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INVITED REVIEW

Polarized targeting of ion channels in neurons

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Abstract Since the time of Cajal it has been understood that axons and dendrites perform distinct electrophysiological functions that require unique sets of proteins [Cajal SR Histology of the nervous system, Oxford University Press, New York, (1995)]. To establish and maintain functional polarity, neurons localize many proteins specifically to either the axonal or the somatodendritic compartment. In particular, ion channels, which are the major regulators of electrical activity in neurons, are often distributed in a polarized fashion. Recently, the ability to introduce tagged proteins into neurons in culture has allowed the molecular mechanisms underlying axon- and dendrite-specific targeting of ion channels to be explored. These investigations have identified peptide signals from voltage-gated Na^+ and K^+ channels that direct trafficking to either axonal or dendritic compartments. In this article we will discuss the molecular mechanisms underlying polarized targeting of voltage-gated ion channels from the Kv4, Kv1, and Na_v1 families.

Keywords Axonal compartment · Somatodendritic compartment · Na^+ channel · K^+ channel

Introduction

In pioneering studies investigating the mechanisms of protein targeting in eucharyotic cells, it was demonstrated that short peptide motifs direct proteins specifically to intracellular organelles, such as the endoplasmic reticulum and the nucleus [1, 12]. More recent work has found that peptide motifs can also target proteins to specific regions on the plasma membrane. This observation was first made in epithelial cells, where many proteins are directed to one of two plasma membrane domains, known as the apical and basolateral regions, that are located at opposite ends of the cell and are separated by a band of tight junctions [21]. Like those of epithelial cells, the plasma membranes of neurons are divided into two regions, the axon and the dendrite, by a barrier that prevents diffusion of proteins between the two compartments [43]. The similarities in cellular structure between neurons and epithelial cells, and the fact that certain proteins express in a polarized manner in both cell types, suggested that targeting mechanisms based on peptide motifs might mediate the localization of proteins in both cell types [7]. When technical advances allowed neurons to be transfected with recombinant DNA constructs, it was confirmed that peptide motifs mediate polarized targeting of proteins in neurons [15, 42].

The ability to express tagged proteins in neurons allowed vesicle trafficking to be observed in axons and dendrites, leading to new insights into the mechanisms underlying polarized targeting. In particular, it has been shown that polarized targeting can be mediated by four distinct mechanisms: polarized vesicular trafficking, compartment-specific endocytosis, compartment-specific vesicle docking, and transcytosis (Fig. 1). (1) In polarized vesicular trafficking, protein is sorted into a subset of vesicles that is transported specifically to a particular subcellular compartment, such as the dendrites (Fig. 1a). Polarized vesicular trafficking was first characterized by studies in which the green fluorescent protein (GFP)-labeled transferrin receptor was expressed in hippocampal neurons in culture. The transferrin receptor was localized to vesicles that were found exclusively in dendrites and that were distinct from those containing exogenously expressed, tagged axonal proteins [2]. (2) Compartment-

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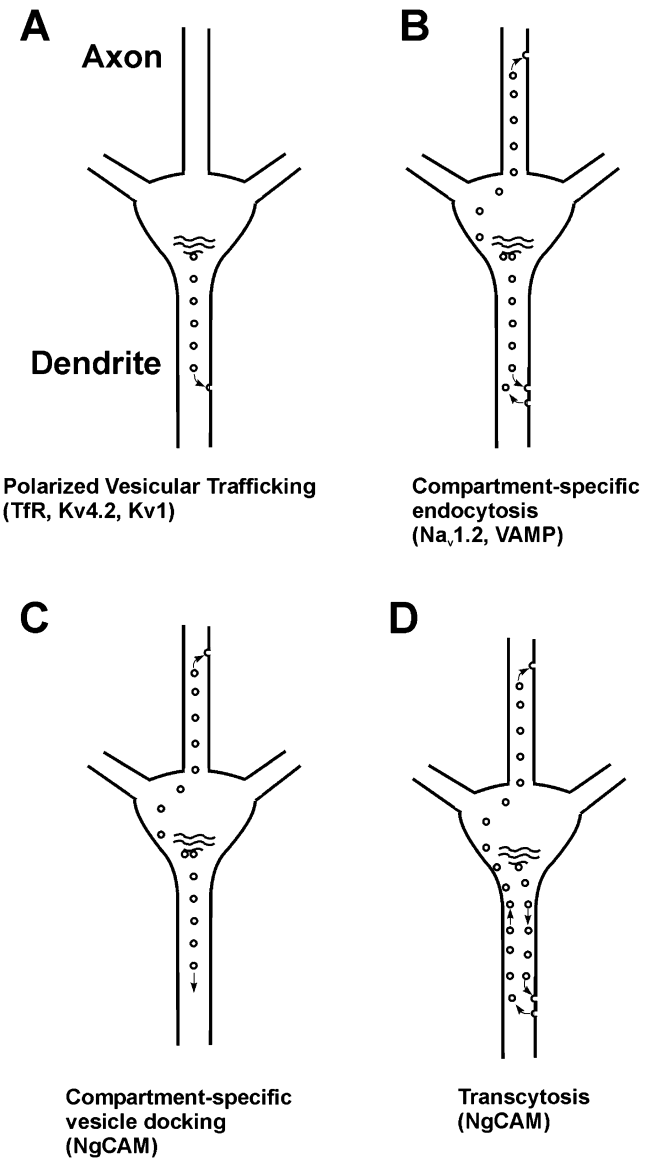

Fig. 1 Mechanisms of polarized targeting of proteins in neurons. Four mechanisms underlying polarized targeting of proteins in neurons are illustrated in the text. **a** Polarized vesicular trafficking. **b** Compartment-specific endocytosis. **c** Compartment-specific vesicle docking. **d** Transcytosis. For clarity, vesicular trafficking is shown in the apical dendrite, but not in secondary dendrites

specific endocytosis, the mechanism by which the snare protein VAMP2 is localized, is characterized by vesicular transport to both axons and dendrites (Fig. 1b) [31]. Following transport, vesicles undergo exocytosis, resulting in the deposition of VAMP2 onto the membranes of both compartments. Subsequently, VAMP2 is endocytosed specifically from the dendritic compartment, resulting in the presence of the protein only on the axonal membrane. (3) Compartment-specific vesicle docking, like compartment-specific endocytosis, involves the nonspecific vesicular transport of protein (Fig. 1c). However, the former mechanism involves the docking of transport vesicles in a single compartment. This mechanism was observed to be responsible in part for targeting of NgCAM, which is present in vesicles that dock specifically at the plasma membrane of the axonal compartment resulting in expression of protein exclusively on the axonal membrane [2, 31]. (4) NgCAM was also found to be localized by transcytosis (Fig. 1d) [44]. This mechanism involves vesicular transport of protein specifically to the dendrites, followed by endocytosis and transport to the axon.

Recent studies have found examples of ion channels that are targeted by polarized vesicular trafficking and compartment-specific endocytosis, but none have been found to be localized by either compartment-specific vesicle docking or transcytosis. In this review we will examine the targeting of three different voltage-gated ion channels: Kv1 and Kv4 channels, which are localized by polarized vesicular trafficking, and Na$_v$1 channels, which are localized by compartment-specific endocytosis.

Dendritic targeting of Kv4.2

Kv4.2 is a voltage-gated K^+ channel found in dendrites that both activates and inactivates quickly, electrical properties that make it ideal for blocking action potential initiation and propagation [32]. Patch clamp recordings of pyramidal neurons from the CA1 region of the hippocampus have revealed that, under normal conditions, action potentials can neither be initiated in dendrites, nor can they back-propagate from the axon to dendrites [14]. Inhibition of Kv4.2, either by exposing a hippocampal slice to the pharmacological blocker 4-aminopyridine or by expressing a dominant negative variant of a Kv4.2 subunit in hippocampal pyramidal cells, allows both initiation and backpropagation of action potentials in the dendrites [3, 14, 16]. Accordingly, the subcellular targeting of this channel defines a region through which action potentials do not travel, and because it is expressed in most CNS neurons, it likely plays a critical role in specifying the electrophysiological characteristics of neurons throughout the brain [27].

Recently, signals involved in targeting of the channel were identified, allowing us to better understand how Kv4.2 is localized to dendrites. Using tagged, recombinant versions of the channel transfected into neurons in cultured slices of rat cortex, its localization on the membrane was assessed using surface labeling [28]. By testing a series of constructs consisting of chimeras between Kv4.2 and the axonal channel Kv1.4, as well as Kv4.2 constructs with sequences either deleted or mutated, a 16-amino acid sequence containing two tandem leucines was identified that is necessary for dendritic targeting of the channel. This single domain is sufficient to direct dendritic targeting, as a fusion of this domain with Kv1 channels, which are found

in axons, as well as with nonspecifically localized, heterologous proteins, such as CD8, directs these proteins to dendrites [28]. This motif is conserved in all K^+ channels that belong to the Shal family, of which Kv4.2 is a member, from *Caenorhabditis elegans* to humans. However, similar motifs are not found in other K^+ channel families.

Whether or not a protein is localized by a mechanism that involves polarized vesicular trafficking can be determined by comparing that protein's intracellular expression pattern with its expression pattern on the cell surface. Proteins targeted by polarized vesicular trafficking have intracellular and surface expression patterns that are both confined to a specific compartment. In contrast, proteins targeted through unpolarized vesicle trafficking followed by a plasma membrane-based sorting process are expressed intracellularly in both compartments, but are present on the plasma membrane in only one compartment. Two observations suggest that Kv4.2 is localized by polarized vesicular trafficking: (1) both cytosolic and surface Kv4.2 is localized to dendrites and (2) the dileucine-containing motif does not mediate endocytosis (Fig. 1) [28]. Polarized vesicular trafficking involves somal sorting of proteins into a subset of vesicles, followed by transportation of these vesicles specifically to dendrites, both of which are hallmarks of dendritic targeting of the transferrin receptor [2]. Clues about the mechanism by which vesicle sorting occurs are suggested by analogy with dileucine-containing motifs that mediate basolateral targeting in epithelial cells and/or endocytosis [38]. These motifs bind to subunits of clathrin adaptor complexes, providing a means of fastening the cytoplasmic domain of a transmembrane protein to a constituent of the protein coat [18]. Thus, transmembrane proteins containing specific motifs can be sorted into vesicles whose coat contains the cognate adaptor protein complex [26]. While this is a plausible mechanism by which targeting motifs can mediate sorting of proteins into a subset of transport vesicles, it is not clear why a vesicle containing a protein with a certain targeting motif should be transported specifically to a particular subcellular compartment. However, previous work has suggested that, in neurons, locomotion of transport vesicles is mediated by kinesin motors, suggesting that kinesins may play a role in specifying the destination to which the vesicles are transported [13].

How could a kinesin motor mediate dendrite-specific trafficking of transport vesicles? One possibility is that certain kinesins autonomously traffic to a specific subcellular compartment, perhaps by recognizing the subset of microtubules that project to the compartment. Evidence for the existence of such proteins, which have been termed smart motors [2], comes from the observation that a number of the 45 kinesins found in mammals are localized specifically to dendrites [20, 23]. The question of whether a kinesin acts as a smart motor when transporting a particular cargo has important implications for the function of targeting motifs on the cargo protein. If a protein is transported by a smart motor, then, by definition, all the information that is necessary for targeting of the cargo is provided by the kinesin. In that case, the only role of a targeting motif on the cargo protein is to attach that protein to the appropriate kinesin.

Kv4.2 is transported by the kinesin Kif17, which is also responsible for dendritic transport of the NMDA receptor subunit NR2B [5, 11, 33]. Using coimmunoprecipitation assays, it was found that Kif17 does not attach to Kv4.2 via the dileucine-containing motif, but rather through the extreme C terminus of the channel [5]. Because the dileucine motif is not involved in attachment to Kif17, but is necessary for dendritic targeting of Kv4.2, it must be influencing Kif17 to traffic to dendrites. In that case, Kif17 cannot be acting as a smart motor when transporting Kv4.2. These results corroborate the finding that a Kif17 mutant lacking the cargo binding domain traffics to both axons and dendrites, indicating that the motor domain by itself is not sufficient to mediate compartment-specific targeting [25]. Accordingly, Kv4.2 must influence Kif17 to traffic to dendrites, likely by signaling through the dileucine motif and proteins with which the motif interacts. This conclusion is consistent with evidence that associated proteins can influence the tendency of kinesins to traffic specifically to axons or dendrites. For instance, the kinesin Kif5B, which transports the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluR2, a dendritic protein, traffics to dendrites when associated with the Psd-95, Dlg, and ZO1 (PDZ) domain-containing protein glucocorticoid receptor-interacting protein (GRIP). In contrast, Kif5B traffics to axons when associated with the adaptor protein JSAP1 [34]. Proteins that interact with the dileucine motif of Kv4.2 could thus play a role analogous to that of GRIP by influencing Kif17 to traffic specifically to dendrites. In summary, the results of previous experiments point to a model whereby the dileucine motif, through its interacting proteins, causes the channel to be sorted into specific vesicles and influences Kif17 to traffic those vesicles specifically to dendrites. Future experiments will be required to test whether this model accurately describes the mechanism underlying dendritic targeting of Kv4.2.

$Na_v1.2$ targeting to axons

The subcellular distribution of sodium channels from the Na_v1 family play a critical role in determining where action potentials initiate in neurons [18]. $Na_v1.2$ is localized to the axon hillock where it responds to depolarization by generating Na^+ currents necessary to trigger action poten-

tials [36]. To identify signals that target this channel to the axon, a series of chimeric constructs consisting of cytoplasmic regions of the channel fused with heterologous proteins were tested in hippocampal neurons in dissociated culture [8]. These studies revealed that the C terminus contains a nine-amino acid, dileucine-containing motif that is sufficient to target heterologous proteins to the surface of the axon. Because these proteins are present intracellularly in both axons and dendrites, this motif mediates targeting through a plasma-membrane-based trafficking mechanism, rather than through polarized vesicular trafficking. In COS cells, the motif mediates clathrin-dependent endocytosis through an interaction with the adaptor protein AP-2, and in neurons it mediates dendrite-specific endocytosis. These results suggest a targeting mechanism whereby proteins with this motif are transported to, and inserted into, the membranes of both axonal and dendritic compartments. Localization to only the axon occurs through prompt endocytosis of the proteins from the dendritic membrane, but not the axonal membrane (Fig. 1b) [8]. Surprisingly, dendrite-specific endocytosis does not appear to be an important mediator of axonal targeting in other Na_v1 channels, despite the presence of peptide regions that are homologous to the dileucine-containing motif in $Na_v1.2$ in the Na^+ channels $Na_v1.1$ and $Na_v1.6$ [8].

While the targeting motif of $Na_v1.2$ is sufficient to mediate targeting to the axon, it does not affect targeting within the axonal compartment. Instead, targeting to the axon initial segment is mediated by a 27-amino acid ankyrin-binding motif found in the cytoplasmic linker between the second and third transmembrane domains of the channel [9, 19]. This motif is also present in $Na_v1.1$, Na_v 1.2, $Na_v1.3$, and $Na_v1.6$, and is sufficient to cause heterologously expressed transmembrane proteins, such as CD4, and even cytoplasmic proteins, such as GFP, to localize to the axon initial segment through ankyrin binding. Thus, the axonal targeting of Na^+ channels appears to involve at least two different mechanisms: direct binding to a cytoskeletal protein and dendrite-specific endocytosis. Whether these mechanisms are the sole determinants of subcellular localization of Na^+ channels, however, remains unclear. These initial studies concentrated on the behavior of targeting motifs fused to heterologous proteins, although it was not determined whether either motif is necessary for axonal targeting of Na^+ channels. Thus, it is not known whether Na^+ channels have additional determinants that might assist in axonal targeting. The larger question is whether axonal targeting might be fundamentally different from dendritic targeting. Several studies have suggested that dendritic targeting involves polarized vesicular trafficking, whereas axonal targeting mechanisms involve nonspecific trafficking of vesicles followed by targeting to the plasma membrane of the axonal compartment through mechanisms that act at the membrane [2, 8, 9, 28, 31].

Kv1 channel targeting to axons

Kv1 channels represent the first example of an axonal channel that is localized through polarized vesicular trafficking. Kv1 channels are voltage-gated K^+ channels that are thought to play a role in repolarizing the action potential [39]. Accordingly, they are localized in axons, although there is evidence that they may also be found in dendrites [40, 41]. When tagged and expressed exogenously in dissociated hippocampal cells in culture, Kv1 channels are found in the entirety of the axon, but in dendrites they are present only in proximal regions [10, 29]. Testing of a series of deletion and chimeric constructs showed that the T1 region, which is located on the intracellular N terminus of Kv1 channels, is necessary and sufficient to mediate axonal targeting [10, 29]. In particular, when the T1 domain of Kv1.2 was replaced with an artificial tetramerization domain based on the leucine zipper protein GCN4, or when the T1 domain of Kv1.3 was mutated, the respective channels localized nonspecifically. Conversely, when the T1 domain of each of Kv1.1–4 was fused to a nonspecifically localized protein, the resulting fusion protein localized specifically to axons [10, 29].

Experiments to identify the region of T1 that is necessary and sufficient for axonal targeting failed to define a short, contiguous axonal targeting signal. However, it is likely that Kvβ, which binds to the T1 domain, plays a role in the targeting of Kv1 channels. Mutating amino acids in the T1 domain that are necessary for interaction with Kvβ also prevents the domain from mediating axonal targeting [10]. This result contrasts with experiments involving $Na_v1.2$, which found that the beta subunit that associates with that channel does not play a role in axonal targeting [9, 10]. Although interaction with Kvβ appears to be necessary for axonal targeting, it is likely not sufficient, as mutating at least one residue that is not involved in binding of Kvβ to T1 prevents the T1 domain from mediating axonal targeting [10]. These results suggest that axonal targeting mediated by the T1 domain depends both on interactions mediated through Kvβ and others that are independent of Kvβ.

In the above experiments, the localization of surface protein was used to assess subcellular targeting. Thus, they do not provide information about the molecular mechanisms underlying axonal targeting mediated by the T1 domain. In particular, they do not address whether the T1 domain mediates trafficking of vesicles, or whether it works through a plasma membrane-based mechanism, such as dendrite-specific endocytosis. To investigate mechanisms by which the T1 domain mediates axonal targeting, the subcellular localization of both surface and intracellular protein for a CD8/T1 fusion protein was assessed following expression in cortical neurons in slices [29]. In this case, both intracellular and surface CD8T1 were targeted specifically to axons, suggesting that the T1 domain

mediates trafficking of vesicles rather than dendrite-specific endocytosis or axon-specific vesicle docking. Further evidence that the T1 domain mediates axonal targeting of vesicles was provided by experiments examining the trafficking of vesicles containing a fusion of the T1 domain with transferrin receptor (TfRT1). When a TfR–GFP construct was expressed in dissociated neurons, the protein was present in 1–2-μm tubulovesicular structures that were confined to dendrites and that moved at high rates of speed both anterogradely and retrogradely [2, 35]. In contrast, TfRT1–GFP localized to vesicles that moved in axons, providing evidence that the T1 domain is sufficient to mediate trafficking of vesicles to axons [29]. These results indicate that trafficking of vesicles is important for axonal targeting of at least one ion channel, and suggests that axonal and dendritic trafficking do not necessarily involve distinct mechanisms. Furthermore, it is possible that mechanisms that have been found to mediate axonal targeting of Na^+ channels at the level of the plasma membrane may, in fact, function in a role that is secondary to axonal trafficking of vesicles.

Additional channels

We have chosen to concentrate on mechanisms underlying polarized targeting of three channels, Kv4, Kv1, and Na_v1 channels, as these have been studied in the greatest detail. However, numerous other ion channels have been found to localize specifically to either the axonal and dendritic compartments, and, in many cases, regions of the channel have been identified that are involved in the localization. For instance, when expressed in dissociated hippocampal cultures, tagged versions of the calcium channel Cav2.2 are targeted either to axons or dendrites, depending on which exon comprises the C terminus of the channel. The long splice variant Cav2.2a is localized to presynaptic sites in axons, whereas the short splice variant Cav2.2b is localized to the soma and dendrites [22]. The potassium channels Kv3.3 and Kv3.1 have been found to localize specifically in the somatodendritic region of pyramidal neurons of the electrosensory lateral line lobe of *Apteronotus leptorhynchus* [6]. Moreover, the coding sequence of Kv3.3 contains a PDZ binding motif at the extreme C terminus that is necessary and sufficient to mediate targeting throughout the dendritic tree of pyramidal neurons in vivo [6]. In contrast, Kv3.1 does not contain the PDZ binding motif and is expressed only in the somata and proximal dendrites. Finally, the AMPA receptor GluR1 contains a motif in its C terminus that is sufficient to direct targeting of a heterologous protein to dendrites [30]. A remarkable finding from these studies, and others that have examined the subcellular targeting of polarized, nonchannel proteins, is that there appears to be no consensus in the primary amino acid structure of motifs that mediate either axonal or dendritic targeting.

Future challenges

Although much has been learned about the mechanisms of polarized targeting of ion channels, many challenges remain. In the case of the dileucine motif that mediates dendritic targeting of Kv4.2, it will be critical to identify interacting proteins. These proteins likely cause Kv4.2 to sort into a specific subset of vesicles and also modify the function of Kif17 such that it transports those vesicles specifically to dendrites. In addition to helping to define the targeting mechanisms of specific channels, the identification of proteins that bind to targeting motifs will make it possible to determine whether other dendritically targeted proteins share a common targeting mechanism. This question is particularly intriguing because all known dendritic targeting motifs appear only in single proteins or in families of proteins that are highly related, suggesting that a multiplicity of mechanisms are involved in dendritic targeting. By disrupting the function of Kv4.2 dileucine motif interactors and determining whether this disruption blocks the localization of other dendritically targeted proteins, it should be possible to determine whether proteins share common mechanisms of dendritic targeting, even if they contain targeting motifs that are not homologous.

To better understand Na^+ channel targeting, it will be important to determine whether polarized vesicle trafficking is involved. While the known mechanisms, which involve nonspecific trafficking of vesicles followed by dendrite-specific endocytosis and/or binding to ankyrin, are sufficient to account for the distribution of Na^+ channels seen in CNS neurons, it has not been shown that other mechanisms are not involved. Indeed, it is likely that a targeting process involving polarized vesicle trafficking would be significantly more efficient than one that relies on the nonspecific trafficking of vesicles. Also, it remains to be explained how dendrite-specific endocytosis is achieved. It is well documented that axonal membranes are capable of mediating endocytosis [37]. Thus, the endocytosis motif found in $Na_v1.2$ must possess distinctive qualities that enable it to direct endocytosis, specifically in dendrites. One possibility is that the motif interacts specifically with adaptor proteins that are active only in dendrites. As with the mechanisms underlying Kv4.2 targeting, unraveling the mechanisms of $Na_v1.2$ targeting will require the identification of proteins that interact with its targeting motif.

For Kv1 channels, defining the role of Kvβ in targeting will be critical. To do this, it will be necessary to identify other proteins in the T1/Kvβ complex. Because vesicle trafficking appears to play an important role in axonal targeting mediated

by the T1 domain, it will also be important to identify any kinesins involved in the transport of Kv1 channels and any adaptor proteins that might connect kinesins to the channel.

The contribution of RNA transport to polarized targeting of ion channels is another area that merits further study. Currently, RNAs encoding many proteins, including ion channels, have been detected in dendrites; however, the significance of RNA transport for subcellular localization is not clear [24]. Perhaps the best documented examples of ion channels that are translated in dendrites are the AMPA receptors GluR1 and GluR2 [17]. Dendritic translation of both channels is induced in response to electrical activity; however, the importance of dendritic translation to subcellular targeting of AMPA receptors is uncertain. The C terminus of AMPA receptors contains a sequence that is sufficient to mediate dendritic targeting of proteins, and so, it is likely that GluR1 and GluR2 would be targeted to dendrites even in the absence of dendritic RNA transport [30]. Accordingly, RNA transport is likely used as a fast way to deliver protein to dendrites in response to a particular stimulus, rather than a mechanism for shaping the overall AMPA receptor distribution. Further study will be necessary to completely characterize the role of compartment-specific translation in defining the subcellular distribution of dendritic ion channels.

In conclusion, ion channels are targeted to specific compartments in neurons by diverse mechanisms that have been uncovered through the identification of peptide-targeting motifs. In the future, the identification of proteins that interact with these motifs will enable further understanding of how neurons create and maintain the complex expression patterns of ion channels that enable generation and processing of electrical signals.

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