GAP-43 in synaptic plasticity: molecular perspectives

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Abstract: The growth-associated protein, GAP-43 (also known as F1, neuromodulin, B-50), participates in the developmental regulation of axonal growth and neural network formation via protein kinase C-mediated regulation of cytoskeletal elements. Transgenic overexpression of GAP-43 can result in the formation of new synapses, neurite outgrowth, and synaptogenesis after injury. In a number of adult mammalian species, GAP-43 has been implicated in the regulation of synaptic transmission and plasticity, such as long-term potentiation, drug sensitization, and changes in memory processes. This review examines the molecular and biochemical attributes of GAP-43, its distribution in the central nervous system, subcellular localization, role in neurite outgrowth and development, and functions related to plasticity, such as those occurring during long-term potentiation, memory formation, and drug sensitization.

Keywords: GAP-43, protein kinase C, axons, development, regeneration, long-term potentiation, memory

Molecular and biochemical characteristics
The growth-associated protein (GAP-43) is associated with presynaptic neuronal outgrowth and neuronal plasticity in general (Figure 1).1 The GAP-43 gene includes three exons.2 The first exon encodes the membrane targeting domain, the second exon encodes a calmodulin-binding domain and a protein kinase C (PKC) phosphorylation site, while the 5′-flanking sequence directs initiation of RNA transcription from several sites.2 Among the various tissue types and cell types that have been examined, GAP-43 mRNA is expressed only in neurons (Figure 2A).3 Once transcribed, GAP-43 mRNA is stabilized by HuD,4 a neuronal-specific RNA-binding protein.5 HuD expression increases during brain development, nerve regeneration, and learning and memory (Figure 2B),6 suggesting that this protein is important for controlling gene stabilization and would therefore also be important for maintaining elevated levels of GAP-43 mRNA during plasticity-associated processes.7 Overexpression of HuD results in a selective increase in GAP-43 mRNA in hippocampal dentate granule cells, neurons in the lateral amygdala, and layer V of the neocortex,8 mimicking a state of high plasticity (Figure 2B). Experiments have shown that GAP-43 mRNA was more stable in brain extracts from HuD transgenic mice than in non-transgenic littermates, indicating that HuD can positively affect GAP-43 mRNA stability in vivo.5

The GAP-43 amino acid sequence is hydrophilic,9 with no membrane-spanning domains and no sites for glycosylation.10 There is a short hydrophobic amino acid sequence segment, indicating that the GAP-43 protein may be anchored on the cytoplasmic side of synaptic plasma membranes (see Figures 1 and 2A).10 Palmitoylation of...
Cys3 and Cys4 may be responsible for localization of the protein on the inner surface of plasma membranes (including growth cones), with initial palmitoylation occurring at the endoplasmic reticulum-Golgi intermediate compartment.

Phosphorylation of GAP-43 is a major factor in its biochemical and physiological activity. The rat GAP-43 protein consists of 226 amino acids with a single phosphorylation site at Ser41 whereby PKC, and specifically, PKC-beta, can phosphorylate the protein (Figure 1). Calmodulin has been shown to inhibit GAP-43 phosphorylation by PKC (Figure 2A) and when the Ser41 site is changed to aspartate to mimic constitutive phosphorylation, calmodulin binding by GAP-43 is absent. GAP-43 is bound by calmodulin when Ca$^{2+}$ levels are low and is released when Ca$^{2+}$ levels rise (Figure 2A and B), suggesting that calmodulin may act as a negative regulator of GAP-43 during periods of low activity in the neurons. One functional outcome of GAP-43 during activity-dependent increases in Ca$^{2+}$ levels, when it is not bound by calmodulin, may be the regulation of exocytosis and endocytosis and synaptic vesicle recycling via interactions with synaptophysin, SNAP-25, and rabaptin-5 (Figures 1 and 2B). When calmodulin dissociates from GAP-43, it is free to interact with CamKII, Ca$^{2+}$/calmodulin-dependent protein II, leading to phosphorylation of cofilin by LIM kinases and neurite outgrowth.

A number of other factors have been shown to modulate the phosphorylation of membrane-bound GAP-43. In the absence of Ca$^{2+}$, arachidonic acid has been shown to exert a modest effect on the phosphorylation of GAP-43, while at Ca$^{2+}$ levels likely to exist in the nerve terminal during enhanced plasticity, arachidonic acid (Figure 2B) can increase the sensitivity of GAP-43 phosphorylation to Ca$^{2+}$ and increase the maximal level of phosphorylation by 50%. The stimulatory effect of arachidonic acid and its synergistic interaction with Ca$^{2+}$ are mediated by PKC (Figure 2B).

**Neuroanatomical localization**

GAP-43 has been shown to be relatively neuron-specific (it has been detected in the plasma membranes of cultured neonatal rat cortical astrocytes with a high density in presynaptic terminals) in both the peripheral and central nervous systems. GAP-43 is expressed ubiquitously in the central nervous system at high levels during the perinatal period, with progressively restricted expression during maturation.

GAP-43 remains present in the mature central nervous system in structures known to exhibit high plasticity, such as the cerebellum (granule cells but not Purkinje cells), neocortex, entorhinal cortex, hippocampus, and olfactory bulb. Dense GAP-43 localization has specifically been shown in layer I of the cortex, the CA1 field of the hippocampus, but not granule cells, and in a subset of subcortical structures including the caudate putamen, olfactory tubercle, nucleus accumbens, bed nucleus of the...
GAP-43 in synaptic plasticity

Neurons containing biogenic amines such as the substantia nigra pars compacta (dopamine), the locus coeruleus (norepinephrine), and dorsal raphe (serotonin) exhibit intense GAP-43 staining. In cholinergic neurons, the medial septum, nucleus basalis magnocellularis, and the vertical limb of the diagonal band express intermediate levels, while the horizontal limb of the diagonal band and the substantia innominata express higher levels of GAP-43.

Within the brainstem and spinal cord (such as spinal motor neurons), GAP-43 is present at all vertebral levels, with higher concentrations in the cervical and thoracic regions. Densely myelinated regions are low in GAP-43, while unmyelinated or lightly myelinated areas, such as the substantia gelatinosa of the spinal cord and the nucleus of the stria terminalis, amygdala, and medial preoptic area of the hypothalamus.

**Figure 2** Functional aspects of GAP-43 in the neuron.

**Notes:** (A) During basal synaptic transmission, the HuD protein is not bound to GAP-43, decreasing its stability and keeping protein levels low. GAP-43 bound of CaM precludes its plasticity-permissive functions. (B) During high-plasticity states, HuD binds to and stabilizes GAP-43 mRNA, thereby elevating protein levels. In the presynaptic terminal, GAP-43 phosphorylation by PKC facilitates neurite outgrowth and vesicle recycling, thereby leading to enhanced neurotransmitter release (glutamate). This enhanced glutamate release liberates post-synaptic AA and DAG which can facilitate pre-synaptic phosphorylation of GAP-43.

**Abbreviations:** AA, arachidonic acid; CaM, calmodulin; DAG, diacylglycerol; LTP, long-term potentiation; PKC, protein kinase C; SNAP-25, synaptosomal-associated protein 25.
the solitary tract, express high levels of GAP-43. The electron microscopic examination has revealed that GAP-43 is localized in small myelinated and unmyelinated fibers and in terminals that make single axodendritic or axosomatic synapses. At this ultrastructural level, GAP-43 labeling is most prevalent in small unmyelinated axons (0.12–0.15 microns in diameter) and small (0.35 microns) axon terminals that contain round vesicles and form asymmetric synapses with thin spines.

**Cellular localization**

GAP-43 is present throughout the neuron but at higher levels in axon terminals and growth cones. GAP-43 is absent from dendrites and myelinated axons, as indicated by double labeling with antibodies against microtubule-associated protein 2 and the large neurofilament protein. When cultured neurons begin to extend neurites, the cell body shows diffuse (decreased) GAP-43 staining while the growing tip of the neurites (ie, growth cone) shows elevated, punctate GAP-43 staining. That GAP-43 is localized to the inner surface of the growth cones was shown on positive immunostaining only after permeabilization of the plasma membrane. Showing its neuron-specificity, the GAP-43 antibody used did not label Schwann cells or fibroblasts.

GAP-43 contributes to neuronal growth and nerve terminal plasticity. GAP-43 appears critical for stimulus-induced nerve sprouting at the neuromuscular junction; an effect that may occur via promotion of F-actin accumulation. GAP-43 accumulates at subplasmalemmal rafts where it can regulate actin activity via modulation of PI(4,5)P2. Utilization of in vivo lentiviral-mediated gene silencing in the olivocerebellar system to downregulate GAP-43 climbing fibers resulted in atrophy of climbing fibers, measured as a decrease in length, branching, and number of synaptic boutons.

**Relationship to development**

In animal studies, expression of GAP-43 during development appears to vary depending on brain region. In the cerebellum, GAP-43 mRNA expression appears to be critical in granule cell differentiation/migration and in parallel and climbing fiber axonal outgrowth and synaptogenesis. During development, cerebellar GAP-43 mRNA expression increases from birth to postnatal day (PND) 7, then gradually declines during maturation. By PND21, GAP-43 mRNA expression is localized to the internal granule layer and the inferior olive, with minimal to no hybridization in the deep cerebellar nuclei and none in the molecular layer (similar to the adult). In the auditory brainstem, high levels of GAP-43 protein are evident in all subdivisions of the cochlear nuclear complex and the superior olivary complex at PND0. Between PND8 and PND12, the GAP-43 staining pattern in these regions became punctate, indicating the formation of presynaptic endings. By PND16, the auditory brainstem nuclei appear mostly devoid of GAP-43 immunoreactivity, with the exception of staining localized to presynaptic terminals. In the cat visual cortex, phosphorylation of GAP-43 increased approximately 10-fold from PND1 to weeks 3–13 (the critical period of synaptic plasticity) then decreased to 2.5-fold of PND1 by week 51. Protein quantification of GAP-43 in the cortex and hippocampus was elevated at PND14 and PND21 during an active period of synaptogenesis. Evidence from this study showed that GAP-43 is highly expressed in immature growing axonal terminals with decreased expression during the maturation process, showing an inverse relationship with synapsin or synaptotagmin expression. In the optic nerve and optic fiber layer of the retina, GAP-43 staining was high at PND0 then disappeared between PND8 and PND16.

At the developmental cellular level, GAP-43 appears to be involved in neurite outgrowth via the amplification of path-finding signals from the growth cone. Six days after plating cells, GAP43 labeling was shown to be clustered in growth cones, and by 10 days cell body labeling was lost, while at 20 days the neuritic and growth cone labeling was reduced. GAP-43 expression in adult olfactory neurons resulted in numerous primary olfactory axons with enlarged endings, providing in vivo evidence for a role of GAP-43 in nerve fiber formation and axon morphology determination. The level of GAP-43 expression and axonal growth during development may be dually controlled by activity-independent transcriptional processes and by activity-dependent, N-methyl-D-aspartate (NMDA) receptor-mediated post-transcriptional mechanisms.

Several factors have been shown to interact with GAP-43 during neurite outgrowth. In PC12 pheochromocytoma cells, induction of a neuronal phenotype by nerve growth factor is accompanied by a marked increase in GAP-43 levels. Induction of GAP-43 by nerve growth factor is apparent after 3 hours of exposure and reaches maximal levels at 24 hours. GAP-43-transfected PC12 cells show an enhanced response to nerve growth factor, suggesting that GAP-43 may respond to extrinsic growth factors to modulate neurite extension. Neural cell adhesion molecule (NCAM)-mediated fibroblast growth factor receptor activation in cerebellar granule cells is associated with increased GAP-43 phosphorylation on Ser41 and neurite...
outgrowth, whereas neither NCAM nor fibroblast growth factor was able to stimulate neurite outgrowth following GAP-43 gene deletion.\textsuperscript{71} Newly formed synapses show a dramatic decrease in palmitoylation of GAP-43 early in the critical period,\textsuperscript{72} which may be sufficient to stop advancing axons, suggesting a developmental switch for GAP-43 S-palmitoylation that may be required to disengage the molecular machinery for axon extension.\textsuperscript{72} GAP-43 has been shown to amplify extracellular signals via an interaction with the GTP-binding protein, Go (Figures 1 and 2B), increasing the sensitivity of Go, thereby altering the predilection for neuronal outgrowth.\textsuperscript{73–75}

**Relationship to regeneration**

GAP-43 synthesis is upregulated in association with nerve regeneration, potentially recapitulating an early developmental program.\textsuperscript{1} While endogenous GAP-43 is downregulated in mouse motor nerves and neuromuscular junctions during the second postnatal week, it is re-expressed during regeneration and potentiates nerve sprouting.\textsuperscript{76} After cutting or crushing the sciatic nerve in adult rats in vivo, it takes 3 days for GAP-43 immunoreactivity to appear in the axotomized dorsal root ganglion cells followed by transport into the newly formed sprouts.\textsuperscript{77,78} The intensity of staining peaks at 21 days and becomes undetectable 9 weeks following crush injury and 36 weeks following cutting of the sciatic nerve.\textsuperscript{79} Downregulation of GAP-43 was reported to result in a significant decrease in newly formed branches in climbing fibers after laser axotomy. GAP-43 mRNA downregulation also hampered the generation of reactive sprouts, showing a requirement for GAP-43 in promoting the initiation of axonal regrowth after axotomy.\textsuperscript{79}

GAP-43 mRNA and protein increase following peripheral deafferentation of the olfactory epithelium and after olfactory bulbectomy, and are associated with formation of immature olfactory receptor neurons.\textsuperscript{80} GAP-43 immunoreactivity was shown to be increased 3 weeks after binocular retinal lesions in adult cats in the part of the dorsal lateral geniculate nucleus that represents this region of the retina.\textsuperscript{81} Following axotomy in the medial forebrain bundle, GAP-43 immunoreactivity is associated with catecholaminergic and serotonergic axonal sprouts that regenerate around the surgical lesion.\textsuperscript{82} Removal of cochlear neurons in adult rats leads to emergence of GAP-43 immunoreactivity in varicose fibers of the ipsilateral ventral cochlear nucleus and cell bodies of the lateral superior olive.\textsuperscript{83} Following stroke, optogenetic neuronal stimulation in the ipsilesional primary motor cortex promotes functional recovery associated with improved cerebral blood flow and increased expression of numerous activity-dependent neurotrophins, such as brain-derived neurotrophic factor, nerve growth factor, and GAP-43.\textsuperscript{84}

In the adult rat brain, levels of GAP-43 immunoreactivity in layer IV of the barrel receptor field are moderate in the interbarrel septa and low within the barrels themselves.\textsuperscript{85} Changes in the pattern of GAP-43 immunoreactivity were analyzed 1–8 weeks after unilateral vibrissectomy to examine the effect and time course of removal of all but the C3 vibrissa on GAP-43 immunoreactivity. The C3 area that was GAP-43-immunonegative showed a decrease in total area from 8.4% 1 week after vibrissectomy to a 12% decrease 8 weeks after surgery relative to the control ipsilateral cortex. This suggests a GAP-43-mediated axonal sprouting process within the barrel cortex, whereby GAP-43-positive terminals encroach on areas of GAP-43 absence.\textsuperscript{85} In a second study, GAP-43 mRNA was analyzed in the barrel cortex in adult rats that underwent unilateral vibrissectomy with sparing of the C3 vibrissa.\textsuperscript{86} GAP-43 levels were elevated by 25% compared with the non-lesioned side for 6 days following surgery, then decreased to 88% at 7 days and returned to control levels by 14 days.\textsuperscript{86}

Reports have revealed alterations in the pattern of GAP-43 protein levels in the hippocampus during reactive synaptogenesis following lesions of the perforant pathway.\textsuperscript{70} Changes in the synthesis and transport of GAP-43 in entorhinal cortex neurons and the perforant pathway were assessed during lesion-induced sprouting and reactive synaptogenesis.\textsuperscript{87} Following unilateral entorhinal cortex lesions in adult rats, there was a 2-fold (100%) increase in transport of newly synthesized GAP-43 to the contralateral or “sprouting” hippocampus. This upregulation occurred between 6 and 15 days after damage and coincided with the growth of presynaptic terminals during sprouting.\textsuperscript{87}

Aside from structural/neuronal damage leading to reactive axonal sprouting and associated elevations in GAP-43, the excitotoxicity process can lead to GAP-43-dependent sprouting independent of neuronal damage. In one such study,\textsuperscript{88} hypersynchronous activity in the hippocampus led to expression of GAP-43 mRNA in dentate gyrus granule cells followed by reactive sprouting in the granule cell axons (also known as mossy fibers). Twelve hours after subcutaneous injection of kainate, GAP-43 mRNA expression was evident in granule cells (where expression is normally absent in the adult brain), and 2 days after treatment (up to 40 days), GAP-43 protein immunoreactivity and mossy fiber sprouting within the supragranular layer were observed. As stated, these events are similar to those seen after neuronal damage.
leading to axonal regeneration, with one difference being that granule cell axons were not damaged by kainite.88

**Relationship to long-term potentiation (LTP)**

One cellular process underlying synaptic plasticity is LTP, which has been shown to engage, and be intricately related to, alterations in GAP-43 levels and phosphorylation.89 In the intact rat hippocampus, there was a selective increase in the in vitro phosphorylation state of GAP-43 5 minutes after induction of LTP that was directly related to the change in synaptic efficiency.90 Low-frequency, non-potentiating stimulation resulted in no change in GAP-43 phosphorylation. In vivo phosphorylation of GAP-43 was increased in the synaptic membranes of the mossy fiber CA3 pyramidal neurons at 1 and 5 minutes after tetanic stimulation, but not at 60 minutes, suggesting a role for GAP-43 phosphorylation in the induction but not maintenance of LTP at the mossy fiber synapse.91 This GAP-43 phosphorylation was shown to occur independent of the NMDA receptor, to be mediated by PKC, and to be inhibited in the presence of calmodulin.91 Dorsal hippocampal tissue extracted from animals 3 days following induction of LTP in granule cells by stimulation of the perforant pathway showed enhanced phosphorylation of GAP-43 compared with tissue from low-frequency stimulated controls.92 Perforant path LTP in the intact mouse hippocampal dentate gyrus increased GAP-43 mRNA in hilar cells 3 days after tetanus, but not in granule cells.93 The LTP-induced GAP-43 mRNA elevation in hilar cells was positively correlated with the level of potentiation and blocked by pretreatment with the NMDA receptor antagonist, DL-aminophosphonovalerate.93 The phosphorylation state of GAP-43 was monitored after induction of LTP in the CA1 field in rat hippocampal slices and revealed increased phosphorylation 10–60 minutes following LTP induction but not after 90 minutes.94 The increased GAP-43 phosphorylation was not observed when LTP was blocked with DL-aminophosphonovalerate or when tetanic stimulation failed to induce LTP.94,95 At 1 hour, but not at 2 hours after LTP, GAP-43, and PKC-gamma mRNA hybridization were increased.96 A related study showed decreased levels of GAP-43 gene expression in the CA3 subfield and both PKC-beta and PKC-gamma 3 days after LTP induction.97 Alterations in GAP-43 mRNA and PKC-gamma were highly correlated. The authors suggested that lowered expression of GAP-43 at 3 days would reduce potential growth, leading to synaptic stabilization in stimulated pathways.97 Injection of monoclonal antibodies that inhibited PKC phosphorylation of GAP-43 was shown to prevent induction of LTP in CA1 pyramidal neurons in rat hippocampal slices.98

Overexpression of a constitutively phosphorylated form of GAP-43 results in an enhancement of LTP in hippocampal slices in the CA1 region associated with an increase in presynaptic paired-pulse facilitation.99 LTP enhancement was not observed in transgenic mice overexpressing a non-phosphorylatable form of GAP-43 nor in GAP-43-deficient mice.99 Others have suggested that presynaptic phosphorylation of GAP-43 may be affected by nearby messenger molecules produced postsynaptically following NMDA receptor activation that diffuse to activate PKC.100 In this regard, application of arachidonic acid at concentrations that produce LTP significantly increased translocation of PKC immuno-reactivity from cytosol to membrane and phosphorylated GAP-43 observed in hippocampal synaptosomes, suggesting that arachidonic acid may contribute to LTP maintenance by activation of presynaptic PKC and phosphorylation of GAP-43 (Figure 2B).100

**Relationship to memory storage**

Given the relationship of GAP-43 with LTP, it is of no surprise that changes in levels of GAP-43 (whether decrements or increments) have been shown to be associated with memory storage processes in adult animals. In one such example, heterozygous GAP-43 knockout mice with GAP-43 levels reduced by one half showed impaired memory for a shock-paired context.101 In this study, there were no decrements in cued shock-conditioning nor decrements on tests of nociceptive or auditory perception, indicating that the contextual memory impairment was not based on impaired sensory or performance factors.101 Using a similar behavioral procedure (contextual fear conditioning), sustained phosphorylation of GAP-43 in the hippocampus was noted for 1.5–72 hours after training.102 At early time points after contextual fear conditioning training (15–90 minutes), PKC-alpha and PKC-gamma translocated to the membrane, while PKC-betaII and PKC-epsilon moved more transiently (15–30 minutes) to the cytosol.102

Three lines of transgenic mice have been developed, each with a particular manipulation of the PKC phosphorylation site:103 G-Phos overexpresses the phosphorylatable and dephosphorylatable form of chick GAP-43 (the native protein; no mutation); G-Perm overexpresses chick GAP-43 that is permanently pseudophosphorylated; and GNonP overexpresses nonphosphorylatable GAP-43. The overall behavioral memory functions associated with each of these lines has been reported;104 G-Phos mice showed enhanced
spatial flexibility on a water maze task; G-Perm mice showed memory persistence as evidenced by their inability to extinguish a classically conditioned fear response; and G-NonP mice showed retention deficits in their ability to recall spatial information on a water maze task. In another study, G-Phos mice alone were more fully tested for their ability on the water maze spatial task. These G-Phos mice could be divided at the behavioral level into “spatial bright” and “spatial dull” groups based on their water maze task performance and GAP-43 protein levels in the hippocampus. G-Phos dull mice showed both acquisition and retention deficits on the fixed hidden platform task, but were able to learn a visible platform task while G-Phos bright mice showed memory enhancement relative to wild-type on a more difficult movable hidden platform spatial memory task. In the hippocampus, the G-Phos dull group showed a 50% greater transgenic GAP-43 protein level and a 2-fold elevated transgenic GAP-43 mRNA level than that measured in the G-Phos bright group. While overexpression of GAP-43 would normally be predicted to enhance memory function, in the case of G-Phos dull mice, it could be the case that high levels of GAP-43 protein aggregate in presynaptic terminals leading to impoverished synaptic vesicle recycling, deficient neurotransmitter release, and impaired memory function.

Two other studies have examined the relationship of GAP-43 levels with neuronal restructuring after memory formation. In the first study, rats that were tested for their retention of a spatial memory task after a 30-day delay exhibited increased GAP-43 labeling in the anterior cingulate cortex as compared with the 1-day retention group. In a second study, mice were trained for 5 days on one of three different versions of the water maze task. High-resolution magnetic resonance imaging analysis revealed structural expansion in the hippocampus of mice trained on the spatial version of the task that was correlated with GAP-43 protein staining.  

Relationship to drug-induced synaptic plasticity

Changes in phosphorylation of GAP-43 have been demonstrated in other forms of behavioral plasticity, such as those associated with drug use. Following one injection of amphetamine, GAP-43 phosphorylation was increased in rat striatum and persisted for 1 week. In cultured PC12 cells, repeated intermittent amphetamine treatment (5 minutes a day for 5 days) induced neurite outgrowth that was associated with an increase in the level of GAP-43 staining. A single exposure to cocaine 20 mg/kg induced locomotor sensitization to an injection of cocaine 10 mg/kg that was observed at 24 hours, 48 hours, and 7 days, with an associated increase in mRNA GAP-43 in the shell and core subregions of the nucleus accumbens and in the ventral tegmental area. Administration of a single intraperitoneal dose of ethanol (2.5 g/kg, 15% in saline) resulted in a decrease in GAP-43 mRNA level 2 hours after administration, with subsequent decreased GAP-43 and phosphorylated GAP-43 immunoreactivity 4 hours after administration in the perforant and mossy fiber pathways.

Conclusion

Activity of GAP-43, whether mRNA elevations or phosphorylation of the protein, is intricately involved in presynaptic plasticity as occurs during developmental outgrowth of neurites, elevated following neuronal injury in the course of axonal regeneration, and tightly linked with memory processes for the period of reorganization of neural networks. These functions are in part due to regulation and distribution of GAP-43 mRNA at the molecular level and PKC-dependent phosphorylation at the biochemical level. Utilization of this knowledge may open up future directions for targeted treatments of developmental disorders, memory dysfunction, and neuronal injury.

Disclosure

The author reports no conflicts of interest in this work.

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