Chapter

Current Advances in Mass Spectrometry Imaging for Insect Physiology and Metabolism

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Abstract

Research regarding the distribution of metabolites is a vital aspect of insect molecular biology. However, current approaches (e.g., liquid chromatography-mass spectrometry or immunofluorescence) have cons like requirement of massive tissues, low efficiency, and complicated operating processes. As an emerging technology, mass spectrometry imaging (MSI) can visualize the spatiotemporal distribution of molecules in biological samples without labeling. In this chapter, we retrospect the major types of *in situ* measurement by MSI, and the application of MSI for investigating insect endogenous and exogenous metabolites and monitoring the dynamic changes of metabolites involved with the interactions between insects and plants. Future studies that combine MSI with other genetic tools can facilitate to better explore the underlying mechanisms concerning insect physiology and metabolism.

Keywords: spatial metabolomics, *in situ* characterization, endogenous metabolites, exogenous metabolites, plant-insect interaction

1. Introduction

Insect molecular biology studies the molecular basis of biological processes in insects, including molecular synthesis, modification, mechanisms, and interactions [1]. Metabolites play key roles among all these aspects of insect molecular biology. Therefore, understanding the distribution of metabolites contributes to revealing the mechanisms of insect biology, including ontogeny, metabolism, and physiology. Research methodologies such as liquid chromatography-mass spectrometry (LC-MS) and immunofluorescence are generally used in visualizing the distribution of metabolites. However, all of them have their shortcomings. LC-MS or gas chromatography-mass spectrometry (GC-MS) usually uses the homogenate of a certain weight of specific tissue(s) or organ(s), resulting in losing *in situ* spatiotemporal information. Insect body sizes are mostly small, let alone certain tissues; so tissue-specific researches, in most case, consume a large number of insect individuals [2–4]. On the other hand, ordinary *in situ* characterization technologies such as immunofluorescence assay and fluorescence *in situ* hybridization (FISH) require labeling at specific biomolecules [5–7]. Hence, operating processes such as synthesizing probes and antibodies are usually time-consuming, inefficient, and limited to only one molecule.

As a new molecular visualization technology, mass spectrometry imaging (MSI) has drawn more and more attention in recent years. MSI can visualize the spatial distribution of molecules in specific samples without any labeling and enable simultaneous evaluation and identification of hundreds of molecules *in situ*. In comparison with LC-MS and GC-MS, MSI requires only one sample for biomolecular localization, which makes it a powerful tool to visualize the changes in organism physiology and biochemistry. The basic principle of MSI is to scan target samples such as tissue slice for desorption and ionization of molecules or ions on the surface of samples by a laser or a high-energy ion beam [8]. Mass analyzer obtains mass-to-charge ratio (m/z) and ion intensity of the molecules or ions from pixels. Mass peaks are obtained from the database of imaging software such as FlexImaging and used to visualize one-dimensional linear profiling, two-dimensional spatial distribution of molecules, or three-dimensional anatomic structure [8]. MSI has been widely applied in life sciences, such as histology [9, 10]; pathology [11, 12]; pharmacology [13, 14]; food science [15]; botany [16–19]; and microbiology [20, 21].

In this chapter, we introduce the major types of *in situ* measurement by MSI and present an example of matrix-assisted laser desorption ionization (MALDI) to elucidate the operating processes. We also discuss the advances of MSI in insect physiology and biochemistry to better promote the research in entomology.

2. Mass spectrometry imaging method

Among all the MSI technologies, we can divide them into two major groups, vacuum ionization mass spectrometry imaging system and ambient ionization mass spectrometry imaging system, based on whether the environment of the instruments is a vacuum [8]. Based on desorption or ionization ion, vacuum ionization mass spectrometry imaging system can be further divided into different categories, namely MALDI [22], secondary ion mass spectrometry (SIMS) [23], surface-assisted laser desorption ionization (SALDI) [24], and laser desorption ionization (LDI) [25]; ambient ionization mass spectrometry imaging system can be further divided into different categories, namely desorption electrospray ionization (DESI) [26], laser ablation electrospray ionization (LAESI) [27], laser electrospray mass spectrometry (LEMS) [28], electrospray laser desorption ionization (ELDI) [28], atmospheric pressure matrix-assisted laser desorption ionization (AP-SMALDI) [29], and air flow-assisted ionization (AFAI) [30]. Among all these above-mentioned technologies, MALDI-MSI is the most popular technology in life science research because it not only can be applied to a wide range from inorganic ion, small molecules to proteins but also has the characteristics of high accuracy and sensitivity [31]. Here, we provide a further explanation of the basic principle of MADLI-MSI and elucidate the workflow for MALDI.

The basic working principle of MALDI is that target analytes on the surface of tissue are crystallized with matrix (e.g., α-cyano-4-hydroxycinnamic acid and 2,5-dihydroxyacetophenone) to form a complex. When the complex is exposed by infrared laser at 2.94 or 10.6 μm and/or ultraviolet laser at 337, 355, or 266 nm, it absorbs the laser energy and converts these analytes into a phase of gas, which causes molecule ionization. The ionized molecules automatically enter a mass spectrometer where the molecules are detected and mapped [19].

A typical experimental workflow for MALDI is as follows (**Figure 1**):

a. Insect tissues are flash-frozen (with or without fixation) in an embedding media with gelatin, carboxymethylcellulose, or water;

b. Each sample is cryo-sectioned at 10–20 μm thickness and mounted onto glass slides coated with indium tin oxide, which is then lyophilized for tissue imaging;

c. The lyophilized slide is subject to three irregular fiducial markings on the surface of each sample for localization;

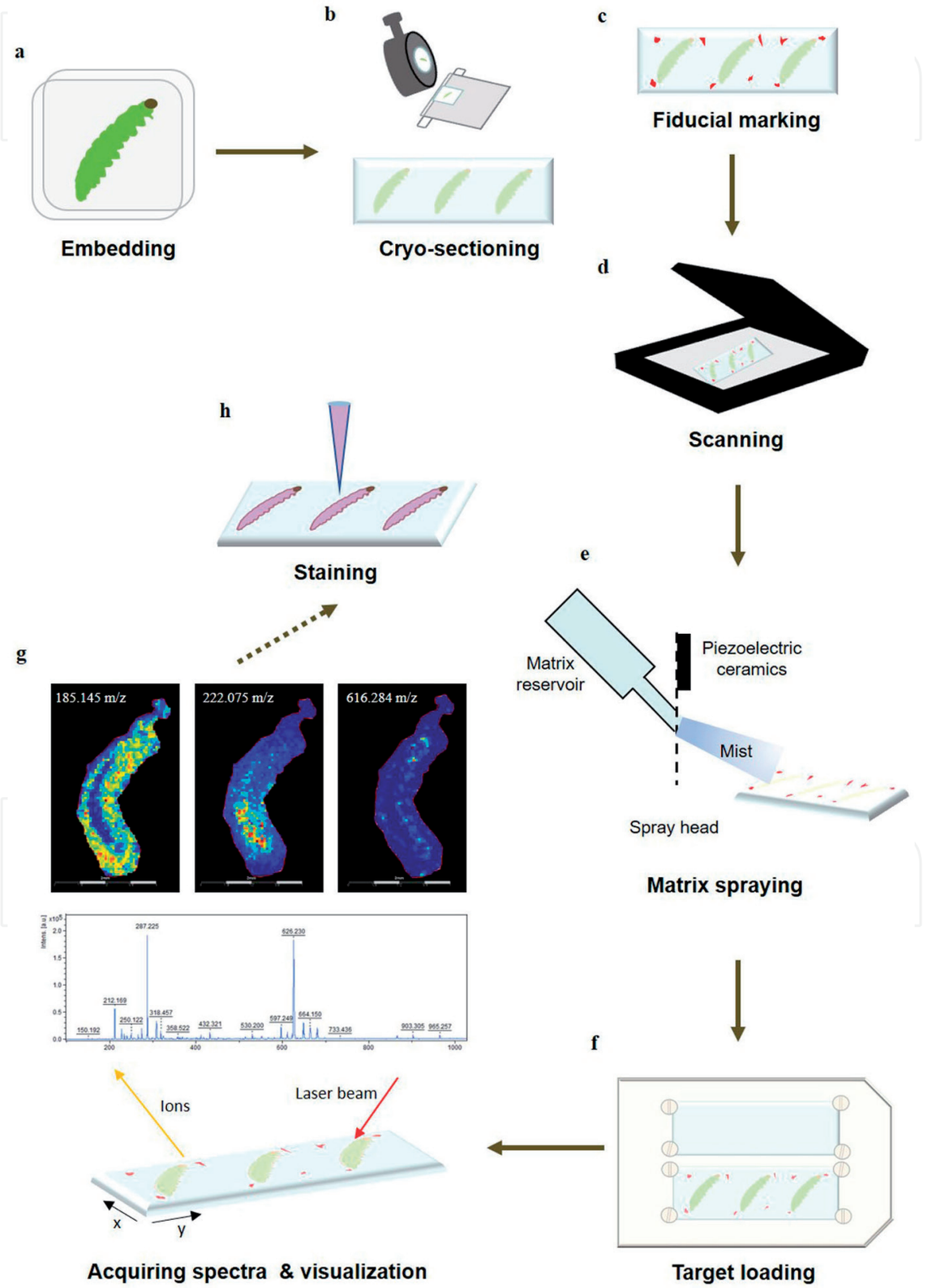

Figure 1.
MALDI-MSI imaging workflow.

d. A digital image of the sample with fiducials is acquired;

e. A chemical matrix is applied to promote desorption and ionization. Matrix is coated by a sprayer/nebulizer or by solvent-free sublimation to acquire homogeneous matrix coverage over the entire tissue surface;

f. After matrix deposition, the target is inserted into the instrument, for which experimental parameters (e.g., laser energy, step size of plate movement, and a selected region of the tissue) are optimized to scan the image;

g. A laser beam is emitted for desorption to acquire mass spectra at every x and y grid points within the scanning area, so to visualize target ions and convert the ion's intensity into a color scheme;

h. Hematoxylin-eosin staining is optional for displaying tissue localization.

3. Application of mass spectrometry imaging in entomological researches

MSI can visualize the spatial and temporal distributions of molecules. Endogenous metabolites, exogenous metabolites, and insect-plant interactions are three main aspects of MSI application to insect tissue section for *in situ* characterization. Endogenous metabolites refer to lipids, neuropeptides, proteins, and defense compounds [32–35]; exogenous metabolites are drugs and insecticides [36, 37]; insect-plant interactions are associated with the fate of plant secondary defense compounds in insects [38]. We summarize the major applications of MSI for a better understanding of insect physiology and metabolism (**Table 1**).

3.1 Insect endogenous metabolites

3.1.1 Lipids

Lipids are basic cell components and play important roles in insect development and reproduction, such as maintenance of cell membrane structure and intra or extracellular signaling [39–41]. For example, glycerophospholipids, phosphatidylcholines, and phosphatidylethanolamines are basic components of cell and lysophospholipids have an important function in inflammation, abiotic stress, and biotic stress signal transmit [42]. MSI has been widely applied in many aspects in model insect *Drosophila melanogaster*, such as the neutral lipids three-dimensional spatial distribution on the surface adults [43, 44], body lipid distribution [45], brain lipid structure [46, 47], wing lipids [34, 44], Malpighian tubule phospholipid distribution [48], and phospholipids in the brain treated with cocaine [49]. Moreover, MSI detected and localized the composition and distribution of triacylglyceride in *Aedes aegypti*, phospholipid and phosphatidylcholine in *Anopheles stephensi* [42], and phospholipids in *Schistocerca gregaria* [37].

3.1.2 Neuropeptides

Neuropeptides, a kind of structurally diverse signaling molecules, can control and regulate fundamental physiological functions such as growth, reproduction, and environmental stress tolerance in animals [50]. MSI detected and localized the distribution of 14 neuropeptides in coronal brain sections in all development stages

Species	Tissue	Major analyte	Method	Embedding medium	Thickness (μm)	Matrix	Ref.
Endogenous metabolites							
Anopheles stephensi	Whole-body	Lipids	AP-SMALDI	5% CMC	20	DHB	[42]
Aedes aegypti	Ovarian follicles	Lipids	3D-SIMS	/	100	/	[41]
Apis mellifera	Brain	Neuropeptides	MALDI	/	14	CHCA	[51, 52]
	Brain	Protein	MALDI	/	12	CA	[32]
	Brain	L-arginine	MALDI	/	12	DHB	[53]
	Venom	Venom toxins	MALDI	/	10	CHCA	[54]
Drosophila melanogaster	Body	Peptide	MALDI	Agarose	10	CHCA	[55]
	Brain	Lipids	SIMS	10% Gelatin	15	/	[47]
	Brain	Phospholipid	SIMS	10% Gelatin	12	/	[49]
	Collar	Lipids	SIMS	10% Gelatin	10	/	[46]
	Brain & head	GABA	MALDI	4% CMC	15	CHCA	[56]
	Malpighian tubule	Lipids	MALDI	5% CMC	12	DHB、DAN	[48]
	Surface	Lipids	MALDI	/	/	LiDHB	[43]
	Whole-body	Neuropeptides	AP-SMALDI	5% CMC	20	DHB	[29]
	Whole-body	Lipids	MALDI	10% Gelatin	20	DHB	[45]
	Wing	Lipids	SIMS	PBS	/	DHB	[34, 44]
Graphosoma lineatum	Head to abdomen	Non-polar compounds	DAPPI	/	/	/	[57]
Paederus riparius	Whole-body	Defensive compounds	AP-SMALDI	10% Tragacanth gum	16	DHB	[35]

Species	Tissue	Major analyte	Method	Embedding medium	Thickness (μm)	Matrix	Ref.
Periplaneta americana	Brain	Neuropeptides	MALDI	Gelatin	14	CHCA	[33]
	Neuro-endocrine tissues	Neuropeptides	MALDI	Paraffin	20	DHB	[58]
Prorhinotermes simplex	Head to abdomen	Non-polar compounds	DAPPI	/	/	/	[57]
Solenopsis invicta	Venom	Venom proteins	MALDI	Gelatin	14	DHB	[59]
Exogenous metabolites							
Drosophila melanogaster	Whole-body	Insecticide	MALDI	10% Gelatin	15	DHB	[36]
Schistocerca gregaria	Whole-body	Drugs	DESI	5% CMC	50	/	[37, 60]
Helicoverpa armigera	Whole-body	Biopesticide	MALDI	/	16	DHB	[61]
Insect-plant interaction system							
Aphis glycines	Feeding leaf	Metabolites	MALDI	/	/	DHB、DAN	[62, 63]
Athalia rosae	Whole-body	Glucosinolates	MALDI	Water	15	CHCA	[64]
Chorthippus dorsatus	Gut, feces	Metabolites	LDI	1% PBS	12	DHB、DAN、CHCA	[38]
Others							
Acromyrmex echinatior	Nest	Microbial	MALDI	/	/	DHB、CHCA	[65]
Ants	Propleural plate	Fungus	MALDI	/	/	DHB	[66]
Bombus terrestris	Whole-body	/	MALDI	pHPMA	12	DHB、SA	[67]

Table 1.
Overview of the application of MSI in insect sciences.

of *D. melanogaster* [29]. These neuropeptides play important roles in physiological processes (e.g., allatostatins and tachykinin-like peptides participate in odor perception and locomotor activity). Neuropeptides can act as transmitters or neuromodulators in the central nervous system [33]. Neuropeptides in the brain of *Apis mellifera* are related to the functional division of the population and their activities. Worker bees' neuropeptide levels at the age of 0–15 d increased with the in-hive activities but decreased with out-hive activities (guarding and foraging) at 15–25 d [51]. Further study proved that allatostatin and tachykinin-related neuropeptides in the brain of worker bees were related to aggressiveness behaviors [52]. Neuropeptides distribution in the retrocerebral complex of *Periplaneta Americana* revealed the differentiation of prohormone processing and the distinctness of neuropeptides-based compartmentalization [33]. These studies proved that MSI has the advantages of sensitivity, which can facilitate to detect peptides in low abundance.

3.1.3 Proteins

As a kind of macromolecules, proteins are fundamental compounds of organisms and take part in important cellular processes, such as DNA replication and metabolisms. MSI can simultaneously and specifically detect the spatial distribution of massive proteins and overcome antibody cross-contamination. MSI system has been used to evaluate the negative impacts in the brain of *A. mellifera* exposing to a sublethal concentration of imidacloprid. The system has successfully visualized the distribution of 24 proteins (e.g., cytochrome P450s, glutathione S-transferases, and heat shock protein 70s). Besides, 8-day exposure to imidacloprid triggered biochemical changes in *A. mellifera* brain (e.g., up-regulated acetylcholinesterase and amyloid precursor-like protein and down-regulated cytochrome P450 and disulfide-isomerase protein). This could influence the well-being of *A. mellifera* (e.g., learning and memory acquisition, maintaining neuronal integrity, detoxification, and apoptosis) [32].

3.1.4 Others

In addition to lipids, neuropeptides, and proteins, MSI can also be used to visualize the distributions of defensive compounds, special proteins (e.g., venom allergens and toxins) and other small molecules (e.g., betaine and amino acids). Defensive compounds (e.g., pederin, pseudopederin, and pederon) were detected and localized in the organs of *Paederus riparius* [35]. Three venom allergens and two venom toxins were mapped in the honeybee [54]. Poison sac was the lactation of main venom proteins in *Solenopsis invicta* [59]. Nonpolar compounds (e.g., (*E*)-1-nitropentadec-1-ene and (*E*)-hex-2-enal) can be detected from the head to the abdomen in two model insects, *Prorhinotermes simplex* and *Graphosoma lineatum*. Gland openings and gland reservoirs were the most active areas in *P. simplex* and *G. lineatum* [57]. Other small molecules (e.g., betaine and amino acids) were detected in *Schistocerca gregaria* [37]. Semiochemicals were mapped on the surface of the adults of *D. melanogaster* [43]. Two male-specific sex pheromones were localized in the ejaculatory bulb of *D. melanogaster* [45]. MSI can also be used as a novel *in situ* metabonomic tool to study the metabolism of L-arginine of the honeybee brain in response to proboscis extension [53].

3.2 Insect exogenous metabolites

3.2.1 Insecticides

MSI can be applied to visualize the distribution of insecticides in insects and their negative influence on the target insects. Imidacloprid was used to study its

distribution and accumulation in *D. melanogaster*. Based on laser irradiation, imidacloprid was found to be converted to guanidine-imidacloprid. It eventually accumulated and spread in the abdominal region [36]. *Azadirachta indica* is an economical tree that can be used to distract a biopesticide component, azadirachtin-A. It was only presented in the midgut of *Helicoverpa armigera* after application [61].

3.2.2 Drug/pharmacological test

Pharmacology model animals are crucial for scientists or pharmacologists to test the side effects of newly developed drugs before clinical trials on human beings. Common pharmacology model animal species include mice, rabbits, dogs, and monkeys. Insects, compared with the above-mentioned animals, have pros such as low costs, high fertility, and moral constraints. Locust has become a new model species for pharmacology test because of its high similarity with mammals. Antihistamine drug terfenadine was tested in locust to study the distribution of secondary metabolites. Terfenadine was gradually degraded from hemolymph to stomach and intestinal wall. However, terfenadine-related chemical compounds such as terfenadine acid, terfenadine glucoside, and terfenadine phosphate were distributed in the unexcreted feces in the intestine, which revealed a rapid discharge of metabolites through defecation [37]. Besides, the spatial and temporal distribution of midazolam was tested in locust. The results showed that midazolam was abundant in 30-min but only found in the feces after a 2-hour application. Midazolam glucoside was found in gut, gastric caeca, and feces after a 2-hour application, indicating that glucose conjugates are a major detoxification pathway to neutralize the effects caused by midazolam in locusts [60].

In addition, *D. melanogaster* was used to test how cocaine, drug removal, and methylphenidate influence the brain lipids. The results showed that cocaine increased the level of phosphatidylcholines and decreased the levels of phosphatidylethanolamines and phosphatidylinositols. Methylphenidate-treated flies failed to rescue the levels of phosphatidylethanolamines and phosphatidylinositols, but enhanced the reversal of phosphatidylcholine levels [49].

3.3 Insect-plant interaction

Plants and herbivorous insects are co-evolved in nature. Plants activate defense reaction by releasing secondary metabolites when they are under attack by herbivorous insects, while herbivores trigger anti-defense systems for adapting and overcoming the side effects of secondary metabolites produced by plants [68]. Illuminating the changes of secondary metabolites during the interactions between insects and plants can contribute to a better understanding of plant resistance and insect adaptability.

Chemical interaction between soybean (*Glycine max*) and aphid (*Aphis glycines*) was studied. The metabolite changes (e.g., phosphorylcholine and amino acid) were detected in the aphid-infested soybean leaves. The results suggested that secondary metabolites were produced by dead cells after aphid infestation. Moreover, other compounds such as pipecolic acid, salicylic acid, formononetin, and dihydroxyflavone were consistently detected in the plant regions infested by aphids [62]. It was also found that isoflavones can be accumulated in mesophyll cells or epidermis but were not present in the vasculature. The results indicated that isoflavones take part in non-phloem defense response [63].

In addition, MSI can be used for physiological studies such as annihilation of the plant secondary metabolites by herbivores. Glucosinolate gradually changes in the distribution and metabolic sequestration were detected in the body of *Athalia*

rosae that fed on host plants after different periods. The glucosinolate sinalbin was accumulated in the hemolymph and eventually circulated the Malpighian tubules. The results indicated that the insect gut plays a crucial role as a regulatory functional organ [64].

Moreover, MSI can be applied to the entire metabolic process of secondary metabolites in the plant-insect-soil system. The fate of the secondary metabolites produced by *Dactylis glomerata* was tracked in the different organs of herbivore *Chorthippus dorsatus*, and finally in the soil solution. After infestation by herbivores, levels of quinic acid, apigenin, and luteolin decreased, while those of flavonoids and rosmarinic acid increased in the leaf wounds of plants in 1 d. Quinic acid can be detected during the digestion process in the grasshoppers' gut and unexcreted feces [38]. Overall, MSI is a useful tool to visualize plant defense and insect defense processes from the responses of plants infested by herbivores to insect defense systems responding to plant-derived metabolites.

4. Conclusion

MSI has been proved to be an effective and powerful tool to visualize molecules' spatial distribution and temporal changes. In this chapter, we introduce the major types of MSI methodologies and describe the typical experimental workflow for MALDI-MSI. We also retrospect three major applications of MSI in insect physiology, for example, endogenous metabolites, exogenous metabolites, and insect-plant interaction. However, MSI still has some technical cons with limited application range that need to be optimized. In addition, MSI can cooperate with other genetic tools (e.g., proteomics, metabolomics, or lipidomics) for a better understanding of sophisticated insect biology.

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Conflict of interest

The authors declare no conflict of interest.

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