance masking the differences between the control and experimental animals.[10] More recent evidence indicates that Daw mediated canonical TGF-β signaling could be functioning in central brain neurons to regulate systemic utilization of energy stores adding a new dimension to this versatile metabolic hormone.[19]

The wide-ranging roles of Daw could be explained by tissue-specific roles of Daw in regulating transcription of target genes. For instance, while Daw signaling regulates expression of multiple red-ox and mitochondrial genes both in the larval fat body and adult gut, in the adult gut Daw signaling has an additional role as it regulates the expression of digestive enzymes.[10,18] Since *daw* itself is expressed in multiple tissues including the brain, muscle and gut, one possibility is that paracrine Daw signaling regulates the tissue-specific roles of Daw in sugar metabolism. However, experimental evidence suggests that Daw is capable of acting as a potent endocrine hormone. Ectopic expression of a *daw* transgene in a wide range of organs like the fat body, muscle, oenocytes and even a small population of neuro-secretory cells (IPCs) can completely rescue all the *daw* mutant phenotypes and the extent of rescue is dependent on the size of the organ that is expressing the ligand. For instance, expression of *daw* in the IPCs of a *daw* mutant can rescue circulating sugar concentration to normal levels,[18] however expression of *daw* in the fat body or muscle of *daw* mutants significantly reduces circulating sugar concentrations below normal levels leading to a hypoglycemic phenotype.[18] Similarly, expressing *daw* in the IPCs or oenocytes of *daw* mutants can rescue fat body p-Smox abundance to normal level, however expressing *daw* in the muscle causes hyper-activation of p-Smox, indicating a dose dependent, hormonal, role of Daw in activating TGF-β signaling in peripheral tissues.[18] Additionally, glucose repression in the gut of adult flies is mediated by Daw that originates from the fly fat body.[10] Taken together these studies indicate that both Actβ and Daw act as metabolic endocrine hormones that regulate sugar metabolism.

Finally, the role of Myo in in glycemic control as yet to be characterized. Muscle-derived Myo remotely suppresses rRNA synthesis and decreases nucleolus size in the fat body via modulating Babo/p38 signaling,[20] suggesting an endocrine role for Myo. Some of our unpublished results also indicate that Myo gain-of-function results in elevated circulating glucose and trehalose levels. Thus, it will be interesting to investigate the metabolic roles of Myo in glycemic control in future studies.

3. *Drosophila* Activins and Lipid Metabolism

The fly fat body, similar to mammalian adipose tissue and liver, is an important metabolic organ controlling lipid storage and mobilization that maintains energy balance in response to nutrient availability and physiological stresses.[21] Recent studies have demonstrated the critical roles of activin signaling in the fat body in the regulation of systemic lipid homeostasis.[18,22] Specifically, intracellular Babo signaling promotes lipid mobilization and decreases TAG content in the larval fat body.[22] Even though modulation of activin signaling in the fat body exhibits changes in mitochondrial function and lipid β-oxidation rate that contribute to lipid mobilization,[22] the molecular mechanisms by which activin signaling regulates lipid metabolism are still unknown. Nevertheless, enhanced activin signaling and impaired lipid homeostasis in the fat body is associated with larval muscle dysfunction, suggesting a muscle-to-fat-body interorgan communication modulated by activins. Specific perturbation of mitochondrial complex I activity in larval muscles strongly induces Actβ and Daw expression. Muscle-derived Actβ, but not Daw, is further characterized to non-autonomously activate Babo/Smox cascade and suppresses mitochondrial activity and lipid mobilization in the fat body.[22] Systemic loss of *daw* also leads to increased total TAG content in feeding third instar larvae.[18] However, whether this phenotype is due to the role of Daw in insulin release or its effect on mitochondrial metabolism has not been studied.

4. *Drosophila* Activins and Mitochondrial Activity and Autophagy

Activin/Babo signaling in the fat body is highly associated with mitochondrial activity, including ATP production, O_2 consumption, as well as β-oxidation.[22] A recent study has illustrated how Actβ mediates synchronized mitochondrial dysfunction between larval muscle and fat body. As mentioned above, larval muscles with impaired mitochondrial complex I produce Actβ to activate Babo signaling and simultaneously suppress mitochondrial activity in the fat body.[22] It is not likely that Actβ impairs mitochondrial activity via down-regulation of general mitochondrial genes because complex I perturbation in larval muscles, which correlates with elevated Actβ production, actually up-regulates the expression of more than half of mitochondrial genes. Thus, this up-regulation may represent a compensatory effect in response to Actβ associated mitochondrial injury.

In addition to the role of Daw in mitochondrial activity mentioned above, Daw also regulates autophagy to control multiple metabolic processes.[9] Smox was found to directly suppress expression of *Atg8*, a LC3 homolog and an essential component of autophagy, and to inhibit autophagy/lysosome activity in adult muscles in response to Daw.[9] Since

autophagy-associated protein degradation in the fly muscle plays an important role in proteostasis and systemic aging, it indicates that perturbation of Daw/Smox signaling robustly promotes autophagy and proteolysis activity in aged adult muscles and significantly slows down systemic aging.[9]

5. Regulation of *Drosophila* Activins by Nutrient Status and Stress Signaling

As endocrine factors controlling systemic energy homeostasis, fly activins are also regulated by nutrient status as well as stress signaling. For example, Actβ induction in larval muscles is regulated by mitochondrial unfolded protein responses (UPR^{mt}).[22] Complex I perturbation in muscle results in reactive oxygen species (ROS) generation and activates Relish (Rel), one of the UPR^{mt} transcriptional factors mounting protective transcriptional responses,[23] to enhance Actβ expression.[22] Either eliminating ROS level via overexpression of an antioxidant enzyme PHGPx or knocking down *Rel* in complex I-perturbed larval muscle is sufficient to decrease Actβ expression, as well as its impact on fat body metabolism.

The mechanism of Actβ induction in the midgut caused by HSD is not well understood. Even though we have attributed the induction of midgut Actβ expression to an increased in EE numbers,[15] whether and how Actβ expression is up-regulated in each EE in response to HSD is still unknown. Thus, it will be of interest to examine whether Actβ expression in the midgut is induced by ROS/Rel signaling as well.

Expression of *daw* is also significantly upregulated in the adult fly fat body in response to HSD indicating the role of a sugar-sensing pathway in regulating *daw* expression.[10] This sugar inducible expression of *daw* is regulated by the combined effects of the evolutionarily conserved inter-cellular glucose-6-phosphate sensing Mondo/Mlx complex and dFOXO signaling pathway.[19,24] While mlx^1 mutants show partial loss of sugar-inducible *daw* expression and $dFoxo^{24}$ mutants still show increased expression of *daw* in response to sugar, mlx^1; $dFoxo^{24}$ double mutants are completely incapable of inducing *daw* expression in response to sugar.[19,24] Downstream of these major sugar responsive pathways, Daw regulates the expression of the *Drosophila* Gli-similar transcription factor, sugarbabe, which then mediates the sugar responsive changes in expression of multiple metabolic enzymes including digestive enzymes, many lipases and lipid synthesis enzymes.[24] *Daw* has also been characterized as a transcriptional target of dFOXO, since dFOXO directly binds to the *daw* promoter and suppresses *daw* expression in both *chico* mutants and IPC-ablated flies.[9] Consistent with the proposed negative regulation of *daw* by dFOXO, *daw* modulates adult muscle proteostasis and systemic aging in a manner opposite to dFOXO.[9]

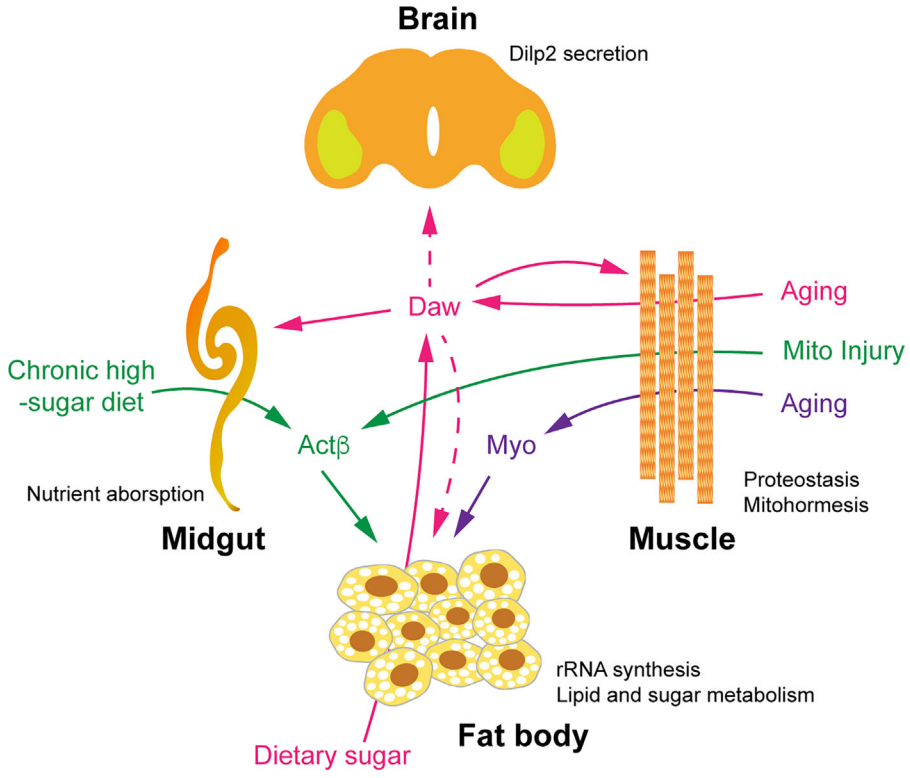

Figure 2. Activin-induced interorgan communication in *Drosophila*. Actβ, which targets the fat body to regulate lipid and sugar metabolism, is produced in the larval midgut upon a chronic HSD or in larval muscles bearing mitochondrial injury. Adult fat body-derived Daw remotely represses sugar absorption in the midgut in response to dietary sugar, while muscle-derived Daw autonomously maintains adult muscular proteostasis and suppresses aging. Note that Daw level affects Dilp2 secretion in the brain and fat body Smox phosphorylation. Muscle-derived Myo regulates aging partially via modulation of rRNA synthesis and nucleolar function in the fat body.

6. Relevance to Human Diseases

Core components of activin signaling share very similar protein sequences, molecular regulations, as well as physiological functions, in both fly and mammals (Figure 1).[1,25] For instance, the regulation of insulin production by activin signaling has been reported in both fly and mammals.[9,18,26] Importantly, studies from *Drosophila* have provided new insights into the role of activin signaling in metabolic disorders. First, elevated TGF-β1 and activin A levels are highly associated with high-fat diet-induced obesity and type 2 diabetes in both mouse and human.[27,28] As shown in *Drosophila*, increased Actβ levels result in Akh enhancement and hyperglycemia. Consistently, TGF- β1 and activin A directly sensitize glucagon response and increase glucose production in mouse cultured hepatocytes,[15] indicating a novel molecular mechanism of glucose imbalance by which activins impact hepatic glucagon signaling and glucose production in an insulin-independent manner. Additionally, similar to Daw, vertebrate Activin A/B has also been implicated in regulating mitochondrial biogenesis by regulating expression of mitochondrial enzymes.[28,29]

Second, autophagy is a fundamental regulator of organelle homeostasis and its dysregulation is highly associated with impaired proteostasis and fibrosis of multiple tissues. Following the report that Daw regulates muscular autophagy to affect muscle proteostasis during aging,[9] two independent studies have demonstrated that TGF-β1 modulates fibrogenesis in atrial myofibroblasts via regulation of autophagy as well.[30] While the molecular mechanisms of autophagy regulation do not appear consistent, probably due to distinct cellular contexts, these studies indicate that activins exhibit conserved organelles regulation to alter tissue physiology across species.

Third, both *Drosophila* and mammalian activin ligands suppress mitochondrial activity and impair lipid metabolism.[18,22,28,31] Consistent with the findings that Actβ is transcriptionally regulated following mitochondrial complex I perturbation in fly muscles,[22] the mRNA levels of *INHBA*, which encodes the activin A subunit, is also increased by ROS/NF-κB signaling in response to mitochondrial complex I perturbation in cultured mouse muscle cells.[22] As a major endocrine organ regulating systemic physiology, muscles may thus produce activin A in response to mitochondrial injury and non-autonomously decrease mitochondrial function and cause lipid accumulation in adipocytes.

7. Concluding Remarks

In this review, we have highlighted the roles of TGFβ/activin-dependent interorgan communications in systemic metabolic regulation in *Drosophila*, focusing on the secreted factors, their physiological roles, as well as their mechanisms of action (**Figure 2**). Despite the knowledge gained, there are still a number of questions that remain to be addressed. In particular, the sites of TGF-β/activin production are not fully characterized. Specifically, unlike Actβ and Myo that are produced in specialized tissues, Daw is ubiquitously expressed in multiple tissues, however, it is possible that the release of Daw from specific tissue(s) is differentially regulated depending on physiological conditions. Further, activin ligand regulation may differ depending on the nature of the stimuli as well, i.e., acute versus chronic. Daw is induced short-term by dietary sugar and suppresses sugar absorption to maintain carbohydrate homeostasis, while Actβ is triggered by a chronic HSD to impair carbohydrate balance. Thus, it will be important to investigate how individual TGF-β/activin ligands are produced and their effects integrated in response to diverse physiological perturbations. Finally, how these generic ligands can achieve specific organ and initiate distinct signaling responses is largely unknown. Actβ prefers to target $Babo_A$, while Daw targets $Babo_C$.[15,32] It is possible that Myo targets $Babo_B$. Previous studies have also demonstrated distinct signaling activities of activin ligands. For example, Actβ and Daw activate Smox, as well as Mad with a less extent, while Myo triggers MAPK activation.[13,20] The tissue specificities and downstream outputs of these ligands appear to be associated with different Babo isoforms. Therefore, investigating expression patterns and downstream signaling activities of individual Babo isoforms and how these crosstalk to other important metabolic responses, like insulin and Akh actions, in multiple organs will be important to clarify.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

Actβ, Daw, *Drosophila*, metabolic regulation, Myo, organ communication, TGF-β/activin

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