Perspective

# The emerging role of non-coding RNAs from extracellular vesicles in Alzheimer's disease

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DOI:10.31083/j.jin.2021.01.360



Submitted: 31 October 2020 Revised: 04 December 2020 Accepted: 12 January 2021 Published: 30 March 2021

Alzheimer's disease is an age-dependent neurodegenerative disease. Recently, different non-coding RNAs (ncRNAs), including microRNAs, long non-coding RNAs, and circular RNAs, have been found to contribute to Alzheimer's disease's pathogenesis. Extracellular vehicles could be enriched in ncRNAs and in their role in mediating intercellular communication. Signatures of extracellular vesicular ncRNAs have shown them to be a potential biomarker in Alzheimer's disease. This perspective discusses the potential role of extracellular vehicle ncRNAs in Alzheimer's disease, providing a theoretical basis for extracellular vesicular ncRNAs in Alzheimer's disease, from pathogenesis to diagnosis and treatment.

*Keywords*

Alzheimer's disease; Non-coding RNA; Extracellular vesicle; Exosome

## 1. Introduction

Alzheimer's disease (AD) is an age-dependent neurodegenerative disease with a prevalence rate of 32% in people aged 85 or older, accounting for 60-80% of all dementia cases. AD is characterized by the occurrence of senile plaques (SPs) and neurofibrillary tangles (NFTs), synaptic dysfunction, neuronal death, chronic inflammation and brain atrophy [1]. SPs are composed of amyloid-beta (A$\beta$) oligomers that interfere with neuronal communication at synapses and lead to synaptic dysfunction and neuronal death. In the amyloidogenic pathway, amyloid precursor protein (APP) is cleaved by $\beta$-secretase (BACE1) and $\gamma$-secretase sequentially to release A$\beta$ in the extracellular space. In contrast, in the non-amyloidogenic pathway, APP is cleaved by $\alpha$-secretase to prevent A$\beta$ generation. NFTs are formed by the hyperphosphorylation of microtubule-stabilizing tau protein, which blocks the intracellular transport of essential molecules, leading to destabilization of microtubules. Older age, family history, genetics, and certain lifestyle factors are the main risk factors for late-onset AD [2].

With the advances in next-generation sequencing (NGS) techniques, several novel classes of non-coding RNAs (ncRNAs) have emerged, including microRNAs (miRNAs), circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) [3]. miRNAs are 17$\sim$22 nucleotides in length and suppress target gene expression via binding to the 3′-untranslated region (3′-UTR) of the target gene, leading to mRNA decay or translation inhibition [4]. A miRNA may target several genes, and similarly, a single gene may be regulated by several miRNAs [5]. lncRNAs are a diverse group of ncRNAs of lengths longer than 200 nucleotides, mainly transcribed by RNA polymerase II. lncRNAs can be grouped into five main categories according to their location relative to coding loci: sense, antisense, bi-directional, intergenic, and intronic. lncRNAs can act as epigenetic modulators and can promote or suppress transcription, splice or translate, through four main mechanisms: (1) guiding specific proteins; (2) binding to and inhibiting a protein target; (3) serving as a scaffold; and (4) acting as a cellular signal [6]. The lncRNA BACE1-AS has recently been found to mediate A$\beta$-induced neuronal injury via autophagy regulation in AD [7]. circRNAs are characterized by their covalently closed circular structure and are expressed in tissue-specific and cell-specific manners. circRNAs can function as miRNA or RNA binding protein (RBP) sponges, enhance particular proteins' function, act as protein scaffolds, recruit specific proteins, or serve as templates for translation [8]. Dysregulation of ncRNAs occurs in AD, possibly even in the early stage [9, 10]. Moreover, all three of these types of ncRNAs are involved in AD pathogenesis [11–14]. Several ncRNAs identified in serum or cerebrospinal fluid (CSF) have been regarded as potential biomarkers for AD [15–19]. Dysregulation of ncRNAs in EVs have been identified in both serum and CSF in AD patients [16, 17, 20–22].

Extracellular vehicles (EVs) are heterogeneous membranous structures of endosomal origin circulating in the extracellular space, considered a novel mode of intercellular communication. EVs comprise a diversity of subpopulations distinguished by their size, morphology, composition, biological origin and function. EVs can be broadly divided into microvesicles (MVs) and exosomes. MVs are 50-500 nm in diameter and are secreted directly from the plasma membrane




Journal of Integrative Neuroscience

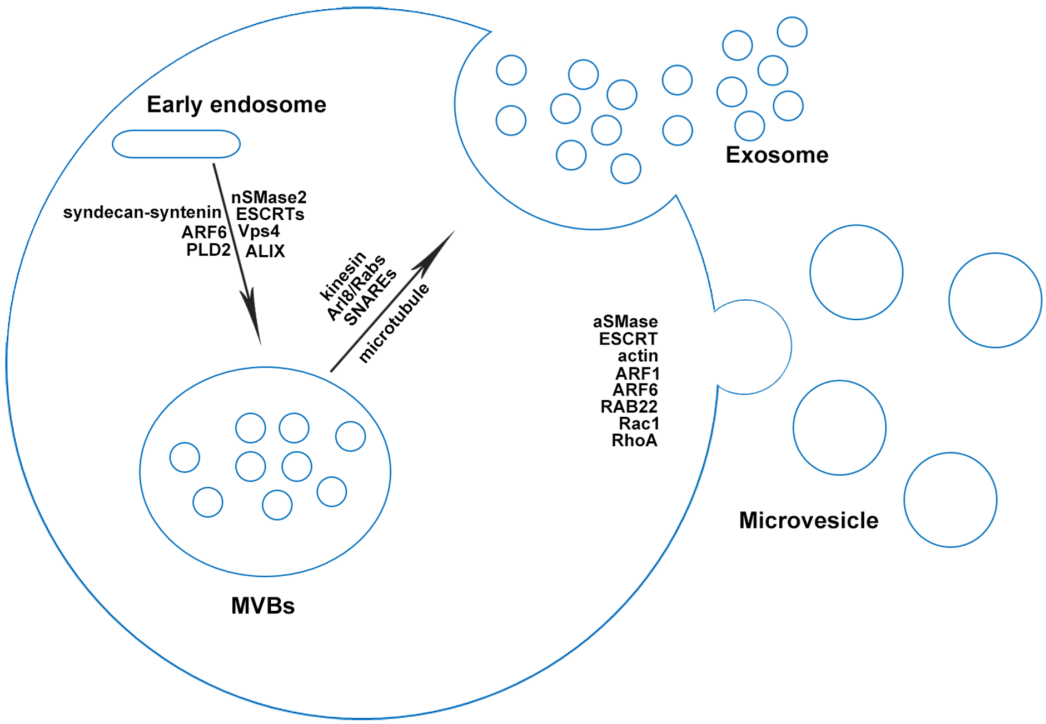


**Fig. 1. Schematic representation of EVs biogenesis and release.** Exosomes are generated within MVBs and transported to the plasma membrane. MVB biogenesis is regulated by nSMas2, ESCRTs, Vps4, ALIX, syndecan-syntenin, ARF6, and PLD2. MVB trafficking, docking and fusion are controlled by kinesins, Arl8, RABs, and SNARE proteins. MVs bud directly from the plasma membrane [26].

by outward budding. Exosomes are 50-150 nm in diameter and are secreted from the plasma membrane through fusion with multivesicular bodies (MVBs) or late endosomes. EVs circulate in various biological fluids and deliver their contents to recipient cells to elicit functional responses. EVs carry specific proteins, lipids or RNA species, which determine their fate and functions in turn. In the brain, several cell types are capable of releasing EVs. EVs derived from microglia, which account for approximately 10% of the brain's cells, are considered part of the inflammatory response. Moreover, oligodendrocytes, neurons, astrocytes, and embryonic neural stem cells have been described to release EVs [23, 24].

## 2. Biogenesis and release of extracellular vesicles

### *2.1 Biogenesis and release of exosomes*

The biogenesis of exosomes starts within the endosomal system. Several cellular steps are needed to release exosomes, including the generation of intraluminal vesicles (ILVs) within MVBs, MVB trafficking along microtubules, and docking and fusion between the plasma membrane and MVBs (Fig. 1). Lipid raft microdomains play a critical role in MVB formation. Neutral sphingomyelinase 2 (nSMase2) mediated generation of ceramide from sphingomyelin hydrolysis induces negative membrane curvature and leads to ILV budding into MVBs [25].

The ESCRT machinery is essential for ubiquitination dependent MVB biogenesis from endosome-derived vesicles. The ESCRT system consists of ESCRT-0 (tumor susceptibility 101, TSG101), ESCRT-I (Signal transducing adapter molecule 1, STAM1), ESCRT-II (Vacuolar protein sorting 25, Vps25), ESCRT-III (Vps20, Vps24, Vps2, and Vacuolar sorting protein, Snf7) and ATPase Vps4 complex. ESCRT-0 and ESCRT-I recognize and retain ubiquitylated transmembrane cargoes on the limiting membrane into MVBs and recruit ESCRT-II/III subcomplexes form a spiral-shaped structure. The ESCRT-III associated ALIX (ALG-2 interacting protein X) affects specific cargo selection [26, 27].

Ubiquitination independent MVB biogenesis has also been extensively described. Syndecan clustering was triggered by heparanase mediated trimming of heparan sulfate chains. Syntenin further binds syndecan to ALIX and participates in exosome formation mediated by ESCRT-III. Selective cargo sorting of CD63 incorporation into exosomes is regulated by the small GTPase ARF6 (ADP ribosylation factor 6) and the effector protein PLD2 (phospholipase D2) [28].

Upon maturation, MVBs can be transported to the plasma membrane along microtubules by multiple kinesin isoforms to secrete exosomes. MVBs transportation, docking, and fusion are regulated by Arl8 (ADP ribosylation factor-like 8), Rabs (RAB7, RAB27, RAB35), and SNARE complexes (YKT6, Syntaxin-1a, Syntaxin-4, Syntaxin-5, synaptotagmin-7, SNAP23, and VAMP7) [29].

**Table 1. Dysregulated extracellular vesicular ncRNAs in AD**

| Source | ncRNA | Change | Ref |
|---|---|---|---|
| plasma EVs | miR-424-5p, miR-3065-5p, miR-93-5p | up | [45] |
| | miR-1306-5p, miR-342-3p, miR-15b-3p | down | |
| plasma EVs | miR-23a-3p, miR-126-3p, let-7i-5p, miR-151a-3p | down | [44] |
| plasma exosome | miR-135a, miR-384 | up | [47] |
| | miR-193b | down | |
| plasma exosome | miR-193b | down | [53] |
| plasma neural exosome | miR-132 | up | [67, 71] |
| | miR-212 | down | |
| CSF exosome | miR-27a-3p, miR-30a-5p, miR-34c | up | [68, 72] |
| CSF exosome | miR-125b-5p | up | [17] |
| | miR-451a, miR-605-5p | down | |
| CSF exosome | miR-193b | down | [53] |
| CSF exosome | lncRNA RP11-462G22.1, lncRNA PCA3 | up | [35] |

### *2.2 Biogenesis and release of microvesicles*

The diameter of MVs are incredibly heterogeneous, ranging from 50 nm to 1,000 nm (up to 10 $\mu$m). MVs are generated through the direct outward budding of the plasma membrane via several distinct mechanisms involved in the biogenesis of exosomes, such as the ESCRT machinery (Fig. 1). Similar to nSMase2, acid sphingomyelinase (aSMase) induces MV production in a ceramide-dependent manner. Another mechanism of MV biogenesis involves non-apoptotic plasma membrane blebs, which expand and retract at the cell surface. These can be released as MVs via actin cytoskeleton and plasmatic membrane rearrangements. Both cargo sorting and MV shedding are tightly controlled by several small GTPases, including ARF1, ARF62, RAB22, Rac1 (Rac family small GTPase 1), and RhoA [26].

## 3. Dysregulated extracellular vesicular ncRNAs in AD

We searched studies on the PubMed database using the following keywords: extracellular Vesicle, EV, exosome, microvesicle, MVB, circular RNA, circRNA, Alzheimer's disease, and AD. EVs mediate horizontal transfer of RNA between donor and recipient cells, as first identified by Valadi *et al.* [30] and Skog *et al.* [31]. ncRNAs are highly enriched in EVs. Pegtel *et al.* [32] reported the exosome-mediated miRNA transfer from Epstein-Barr virus-infected cells to uninfected recipient dendritic cells. These transferred miRNAs can regulate the gene expression of recipient cells [32]. RNA sequencing of EVs has revealed abundant lncRNA and circRNA in human blood [33]. Dysregulation of extracellular vesicular ncRNA has been identified in several neurodegenerative disorders, including AD. Two lncRNAs, PCA3 and RP11-462G22.1, were increased in Parkinson's disease (PD) leukocytes [34]. Similarly, Gui *et al.* [35] found that these two lncRNAs were also elevated in CSF exosomes in AD and PD. Known dysregulated miRNAs and lncRNAs verified by RT-PCR from serum EVs, serum exosomes, and CSF exosomes are summarized in Table 1.

## 4. Role of extracellular vesicular ncRNAs in AD

Extracellular vesicular ncRNAs are shuttled between donor and recipient cells and function actively in recipient cells, suggesting a novel mechanism of intercellular communication [36]. Since the first discovery of EVs in AD physiopathology, their multifaceted roles in this setting have been explored [37], including their role in mediating neuroinflammation [38]. Exosomal miRNAs occurring in the blood have been investigated, and those from the central nervous system (CNS), including neurons, astrocytes, and CSF. They are considered promising diagnostic biomarkers in AD, as detected by RT-PCR or deep sequencing [20, 39–41]. Moreover, exosomes derived from the CNS have also been isolated in the blood (termed plasma-derived neural exosomes), furthering their appeal as target biomarkers in AD [42].

EV miRNAs may be potential biomarkers for the differential diagnosis of AD (Fig. 2). Lugli *et al.* [40] applied NGS to investigate the differently expressed serum exosomal miRNAs in AD relative to controls, identifying 20 miRNAs. miR-342-3p was highlighted particularly, given that its downregulation has also been reported in previous studies. Cheng *et al.* [43] explored serum exosomal miRNA expression in AD from the AIBL cohort and identified 17 dysregulated serum exosomal miRNAs. Both [40] and [43] support the potential biomarker capability of miR-342-3p. Another two miRNAs, miR-21-5p and miR-451a, were found to be decreased in plasma EVs in AD relative to those in dementia with Lewy bodies (DLB), with area under curve (AUC) values of 0.93 and 0.95, respectively, suggesting these could be potential biomarkers to discriminate these diseases [44]. Li *et al.* [45] examined the expression of 18 miRNAs in plasma EVs in vascular dementia (VD), AD, and mild cognitive impairment (MCI). They found that among the three miRNAs found to be decreased in AD compared to healthy control, only miR-1306-5p was differentially expressed between AD, MCI, and VD.

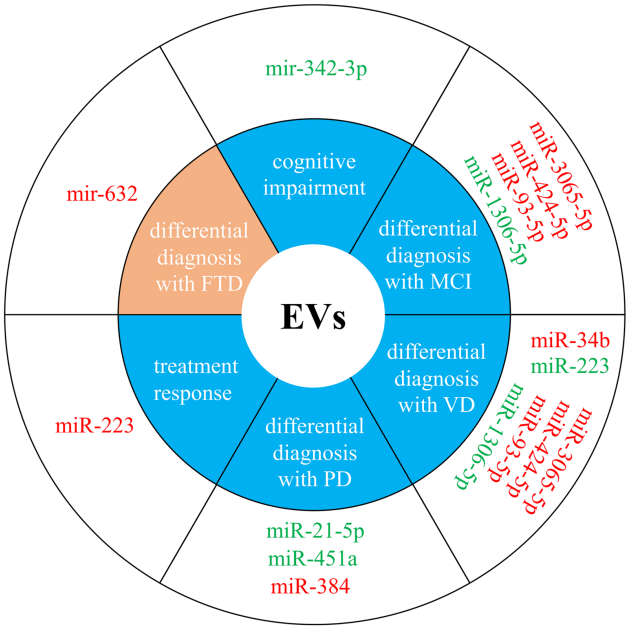


**Fig. 2. Role of EV miRNAs in the differential diagnosis of AD.** Blue color represents serum EVs, and orange color represents CSF EVs. EV miRNAs in red color were upregulated in AD, while EV miRNAs in green were downregulated in AD.

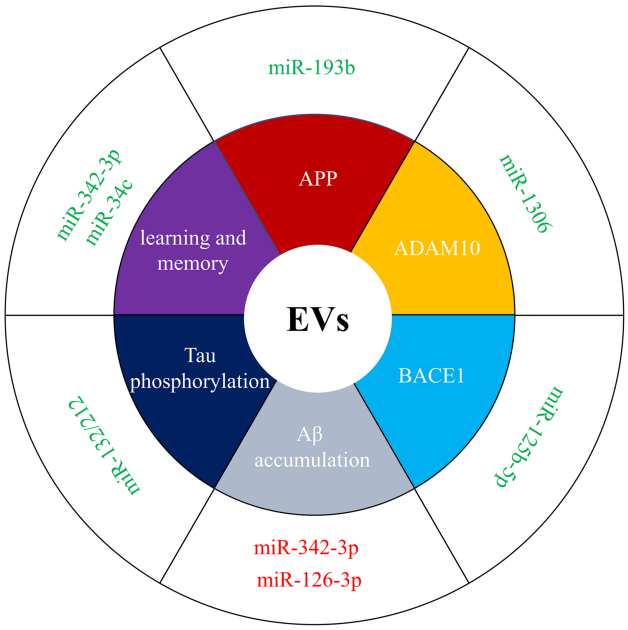


**Fig. 3. Role of EV miRNAs in the pathogenesis of AD.** EV miRNAs in red color were promotive, while EV miRNAs in green color were suppressive for the complementary aspects.

Moreover, upregulation of miR-424-5p, miR-93-5p, and miR-3065-5p might predict AD over other forms of dementia and healthy control [45]. Barbagallo *et al.* [46] found that miR-34b in serum exosomes was higher in AD than VD. Yang *et al.* examined the expression of miR-193b, miR-135a, and miR-384 in plasma exosomes from MCI, AD, PD, and VD patients, finding that miR-384 may be the best miRNA discriminating AD, PD, and VD [47]. Wei *et al.* [48] examined three miRNAs in plasma exosomes from dementia and controls, finding that miR-223 in AD was lower than in VD. Moreover, the miR-223 in untreated AD patients was significantly lower than those who had already received medical care. Schneider *et al.* [49] examined the expression of 752 miRNAs in CSF exosomes in the GENFI AD cohort and sporadic frontotemporal dementia (FTD). mir-632 was significantly increased in AD compared with sporadic FTD, with an AUC value of 0.88.

Dysregulated EV ncRNAs have been linked to AD pathogenesis (Fig. 3). miR-15b-3p, miR-342-3p, and miR-1306-5p from plasma EVs are decreased in AD patients [43]. miR-1306 suppresses the expression of $\alpha$-secretase ADAM10 in SH-SY5Y cells [50]. Deregulation of miR-342-3p reduces A$\beta$ plaques and ameliorates learning and memory deficit in AD [51]. miR-126-3p, which is decreased in AD in plasma EVs, targets TOM1 (target of myb1 membrane trafficking protein) and regulates neuronal accumulation of A$\beta$ oligomers [52]. miR-193b was found to inhibit mRNA and protein expression of APP [53]. Inhibition of miR-132/212 impairs S-nitrosylation balance and induces NOS1-dependent tau phosphorylation in AD [54]. MiR-34c, which is increased in AD in CSF exosomes, induces synaptic impairment by targeting Synaptotagmin 1 via the ROS-JNK-p53 pathway in AD [55]. miR-125b-5p ameliorates A$\beta$-induced neurotoxicity by targeting BACE1 [56]. Given these findings, changes in ncRNA levels associated with AD may give rise to the various phenomena witnessed in the disease course, such as neuronal death, synaptic impairment, and inflammation.

## 5. The therapeutical potential of EV ncRNA for AD

The blood-brain barrier comprises specialized endothelial cells that interface with astrocytes and pericytes to keep an optimal environment for neuronal function by supplying nutrients and other metabolic requirements while eliminating toxic substances. The blood-brain barrier makes the delivery of therapeutics to the CNS challenging, however. Efficient delivery of drugs to the CNS is limited to lipophilic compounds of no more than 400 Da [57]. Rabies virus glycoprotein (RVG) can target the brain specifically, as demonstrated in previous studies in which RVG was engineered to localize at the surface of EVs by fused protein RVG-Lamp2b (lysosome-associated membrane glycoprotein 2b) [58]. Yang *et al.* [59] co-transfected RVG-Lamp2b and circSCMH1 overexpressing plasmids into HEK293T cells to collect EVs containing circSCMH1. These collected EVs were labeled with Dil and injected into mice via the tail vein. In the brain, the $Dil^+$ particles were observed in neurons, astrocytes, and microglial cells. EV delivery of circSCMH1 resulted in improved brain plasticity after stroke in monkeys. This study implies that engineered EVs may have therapeutic potential in delivering ncRNAs in neurological disorders [59]. So far,

attempts to design drugs to target A$\beta$ or tau have not been decisive. Mesenchymal stem cell (MSC) derived EVs were considered an alternative AD treatment approach [60–62]. Cui *et al.* [63] found that exosomes from hypoxia-preconditioned MSCs (PC-MSCs) could rescue cognition and memory impairment of APP/PS1 mice via the regulation of inflammatory responses and the restoration of synaptic dysfunction through increasing miR-21 level. Moreover, they also used RVG to target MSC derived exosomes, delivering exosomes into APP/PS1 mice's brain for AD treatment [64].

## 6. Conclusions and perspectives

There has been an exponential increase in studies of the roles of EVs and extracellular vesicular ncRNAs in the pathogenesis of AD and their biomarker potential. Extracellular vesicular ncRNAs appear to be attractive novel biomarkers for diagnosing and discriminating AD, VD, and MCI. Biomarkers based on serum EV ncRNA deserve further investigation. Recent studies investigating EV ncRNAs mainly focused on miRNAs. The roles of EV related lncRNA and circRNA are as yet rarely explored.

Some challenges remain, however. Microglial EVs play a beneficial role in the early stage of AD while having a detrimental action in the later stages [65, 69]. The detailed roles of EVs from different sources and at different stages of AD are still unknown. Moreover, the different sorting mechanisms of MVB biogenesis determine the incorporation of specific cargo, but the detailed mechanisms involved in the selective sorting of ncRNAs remain unclear. Riancho *et al.* [66] compared miRNA levels in exosome-enriched CSF fractions with miRNAs in raw CSF samples, finding that miR-598 and miR-9-5p were shifted from raw CSF to exosome-enriched CSF fractions in AD, indicating that the changes of exosomal miRNAs may be caused by altered exosome trafficking. [70] circRNAs from EVs in AD has not yet been reported, while miRNAs have been widely studied. Exosome-mediated delivery of ncRNAs for the treatment of AD also deserves further investigation. Further studies may improve our understanding of the role of EVs and extracellular vesicular ncRNAs in both the etiology and progression of AD.

## Abbreviations

3′-UTR, 3′-untranslated region; A$\beta$, amyloid-beta; AD, Alzheimer's disease; ALIX, ALG-2 interacting protein X; APP, amyloid precursor protein; ARF6, ADP ribosylation factor 6; Arl8, ADP ribosylation factor-like 8; AUC, area under curve; circRNA, circular RNA; CNS, central nervous system; CSF, cerebrospinal fluid; DLB, Lewy body; EV, extracellular vehicle; FTD, frontotemporal dementia; ILV, intraluminal vesicle; Lamp2b, lysosome-associated membrane glycoprotein 2b; lncRNA, long non-coding RNA; MCI, mild cognitive impairment; miRNA, microRNA; MSC, Mesenchymal stem cell; MV, microvesicle; MVB, multivesicular body; ncRNA, non-coding RNA; NFT, neurofibrillary tangle; NGS, next-generation sequencing; nSMase2, neutral sphingomyelinase 2; PD, Parkinson's disease; Rac1, Rac family small GTPase 1; RBP, RNA binding protein; RVG, Rabies virus glycoprotein; SP, senile plaque; STAM1, Signal transducing adapter molecule 1; VD, vascular dementia.

## Author contributions

X.W. and Y.H. conceived and designed the study; Y.X. and M.C. collected data; X.W. wrote the paper.

## Ethics approval and consent to participate

Not applicable.


## Acknowledgment

We thank two/three anonymous reviewers for excellent criticism of the article.

## Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 81801069 to Yu Hu and No. 81500925 to Xiong Wang).


## Conflict of interest

The authors declare no conflict of interest.

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