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Review

Do Amyloid β-associated Factors Co-deposit with Aβ in Mouse Models for Alzheimer's Disease?

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Abstract. Senile plaques and cerebral amyloid angiopathy in Alzheimer's disease (AD) patients not only consist of the amyloid-β protein (Aβ), but also contain many different Aβ-associated factors, such as heparan sulfate proteoglycans, apolipoproteins, and complement factors. These factors may all influence Aβ deposition, aggregation, and clearance and therefore seem important in the development of human Aβ deposits. To study AD pathology and test new therapeutic agents, many different mouse models have been created. By transgenic expression of the amyloid-β protein precursor, frequently in combination with other transgenes, these animals develop Aβ deposits that morphologically resemble their human counterparts. Whether this resemblance also applies to the presence of Aβ-associated factors is largely unclear. In this review, the co-deposition of factors known to associate with human Aβ deposits is summarized for several different AD mouse models.

Keywords: Acute-phase proteins, Alzheimer's disease, amyloid-β, apolipoprotein E, complement, heparan sulfate proteoglycans, transgenic mice

INTRODUCTION

To investigate disease mechanisms and test new therapeutic agents, animal models are necessary tools. Even though the use of animals is ethically controversial, there are no other models available that are capable of reproducing the complex nature of human physiology. However, mimicking disease is not easy in animal models, since the biological pathways in animals are often not identical to those in humans. Therefore, most models are created as transgenics, expressing (mutated) human proteins implicated in human disease. But even in transgenic models, it remains difficult to accurately model symptoms and pathology of a human disease.

Alzheimer's disease (AD) is pathologically characterized by accumulation of the amyloid-β (Aβ) protein in senile plaques and cerebral amyloid angiopathy (CAA) [1,2] and by accumulation of hyperphosphorylated tau protein [3]. One of the earliest brain regions affected is the hippocampus, a brain region involved in memory formation. Indeed, memory impairment is one of the main symptoms of AD [4,5]. The importance of Aβ in the pathogenesis of AD, has been emphasized by the discovery of multiple causative muta-

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tions in Aβ-related genes (amyloid-β protein precursor (AβPP) and presenilin genes) in familial AD [6].

Aβ is a cleavage product of AβPP and generally comprises either 40 ($A\beta_{40}$) or 42 ($A\beta_{42}$) amino acids. AβPP is a transmembrane protein that can be cleaved by several different secretases to release Aβ and a number of other cleavage products [7–9]. In the case of Aβ, AβPP is first cleaved by a γ-secretase [10] and then by a β-secretase [11,12]. Many of the mutations in AβPP that cause the familial forms of AD are found close to these AβPP cleavage sites [6]. Aβ is normally cleared from the brain, but when this clearance process becomes impaired, for example due to aging, Aβ can start to oligomerize and eventually form fibrils. This fibrillization can then result in the formation of Aβ deposits throughout the brain. Besides mutations in AβPP, pathogenic mutations have also been found in presenilin-1 (PS1) and, to a lesser extent, presenilin-2 [6,13]. Both these presenilins are part of the γ-secretase complex responsible for Aβ cleavage from AβPP [10,14]. These mutations increase Aβ production, in particular of the more fibrillogenic $A\beta_{42}$ variant [15].

In more than 99% of AD cases, aging is the most important risk factor to develop AD, whereas in less than 1% of cases the disease can be related to gene mutations. However, there are only a few animal species, including dogs and primates, which naturally develop AD-like pathology with advanced age [16]. To create models for AD with a faster development of Aβ pathology, transgenic (Tg) mouse models have been generated based on the introduction of human (mutated) AβPP, either alone or in combination with (mutated) presenilin genes [17]. Mice are usually the animals of choice for creating transgenics, since they are not only very susceptible to genetic manipulation, but are also relatively easy and cheap to maintain.

The first AD mouse model that was developed was the PDAβPP model [18]. In this model, starting at an age of 6–9 months immunohistochemically detectable Aβ deposits developed, that become more dense upon aging and finally morphologically resemble Aβ deposits found in humans. Furthermore, as seen in humans, the Aβ deposits in the PDAβPP mice are surrounded by activated astrocytes and microglia and accompanied by a loss of synaptic density. PDAβPP mice also develop cognitive deficits including memory impairment [19], further demonstrating their similarity to human AD patients.

Many more AD mouse models with Aβ pathology have been created since this first model [17], all characterized by deposition of plaque-like Aβ and most of them developing cognitive impairment [17]. Due to the different gene combinations and mutations used to create these mice, there are, however, many differences between models. For example, the age of pathology onset in AD models varies greatly. In some mice, such as the TgCRND8 model, deposition starts at an age of 3–6 months [20], while in others, such as the Tg2576 model, it starts at an age of 9–12 months [21]. In general, co-expression of PS1 lowers the age of onset [22], because AβPP in these mice is more readily cleaved by the γ-secretase [23]. Plaque morphology can also vary greatly between different models. For example, AβPP23 mice accumulate mostly compact deposits [24], while parenchymal Aβ deposits in TgSwDI mice are mostly diffuse [25]. Contrary to AβPP23 mice, which develop CAA next to parenchymal deposits [26], TgSwDI mice, but also AβPPDutch mice, accumulate Aβ mainly in the brain vasculature with limited parenchymal deposition [25,27]. This characteristic makes these latter models suitable models for familial and sporadic cases of vascular amyloid pathology. AβPP and AβPP/PS1 models do not develop tau pathology, but tau transgenic mice have been developed [28,29]. There is also a triple transgenic mouse model available, overexpressing tau, AβPP, and PS1 [30].

Even though most AD mouse models demonstrate Aβ pathology that morphologically resembles human AD pathology, the Aβ deposited in mice is chemically different from human Aβ. In humans, Aβ species undergo posttranslational modifications, such as N-terminal degradation, cross-linking, and isomerization. These modifications are either not found in AD mouse models or differ from the human situation [31–33]. Furthermore, Aβ deposits in mice are usually less compact than in humans, allowing mild extraction buffers to more easily extract Aβ from mouse brains relative to human brains. Finally, progressive AD pathology in humans is characterized by neurodegeneration, a characteristic that is rarely reproduced in AD mouse models [17]. Only the AβPP23 mouse model has been shown to have some neuronal loss in the CA1 region of the hippocampus [34].

In humans, Aβ deposits not only contain the Aβ protein, but immunohistochemical analyses also demonstrated many other proteins [35–37]. It is thought that because of their close association, these co-deposited molecules contribute to Aβ aggregation and deposition. Although this has not been proven for all co-depositing proteins, *in vitro* studies showed that heparan sulfate proteoglycans (HSPG), apolipoprotein E (ApoE) and

α1 chymotrypsin (α1-ACT), can indeed bind to Aβ, affect its aggregation, stabilize Aβ deposits and protect deposits against proteolytic degradation [38–40]. Because of their tight association with Aβ, these (glyco-) proteins may greatly influence the outcome of therapeutic intervention in humans, aimed at reducing Aβ aggregation and deposition. Since all new potential AD therapeutics are first tested in mouse models, it is important to know if these Aβ-associated factors are present in the various mouse models. In this review, we aim to examine the validity of AD mouse models with respect to the presence of the main Aβ co-depositing factors HSPG, ApoE, complement factors, acute-phase proteins, intercellular adhesion molecule (ICAM)-1, cystatin C, and collagenous Alzheimer amyloid plaque component (CLAC). An overview of the co-deposition of these factors in transgenic AD models is shown in Table 1 and discussed below.

Heparan sulfate proteoglycans

More than twenty years ago, close association of HSPG with Aβ deposits in humans, both parenchymal and vascular, was first described [41]. HSPG consist of a protein core with several highly sulfated glycosaminoglycan chains attached. These glycosaminoglycan chains consist of repeating disaccharide units. There are several different HSPG species, some membrane-associated (glypicans and syndecans) and some associated with the extracellular matrix (agrin, collagen XVIII, and perlecan). HSPG are thought to be involved in numerous (developmental) processes, including neurogenesis, angiogenesis, and blood brain barrier permeability [39]. Of the different HSPG species, agrin, and glypican-1 are the only two HSPG found in association with all different types of Aβ deposits [42–44]. The association of perlecan with Aβ deposits is controversial, as one study described this HSPG to have the strongest association [41], whereas we have been unable to detect perlecan in CAA or senile plaques [42,44]. Overall though, HSPG are key components of human Aβ deposits. Indeed, *in vitro* analysis has demonstrated that HSPG can bind Aβ with high affinity, mainly through their glycosaminoglycan chains [45]. Through this binding, HSPG can enhance Aβ deposition [39,43,46], a process that seems to involve HSPG sulfate moieties [47].

There are only a few studies describing co-deposition of HSPG with Aβ deposits in mouse models. In a characterization study of AβPP23 mice, it is very briefly mentioned that HSPG co-localize with Aβ deposits [48]. A more elaborate study was done using 20-month-old Tg2576 mice [49]. It was demonstrated that antibodies against heparan sulfate stained more than 95% of the "doughnut"-shaped Aβ deposits visible in this model. Whereas in the AβPP23 mice no distinction was made between individual HSPG species, in the Tg2576 mice it was discovered that glypican-1 and syndecan-3, but not agrin, perlecan, syndecan-1 and -2, were found in the Aβ deposits. Therefore, in this mouse model the extensive co-deposition of HSPG seen in humans could only partly be reproduced. For example, agrin, a HSPG that is abundantly present in AD senile plaques, was absent from Aβ deposits in mice, demonstrating that the association of individual HSPG species with Aβ pathology may not always match the human situation.

We recently studied deposition of several HSPG species in the AβPPswe/PS1dE9 mouse model [50]. In this model, in general HSPG were associated with approximately 30% of Aβ deposits. Furthermore, co-localization of the different HSPG species (agrin, glypican-1, and perlecan) occurred in less than 10% of Aβ deposits for each of the species. Therefore, HSPG co-deposition was much less pronounced in this model than in the Tg2576 model or in humans. Due to a low number of detectable vascular deposits, co-deposition of HSPG with CAA vessels could not be determined in this model, nor was it investigated in the Tg2576 model.

Overall, the mouse models seem to differ from human AD pathology in either the type of HSPG species that co-deposit or the number of plaques in which HSPG co-deposition is observed. As previously hypothesized [51], it may be that the lack of glycoproteins, like HSPG, in Aβ deposits in mice is the reason that these deposits are generally less compact and more easily dissolved. It is important to note that HSPGs are an invariant component of all known human amyloidoses, both cerebral and in peripheral organs, pointing to a very important role in amyloidosis in general [52], a property that is apparently partly lacking in Tg mouse models for AD. As far as we know, co-deposition of HSPG has not been studied in mouse models for other types of amyloidosis.

Apolipoprotein E

ApoE is the most prominent apolipoprotein in the central nervous system and mainly produced by astrocytes [53], although other cell types such as pericytes and smooth muscle cells also contribute to cerebral lev-

els [54,55]. ApoE binds to lipoproteins and mediates their interaction with lipoprotein receptors and endocytosis and in this manner regulates cholesterol homeostasis [56]. There are three isoforms of ApoE (ApoE2, 3, and 4), and the ApoE4 isoform is a well-known risk factor for AD [57], whereas the ApoE2 isoform seems to be protective for the development of AD. The exact role of ApoE in AD, however, is still elusive [58]. *In vitro* studies have demonstrated a direct interaction between Aβ and ApoE [57] and it is suggested that through this interaction ApoE can influence Aβ aggregation and mediate Aβ clearance [38,40,58].

The fact that ApoE can bind Aβ *in vitro*, suggests an interaction of these two proteins *in vivo*. Indeed, clear co-deposition of ApoE with human Aβ deposits has been shown. ApoE immunoreactivity is observed in all senile plaques in AD brains, including in diffuse plaques and in the core of amyloid plaques [35,59–61], although some authors described the co-deposition with diffuse deposits to be minor [62]. In these studies, ApoE was also found in CAA vessels [35] and in dystrophic neurites surrounding plaques.

Co-deposition of ApoE with Aβ deposits in mouse models has been well studied and in several models ApoE was found co-localized with Aβ. Immunohistochemical analysis of deposits in Tg2576 [63, 64], AβPP23 [48,65], PS/AβPP [66], and AβPP-YAC mice [67] revealed staining for ApoE mostly in (Thioflavin-S positive) fibrillar deposits, with staining of some diffuse deposits. Furthermore, ApoE colocalized with astrocyte markers [64], demonstrating that in mice, as in humans, astrocytes are likely responsible for ApoE production. In the TgSwDI mouse model, that specifically deposits Aβ in the brain vasculature, ApoE is found in close association with these vascular deposits [33]. All these findings in mice are therefore in concurrence with the ApoE co-deposition found in humans.

Based on studies using brain material of Down syndrome patients, it was suggested that ApoE contributes to plaque maturation [68]. In these patients, $A\beta_{42}$ was the first Aβ species to accumulate and ApoE colocalization could be detected in these deposits before $A\beta_{40}$ accumulates. In the Tg2576 model [64], $A\beta_{42}$ also seems to be the initial Aβ species that deposits, with $A\beta_{40}$ only visible in more mature deposits. Using the Tg2576 model, it was found that all Aβ deposits positive for ApoE contained $A\beta_{42}$, with only some containing $A\beta_{40}$. Therefore, since ApoE co-deposition in mice resembles that in humans, this suggests that ApoE has a similar role in plaque maturation in mice as it has in humans. The function of ApoE in Aβ deposition has also been investigated using ApoE knockout mouse models. By crossing these knockout mice with AD mouse models [69,70], it was discovered that ApoE expression is key in developing Aβ deposition.

Recently, an increased risk for AD was linked to another apolipoprotein, ApoJ [71,72]. Indeed, this apolipoprotein, but also ApoD, is known to co-deposit with Aβ [60,73] and furthermore ApoJ could decrease Aβ aggregation *in vitro* [74] and *in vivo* [75]. Co-deposition of ApoD has not been studied in AD mouse models. In contrast, in the AβPP-YAC mouse model [67] co-deposition of ApoJ with Aβ has been described. Therefore, in general, it appears that co-deposition of apolipoproteins with Aβ is well replicated in Tg mouse models for AD.

Complement factors

The complement system consists of a cascade of factors that can become activated as part of the innate immune response of the body. Although the liver is the main source of complement, brain glial cells can also produce complement factors [76]. The cascade is triggered when either factor C1q (classical pathway), C3 (alternative pathway), or lectins become activated. Ultimately, a membrane attack complex (MAC), consisting of factor C5b-9, is formed that lyses cells by forming a membrane pore. Besides foreign intruders, the Aβ protein is also known to trigger the complement system by binding to C1q or C3 [77,78]. Indeed, in AD brain, expression of the complement system is upregulated [79] and recently a complement component receptor (CR1) was identified as a risk factor for AD [72]. Furthermore, several components of the complement system, including C1q, C3, and C5b-9, can be found clearly associated with Aβ deposits [80] and then mostly with fibrillar plaques [81]. Activation of the complement system in turn can stimulate Aβ aggregation [82]. Although the activated complement system can accelerate (Aβ-induced) neurodegeneration [83], there is also evidence that it can protect the brain from Aβ-induced damage [84].

Co-deposition of complement factors in mouse models has been well-studied. In two studies, C1q, C3, and C4 co-deposition in the Tg2576 model was investigated [85,86]. Strong co-deposition of these three complement factors was found with (Thioflavin-S positive) Aβ deposits. C1q was also strongly expressed in Aβ deposits of PS/AβPP mice [87] and with the vascular deposits of TgSwDI mice [88]. In AβPP23 mice, not

only was co-deposition of C1q studied, but also that of many more complement factors (C3, C3d, C4, C4d, C7, C9) [65,89]. With the exception of C1q, C3, and C3d, co-deposition of these factors was weak, with co-deposition of complement factors further down in the cascade (C7, C9) being almost absent.

In summary, it appears that, unlike in human Aβ deposits, only the early components of the complement cascade (C1q, C3) can be detected in Aβ deposits of Tg mouse models. Although these early complement factors co-deposit with Aβ in mice, functionally they differ from their human equivalents. For example, it is known that mouse C1q does not bind human Aβ as efficiently as human C1q does [90] and that mouse C4 cannot activate C5 convertase [91]. Consequently, subsequent activation of the complement system in mice is also less efficient. Since complement can induce neurodegeneration [83], the less efficient activation of this system may explain the relatively low degree of neurodegeneration seen in mouse models [17]. Furthermore, the difference in complement co-deposition and activation between humans and mice may also provide some explanation for the different results that have been found in immunization studies in humans and mice aimed at finding new therapeutic agents [92,93]. Despite the incomplete activation of the complement pathway in mice, it may still play a critical role in Aβ pathology in mouse models as was demonstrated by a reduction of inflammation and neurodegeneration in an AD mouse model crossed with complement knockout mice [85].

Acute-phase proteins

In AD brains, several acute-phase proteins co-deposit with Aβ, such as serum amyloid P (SAP), α2-macroglobulin (α2M), and α1-ACT [35,94]. Acute-phase proteins are proteins that become acutely upregulated in plasma in response to inflammation.

SAP is a glycoprotein that is closely associated with all Aβ deposits in humans [35,95]. The proposed role for SAP in senile plaques is to protect Aβ fibrils from proteolysis [96]. Besides, SAP may activate the complement system by binding C1q [97]. Only two studies on SAP co-deposition in mouse models have been performed. In one AD mouse model (C57B6/SJL overexpressing AβPP), SAP did not co-localize with amyloid deposits [98,99]. It was postulated that SAP, with a molecular weight of ∼250 kDa, failed to readily pass the blood brain barrier (BBB). Only when SAP was administered intranasally to transgenic AβPP mice, could SAP be detected in association with Aβ deposits [99]. Since in humans, SAP can be detected in the AD brain and its production is exclusive to the liver, it was suggested that the integrity of the human BBB must be disturbed. In the AβPP23 model [65], SAP was also not detected in Aβ deposits, although staining in the periphery of deposits was visible. Therefore, functional disturbance of vessels of AβPP23 mice was apparently also not severe enough to allow BBB crossing of SAP into the brain [100]. The reduced transport of complement activator SAP into the brain may in turn contribute to the relative lack of complement activation in AD mouse models [65].

α2M, a protease inhibitor, co-localized with senile plaques in humans [101] and is thought to prevent accumulation of Aβ [102]. In a study aimed at investigating the role of α2M in AD, the co-localization of this protein in the PS/AβPP mouse model was characterized [103]. Starting from 3 months of age, these mice demonstrated Aβ deposition, with some deposits positive for α2M. The number of α2M -positive plaques then increased with age, with α2M mainly depositing in Thioflavin-S positive fibrillar senile plaques. This demonstrates that α2M co-deposition resembles the human situation.

Finally, the acute-phase protein α1-ACT is a serine protease inhibitor that co-deposits with human Aβ deposits [94]. Whereas some describe α1-ACT to enhance Aβ aggregation [104], others demonstrated an inhibition of fibril formation [38], possibly reflecting different effects of α1-ACT depending on the molar ratio between α1-ACT and Aβ [105]. However, as mice do not possess an α1-ACT homologue [106], co-deposition of this acute-phase protein in AD mouse models is not to be expected. Only by creating double transgenic mice for both human α1-ACT and AβPP, it was possible to study the *in vivo* role of α1-ACT on Aβ aggregation [106]. Thus, it was demonstrated that α1-ACT increased Aβ levels and plaque load in these mice.

Thus, co-deposition of acute-phase proteins has been studied in a few mouse models only and in these models co-deposition with Aβ was restricted to α2M.

ICAM-1, cystatin C, and CLAC

Besides the above mentioned factors, there are several more proteins that co-deposit with human Aβ, including ICAM-1, the cysteine protease inhibitor cystatin C, and CLAC. ICAM-1 is closely associated with Aβ deposits in human AD brains, where it can be found

Table 1
Association of proteins that co-deposit with senile plaques in AD with Aβ deposits in different mouse models for AD

Co-depositing factor	AD mouse models						Reference
	Tg2576	PS/AβPP	AβPP23[a]	TgSwDI[b]	AβPPswe/PS1dE9	AβPP-YAC	
Heparan sulfate proteoglycans			+				[48–50]
HS GAG	+				±		
Perlecan[c]	−				±		
Glypican-1	+				±		
Agrin	−						
Syndecans	±						
ApoE	+	+	+	+		+	[33,63–67]
ApoJ						+	
Complement							[65,85–89]
C1q	+	+	±/+	+			
C3	+		±/+	+			
C3d			±/+				
C4			−				
C4d	+		−	+			
C7			−				
C9			−/±				
Acute phase proteins[d]							[65,103]
Serum amyloid P			−				
α2-macroglobulin		+					
ICAM-1	+						[109]
Cystatin-c	+						[110]
CLAC		+[e]					[116]

(− no co-deposition; ± weak co-deposition; + strong codeposition).
[a]only compact Aβ deposits visible; [b]co-deposition with vascular Aβ; [c]expression in senile plaques in AD is controversial; [d]mice do not possess an α1-antichymotrypsin homologue [106]; [e]co-deposition with Aβ40- and Thioflavin S-negative plaques.

in both classic and diffuse senile plaques in the cerebrum [107] and, specifically, in classic deposits in the cerebellum [108]. Similarly, co-deposition of ICAM-1 has been described in Tg2576 mice [109], although its expression is restricted to Thioflavin-S positive deposits.

Cystatin C has been found in both vascular and parenchymal Aβ deposits in AD [110]. On its own, cystatin C can form vascular amyloid deposits, as demonstrated in Icelandic patients suffering from hereditary cerebral hemorrhage with amyloidosis (HCHWA) [111]. Immunohistochemical analysis of 2-year old Tg2576 mice revealed that, similar to the human situation, cystatin C was detected in Aβ deposits of this model [110]. Other AD mouse models have not yet been investigated for the expression of cystatin C, but the role of cystatin C in Aβ deposition has been studied by creating AD mouse models that overexpress human cystatin C. In these double transgenic mice, cystatin C reduced Aβ deposition [112,113]. However, a reduction was also found when cystatin C was ablated [114].

By raising antibodies against extracted amyloid deposits, the co-deposition of CLAC with Aβ was discovered [115]. A subsequent *in vitro* study demonstrated that CLAC can bind Aβ, but only when Aβ is aggregated [115], making CLAC seemingly more selective in its binding than co-depositing proteins such as HSPG and ApoE. In brain material of AD patients CLAC was found co-deposited with $A\beta_{42}$-positive, but not with $A\beta_{40}$- and Thioflavin S-positive plaques [116]. In the same study, a similar co-deposition of CLAC was found in PS/AβPP mice and it was suggested that CLAC co-deposited with Aβ in more AD mouse models (unpublished data).

CONCLUSION

Some proteins known to co-deposit with human Aβ also strongly associate with Aβ in mice, with ApoE being the most prominent. However, there are also many factors that only partly co-deposit with Aβ in mice or that do not co-deposit at all, in contrast to the situation in AD brains (Table 1). For example, HSPG can be detected in Aβ deposits in mice, but their expression in Tg mouse brains is much more restricted than in humans. Since HSPG are known to stimulate Aβ aggregation and stabilize Aβ deposits, it seems likely that their limited association with Aβ deposits in mice is one of the reasons that these deposits are less compact

and easier to dissolve than their human equivalents. Of the complement factors, only the early factors of the cascade co-deposit with Aβ in mice, resulting in a less efficient activation of the complement system and the lack of formation of the MAC. The absence of a robust complement activation, in turn, may explain the relative absence of neurodegeneration in mouse models for AD. In conclusion, although several transgenic mouse models are far from extensively studied for the association of Aβ-associated proteins with plaques, it appears that the composition of Aβ deposits in transgenic mice is markedly different from human Aβ deposits.

The less pronounced association of the above-mentioned factors may be a consequence of the rapid development of AD pathology in mouse models as compared to human AD patients. Indeed in transgenic mice individual plaques can form within weeks [117] or even within 24 h [118], therefore, there is probably simply not enough time to allow co-deposition of these Aβ-associated proteins in the relatively constrained time period it takes for Aβ to accumulate in mice. The incomplete replication of the expression of Aβ-associated factors in mice and thus, the molecular composition of Aβ deposits in mice, may in turn imply that the results of Aβ-targeted therapeutics will likely be different in mice than in men. Indeed, there are many discrepancies in the outcomes of therapeutic interventions in mice and humans, with many human trials demonstrating side-effects not seen in mice [92]. Therefore, when using AD mouse models to study Aβ deposition or the effects of therapeutic agents on Aβ deposits, it is necessary to consider the differences in Aβ deposit composition between mice and humans when translating findings in mouse models to the human situation.

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