Molecular Mechanisms, Biological Actions, and Neuropharmacology of the Growth-Associated Protein GAP-43

John B. Denny*

Department of Ophthalmology, University of Texas Health Science Center, San Antonio, Texas, USA

Abstract: GAP-43 is an intracellular growth-associated protein that appears to assist neuronal pathfinding and branching during development and regeneration, and may contribute to presynaptic membrane changes in the adult, leading to the phenomena of neurotransmitter release, endocytosis and synaptic vesicle recycling, long-term potentiation, spatial memory formation, and learning. GAP-43 becomes bound *via* palmitoylation and the presence of three basic residues to membranes of the early secretory pathway. It is then sorted onto vesicles at the late secretory pathway for fast axonal transport to the growth cone or presynaptic plasma membrane. The palmitate chains do not serve as permanent membrane anchors for GAP-43, because at steady-state most of the GAP-43 in a cell is membrane-bound but is not palmitoylated. Filopodial extension and branching take place when GAP-43 is phosphorylated at Ser-41 by protein kinase C, and this occurs following neurotrophin binding and the activation of numerous small GTPases. GAP-43 has been proposed to cluster the acidic phospholipid phosphatidylinositol 4,5-bisphosphate in plasma membrane rafts. Following GAP-43 phosphorylation, this phospholipid is released to promote local actin filament-membrane attachment. The phosphorylation also releases GAP-43 from calmodulin. The released GAP-43 may then act as a lateral stabilizer of actin filaments. N-terminal fragments of GAP-43, containing 10-20 amino acids, will activate heterotrimeric G proteins, direct GAP-43 to the membrane and lipid rafts, and cause the formation of filopodia, possibly by causing a change in membrane tension. This review will focus on new information regarding GAP-43, including its binding to membranes and its incorporation into lipid rafts, its mechanism of action, and how it affects and is affected by extracellular agents.

Key Words: synaptic plasticity, neuromodulin, neurotransmitters, growth cones, neurotrophins.

INTRODUCTION

The neuronal growth-associated protein GAP-43 is also known as neuromodulin, B-50, P-57, F1 and pp46 [9]. The structure and function relationships of this protein have been reviewed previously [9]. The protein was initially found to be associated with nerve growth [85] and was subsequently purified and characterized [20]. The importance of GAP-43, in general, is shown by the fact that deletion of the GAP-43 gene in mice results in death early in the postnatal period [89]. In another study, it was found that neuronal growth cones that were depleted in GAP-43 were deficient in persistent spreading, branching, and adhesion [3]. GAP-43 was found to interact with heterotrimeric G proteins and activate G protein-coupled receptor transduction [88]. Finally, GAP-43 is one of the main substrates for protein kinase C in the brain [15] and was found to participate in long-term potentiation [56]. These results suggest necessary roles for GAP-43 in the nervous system. Much effort has been directed toward understanding how GAP-43 produces its effects. This review will present recent information regarding the molecular mechanisms, biological actions, and neuropharmacology of GAP-43. It will include a discussion of the GAP-43 gene and how GAP-43 is synthesized and bound to the cytosolic face of membranes of the early secretory pathway, the incorporation of GAP-43 into lipid rafts, and the sorting of GAP-43 to the plasma membrane. This will be followed by a discussion of the way in which GAP-43 is affected by GTP-binding proteins, the specific mechanisms by which GAP-43 may act, and by new evidence regarding the proteolysis of GAP-43 by purified 20S proteasome and by the ubiquitin/proteasome system. Finally, the biological actions of GAP-43 will be considered in detail, including its locations in the central and peripheral nervous systems, the effects of neurotrophins and cell adhesion molecules on GAP-43, and the effects of neurotransmitters and pharmacological agents. The role of GAP-43 in long-term potentiation and learning will also be discussed.

*Address correspondence to this author at Department of Ophthalmology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78229, USA; E-mail: Denny@uthscsa.edu

SYNTHESIS OF GAP-43

The GAP-43 gene is located on chromosome 3 in humans and chromosome 16 in mice [9]. It contains three exons, with the first exon coding for only the N-terminal 10 amino acids of GAP-43, while the second exon contains most of the coding region, and includes Ser-41, the site phosphorylated by protein kinase C [9]. A 230 base pair region located in the GAP-43 gene, immediately upstream of the protein coding sequence, has been proposed to contain a TATA-less promoter [27]. The most common site of transcription initiation is located 52-56 nucleotides 5' of the initiation codon [9]. In addition, more distal CCAAT and TATA consensus sequences are present [27]. The rat GAP-43 promoter region has also been described as having seven E-boxes that are organized in two clusters, one distal (E3 to E7) and one proximal (E1 and E2) [19]. Only the most proximal box, E1, was found to significantly affect GAP-43 promoter activity [19]. Basic helix-loop-helix transcription factors were found to regulate the expression of the GAP-43 gene [19]. The E1 box is therefore a *cis*-acting element that can positively or negatively affect promoter activity depending on which basic helix-loop-helix transcription factor binds [19]. A repressive

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element that contributes to neuron-specific gene expression has been described that is downstream of the TATA box in the genes for nitric oxide synthase, the protein SNAP-25 (synaptosome-associated protein of 25 kDa), and GAP-43 [96].

The RNA binding protein HuD is required for GAP-43 mRNA stability and GAP-43 gene expression [65]. HuD is an ELAV (embryonic lethal abnormal vision) protein, and is in a group that includes the proteins HuB and HuC [70]. HuD binds to the 3' untranslated region of GAP-43 mRNA in a poly(A) tail length-dependent manner [8]. The stability of GAP-43 mRNA is dependent on the activity of protein kinase Cα [70]. Genetic or pharmacological inactivation of PKCα abolished the HuD-induced stabilization of GAP-43 mRNA and the associated increase in GAP-43 protein [70]. Previous work had shown that NGF (nerve growth factor)-induced increase in GAP-43 mRNA levels in PC12 cells were mimicked by the addition of phorbol esters, which activate protein kinase C [72]. The phorbol ester-induced stabilization of GAP-43 mRNA was blocked by the protein kinase inhibitor polymyxin B [72]. Ribosomes and mRNA have been localized in mammalian axons and growth cones [14]. Although ribosomes, HuD, and GAP-43 mRNA have been detected in axonal growth cones [86], this may be a secondary site of synthesis, as axonal protein synthesis does not appear to be necessary if protein synthesis in the cell body is available [105]. It is nevertheless possible that local protein synthesis is necessary to provide growth-related proteins that sensitize the growth cone to guidance cues [35]. It has been shown that inhibitors of protein synthesis prevent the turning of retinal growth cones in response to chemotropic agents such as netrin-1 [14].

Rat GAP-43 contains 226 amino acids [7]. The true molecular weight of about 25 kDa is much lower than the apparent molecular weight of 43 kDa that is observed on SDS-polyacrylamide gels, and this occurs because the highly charged nature of GAP-43 causes it to bind less than the average amount of SDS per amino acid, and because the protein has an elongated structure [9]. The protein contains many charged and few hydrophobic residues. It contains only one Phe, 2 Leu, 3 Ile, 7 Val, and no Trp or Tyr [7]. Met is present at positions 1 and 5 only. By contrast, it contains 30 Lys, 5 Arg, 3 His, 35 Glu, and 22 Asp, as well as an unusually large number of Pro at 17 and Ala at 45. One-fifth of the total residues are alanine. The protein has little secondary structure [39], which may in part be attributable to the large number of Pro residues. In the absence of nonionic detergent, GAP-43 will not sediment into a sucrose gradient at a potassium concentration similar to that existing in the cytosol, consistent with it being primarily unfolded (J. B. Denny, unpublished results). The addition of nonionic detergent appears to induce the protein to fold into a more compact structure that will sediment into a sucrose gradient.

PALMITOYLATION AND BASIC RESIDUES ARE REQUIRED FOR THE INITIAL BINDING OF GAP-43 TO MEMBRANES

GAP-43 was suggested to bind to membranes as a result of palmitoylation [84]. The protein was subsequently shown to be palmitoylated at Cys-3 and Cys-4, and mutation of these residues to either threonine or glycine eliminated the binding of GAP-43 to membranes [55,106]. The N-terminal 20 amino acids of GAP-43 in rat corresponds to MLCCM RRTKQVEKNDEDQKI [7]. The fatty acid that is added is mainly palmitate, although some acylation with stearate also occurs [53]. At steady-state, most of the GAP-43 in COS-1 or PC12 cells was found to be depalmitoylated, but nevertheless membrane-bound [53]. These results indicate that the palmitate chains of GAP-43 do not serve as a permanent membrane anchor [53]. A cytosolic acyl protein thioesterase could potentially carry out the depalmitoylation of GAP-43 [25,54,79]. When the polybasic region containing Arg-6, Arg-7, Lys-9, and Lys-13 was present, GAP-43 was palmitoylated and membrane-bound [53]. The protein was then depalmitoylated but was nevertheless retained at the membrane, apparently by interaction with other membrane components [53]. If the four residues were replaced by uncharged residues, the protein was palmitoylated, membrane-bound, and depalmitoylated, but was not retained at the membrane [53]. Removal of the palmitates with hydroxylamine did not release native GAP-43 from the membrane [53]. Since the N-terminal 10 amino acids [106] and N-terminal 11 amino acids [60] of GAP-43 are sufficient to bind a reporter protein to the membrane, this suggests that the three basic residues, Arg-6, Arg-7, and Lys-9, are sufficient to complete the GAP-43-membrane interaction that is initiated by palmitoylation, and that Lys-13 is not necessary. Because mutation of Cys-3 and Cys-4 block the membrane binding of GAP-43 [55,106], this suggests that the palmitoylation of GAP-43 is the initial event and is followed by the interaction of the three basic residues with the membrane. Although the palmitoylation consensus sequence of GAP-43 is not clear, the presence of Met-1 and Leu-2 next to Cys-3 and Cys-4 appear to be part of what is recognized by the palmitoyltransferase [54,79], which is also called a protein acyl transferase (PAT) [54]. These results suggest that one purpose of palmitoylation is to bring Arg-6, Arg-7, and Lys-9 of GAP-43 into alignment with the membrane, so that binding can take place. Once this has been accomplished, the palmitate chains appear to be no longer needed and can be removed. It is not yet clear to what extent additional membrane binding of GAP-43 may take place downstream of the three basic residues, except for the proposed interaction between the GAP-43 effector domain and the phospholipid phosphatidylinositol 4,5-bisphosphate, as discussed below.

GAP-43, in its unpalmitoylated form, was found to bind *in vitro* to liposomes which contained 20% phosphatidylserine and 80% phosphatidylcholine [39]. However, these experiments were carried out at a GAP-43 concentration of 1 μM, which may be much greater than the concentration of non-membrane-bound GAP-43 that exists in the neuron at any moment. A high concentration of GAP-43 may lead to interactions that may not occur at lower concentrations of the protein. Although GAP-43 exists at a high concentration in growth cones [40], this is primarily the concentration of membrane-bound GAP-43 that exists at steady-state. The concentration of free GAP-43 that is emerging from ribosomes immediately prior to targeting would be expected to be much lower than this. When *in vitro* experiments were carried out at a 10,000-fold lower concentration (100 pM) of GAP-43, the protein was found to become membrane-bound

when incubated with isolated microsomes containing the ER-Golgi intermediate compartment [61]. This association was blocked by agents that inhibit palmitoyltransferases, such as dithiothreitol, tunicamycin, and low temperature [61], suggesting that enzymatic palmitoylation of GAP-43 is required for GAP-43 to become membrane-bound. Therefore, the palmitoylation of GAP-43 has at least one role, and that is to initiate the binding of the cytosolic protein to the membrane. Additional roles, including the incorporation of GAP-43 into lipid rafts, the possibility of dynamic palmitoylation at the plasma membrane, and the role of palmitoylation in the maintenance of filopodia, will be discussed below.

BINDING OF GAP-43 TO MEMBRANES OF THE EARLY SECRETORY PATHWAY

In the neuronal cell body, GAP-43 is synthesized on free ribosomes but the protein does not go directly from synthesis to the plasma membrane. Instead, it first binds to membranes of the early secretory pathway, which includes the ER-Golgi intermediate compartment (ERGIC), *cis*-Golgi network (CGN), and *cis*-Golgi [4], and then travels by vesicle transport through the secretory pathway to the plasma membrane. This has been shown using the compound brefeldin A, which disrupts the secretory pathway [36]. SNAP-25 (synaptosome-associated protein of 25 kDa) associates with membranes following its palmitoylation in a manner similar to that described for GAP-43 [36]. SNAP-25 is a SNAP receptor, or SNARE, for the soluble NSF attachment proteins (SNAPs) that are required for the vesicular transport of proteins [36]. NSF refers to the N-ethylmaleimide-sensitive factor [36]. It was shown that an intact secretory pathway is required for SNAP-25 to be targeted to the membrane [36]. When the secretory pathway was disrupted by brefeldin A, no palmitoylation or membrane association of SNAP-25 took place [36]. The palmitoylation of GAP-43 was also inhibited under these conditions but not the palmitoylation of heterotrimeric G proteins [36]. This mechanism allows GAP-43 to be sorted onto vesicles which can travel down the axon by fast axonal transport, as discussed below, to ultimately reach the growth cone plasma membrane or presynaptic membrane. Additional evidence has been provided by transfection experiments. When GAP-43 cDNA was transfected into COS-7 cells, prominent GAP-43 immunostaining was seen at the Golgi apparatus [55]. Mutation of Cys-3 and Cys-4 to glycine eliminated this Golgi localization and the mutated GAP-43 showed a cytosolic distribution [55]. Binding of GAP-43 was therefore suggested to take place on the cytosolic surface of the secretory pathway, with the protein becoming membrane-bound due to its affinity for the Golgi palmitoyltransferase [55]. In agreement with these results, *in vitro* experiments showed that GAP-43 was palmitoylated, using a Triton X-114 extraction method, by isolated microsomes containing the ER-Golgi intermediate compartment, in a preparation that was free of Golgi apparatus, as well as by purified Golgi apparatus [61]. The *in vitro* results with GAP-43 and ERGIC membranes support the proposal that protein palmitoylation occurs early in the secretory pathway [61]. Protein palmitoylation in intact cells has been shown to occur at these early sites in the secretory pathway, specifically at an ER-Golgi intermediate compartment and at the *cis*-Golgi, but clearly not at later sites such as the *trans*-Golgi or *trans*-Golgi network (TGN) [11]. Recent work has shown that the palmitoylation motif of GAP-43 could redirect a PSD-95 chimera to axons [29] and could localize stathmin-related proteins to the Golgi if present in conjunction with a Golgi-specifying sequence [17].

INCORPORATION OF GAP-43 INTO LIPID RAFTS AND SORTING OF THE PROTEIN TO AXONS AT THE *TRANS*-GOLGI NETWORK

At some point following its initial binding to membranes and prior to its sorting to the plasma membrane, GAP-43 becomes part of lipid rafts, which are called detergent-resistant membranes or DRMs once they have been isolated [5,13,60]. The N-terminal 11 amino acids of GAP-43 are sufficient to direct green fluorescent reporter protein (GFP), in COS-7 cells, to cholesterol and sphingolipid-enriched membranes and to the Golgi apparatus and plasma membrane, but are not sufficient to direct GFP to lipid rafts [60]. Therefore, it was suggested that parts of GAP-43 beyond the N-terminal 11 amino acids are responsible for directing GAP-43 to rafts [60]. It was suggested that protein-protein interactions may be required to localize a protein to lipid rafts [60]. The N-terminal 20 residues of GAP-43 have been shown to target β-galactosidase to detergent-resistant membranes in PC12 cells, and the addition of two palmitates was required for this to take place [5]. The G protein G_0 was also enriched in these DRMs [5]. While it is true that GAP-43 cannot become part of a raft without first being palmitoylated [5], this does not necessarily mean that the palmitates must be present in order for the GAP-43-raft association to be maintained. The palmitates may not be necessary if GAP-43, once drawn to the raft by the palmitate chains, has bound to other proteins and lipids that are associated with the raft.

Like SNAP-25, GAP-43 is sorted onto vesicles in the *trans*-Golgi network, which then travel by fast axonal transport down the axon [36,84]. The raft formation described above has been suggested to aid in the sorting of GAP-43 into axonally directed vesicles in the *trans*-Golgi network [5,13]. Raft formation appears to occur in membranes of the late secretory pathway because these membranes, like the plasma membrane and membranes of endocytic compartments, are rich in cholesterol and sphingolipids [13]. Rafts seem to have a preferential association with caveolae at the plasma membrane, but the relationship is not clear, since rafts are not restricted to caveolae and are abundant in cells that lack caveolae [13].

GAP-43 AT THE PLASMA MEMBRANE

At steady-state, GAP-43 is highly concentrated at the growth cone plasma membrane, at concentrations estimated at 50-100 μM [40]. It has been proposed that GAP-43 accumulates at detergent-insoluble, cholesterol-dependent rafts at the inner leaflet of the plasma membrane that are enriched in the acidic phospholipid phosphatidylinositol 4,5-bisphosphate, which is also designated PI (4,5) P_2 [15,51]. The addition of the cholesterol-sequestering agent cyclodextrin diminished the presence of GAP-43 in these structures [51]. GAP-43 is proposed to associate electrostatically with PI (4,5) P_2 [51] *via* the net positively charged region that extends from Ile-38 to Lys-56, and which has been called the effector domain

[51] or IQ domain [16]. The sequence of this region in rat GAP-43 corresponds to IQASFRGHITRKKLKDEKK [16]. This region also contains the protein kinase C phosphorylation site, Ser-41, which can be seen in the sequence. Because GAP-43 contains multiple positive and PI (4,5) P_2 multiple negative charges, the interaction of the two molecules can lead to extensive crossbridges and cluster formation [51]. In agreement with this proposal, lateral clustering of PI (4,5) P_2 in membranes has been achieved by the addition of basic peptides [93]. GAP-43 has been shown to completely colocalize with the GPI (glycosylphosphatidylinositol)-linked raft marker protein Thy-1 in primary cultures of hippocampal neurons [1]. GPI-linked NCAM (neural cell adhesion molecule)-120 is also found in the same lipid rafts as GAP-43, while the transmembrane isoforms NCAM-140 and NCAM-180 are not [51]. This is consistent with the finding that transmembrane proteins are usually not present in lipid rafts [13]. It is not yet clear how rafts in the outer leaflet of the plasma membrane, containing sphingolipids, Thy-1, and NCAM-120, and rafts in the inner leaflet, containing GAP-43, are held together [13].

When isolated growth cones were provided with radioactive palmitate, label was incorporated into GAP-43 [84], which is puzzling if the only function of the palmitate chains is to initially bind GAP-43 to membranes. This suggested that palmitoylation is dynamic in the growth cone and that GAP-43 passes through cycles of palmitoylation and depalmitoylation at the plasma membrane, possibly in response to the binding of extracellular compounds. However, when various pharmacological agents were applied, including carbachol, isoproterenol, and forskolin, no change in the palmitoylation of GAP-43 was detected [6]. Because palmitoyltransferases are present at the plasma membrane, it may always be possible to palmitoylate plasma membrane-bound GAP-43 if Cys-3 and Cys-4 are available and an excess of palmitoyl CoA is provided. Depalmitoylation by an acyl protein thioesterase would follow. Thus, cycles of GAP-43 palmitoylation and depalmitoylation could occur at the plasma membrane without necessarily having any biological effect. Nevertheless, it cannot be ruled out that palmitoylation and depalmitoylation control GAP-43 function in some way at the plasma membrane. As an example, palmitoylation of the postsynaptic density protein (PSD-95) at the synapse was found to be regulated by glutamate receptor activity [30]. The interaction of PSD-95 with other proteins at the plasma membrane in a non-neuronal model system was regulated by the ongoing palmitoylation of PSD-95 [30]. GAP-43 has also been shown to be ADP-ribosylated, and primarily at Cys-3 and Cys-4 [76]. This would be expected to occur after GAP-43 targeting, since modification of Cys-3 and Cys-4 would block palmitoylation.

GAP-43 at the axonal growth cone plasma membrane is associated with the formation of filopodia, which are finger-like projections that contain three functionally distinct adhesions [87]. The tip adhesion is involved in signal reception, the shaft adhesion controls lamellar extension, and the basal adhesion is associated with filopodial emergence [87]. Formation of filopodia, in response to extracellular cues, is thought to be the first step in the process of neuronal branching [31]. GAP-43 has also been associated with neurotransmitter release and with endocytosis and synaptic vesicle recycling at the presynaptic plasma membrane. As will be discussed below, all of these effects are associated in various ways with GTP-binding proteins.

GAP-43, G PROTEINS, SMALL GTPASES, AND FILOPODIA

In early studies, GAP-43, *via* only its N-terminal 10 residues, was found to activate the heterotrimeric GTP-binding protein G_0 and cause filopodial extension [90]. GAP-43 mutated at Cys-3 and Cys-4 did not bind to membranes and was inactive [90]. Mutants of GAP-43 at Arg-6 or Lys-9 were also inactive, again implicating these basic residues in GAP-43 function.

GAP-43 function is also associated with certain members of the Ras superfamily of small GTPases, including Rab, RalA, RalB, and the Rho GTPase family members RhoA, Rac1, and cdc42. The Rho GTPase family is extensively involved in neuronal development [37]. For example, the formation of lamellipodia is promoted by Rac1, while the formation of filopodia is promoted by cdc42 [37]. Some initial findings regarding GAP-43 and small GTPases were obtained when proteins were sought which interact with GAP-43 in the presence of calcium [67]. It was shown that GAP-43 interacts with rabaptin-5, which is an effector of the GTPase Rab5. This GTPase mediates membrane fusion in endocytosis. Through this interaction GAP-43 appears to modulate endocytosis and synaptic vesicle recycling [67]. GAP-43 was shown to colocalize with rabaptin-5 in the synaptosome-enriched fraction obtained from primary cultures of rat cortical neurons. Overexpression of GAP-43 in neurons was found to decrease the size of Rab5-containing endosomes, and this effect was altered by mutations affecting the ability of GAP-43 to bind calmodulin [67].

When Rat-1 fibroblasts expressed GAP-43, filopodial extensions were formed during spreading [2]. These effects were found to depend on the presence of Rho GTPase activity [2]. Deletion of the C-terminal residues 177-226 of GAP-43 did not alter these effects, nor did mutations at Ser-41, while mutations at Cys-3 and Cys-4 eliminated them [2]. RalA and RalB both promote filopodial formation and neurite branching and both promote the phosphorylation of GAP-43 by protein kinase C, but RalA acts through the exocyst complex while RalB acts through phospholipase D [50]. Phospholipase D cleaves phosphatidylcholine with the release of phosphatidic acid, which can be converted to the protein kinase C activator, diacylglycerol (DAG) [50]. These pathways are shown in Fig. (**1**). Inhibition of phospholipase D2 impaired L1-stimulated neurite outgrowth in cerebellar granule neurons [95]. The exocyst refers to a 734 kDa complex, consisting of at least eight subunits, that is required for exocytosis [94]. RalA is also associated with refilling the readily releasable pool (RRP) of synaptic vesicles [77], and has been shown to bind to the actin-binding protein filamin and thereby induce formation of filopodia [69].

Overexpression of a constitutively active form of cdc42 in COS-7 cells resulted in about a fivefold increase in the number of cells with filopodia, and cotransfection with a fusion protein containing the N-terminal 14 residues of GAP-43 produced a further increase in the number of cells

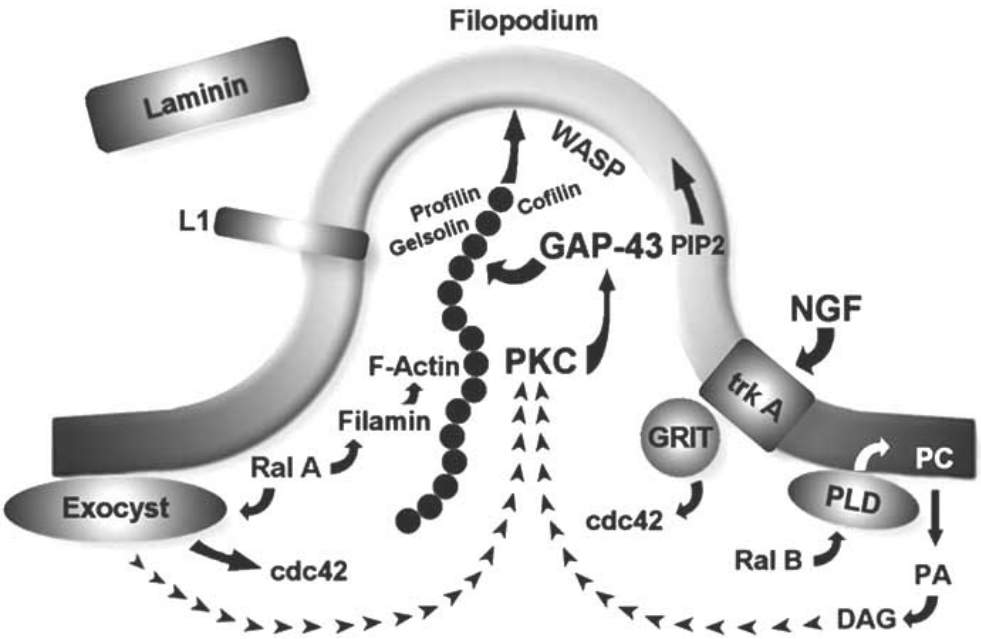

Fig. (1). Summary of some proposed mechanisms involved in filopodial formation. These pathways are discussed in detail in the text. The axonal growth cone plasma membrane is shown protruding as a filopodium begins to form. The extracellular matrix protein laminin is shown to the left, near the transmembrane cell adhesion protein, L1. The three actin-binding proteins, gelsolin, profilin, and cofilin, are shown near the F-actin filament, where they have promoted filament dynamics. At this point they are not inactivated by being bound at the plasma membrane to the acidic phospholipid phosphatidylinositol 4,5-bisphosphate, which is designated PI (4,5) P_2 in the text and PIP2 in the diagram, because prior to local stimulation PIP2 is bound to GAP-43, as shown. Upon local stimulation, which in this case is the binding of nerve growth factor (NGF) to its high affinity receptor trk A, as shown at right, a series of events are set into motion. The Rho-GTPase activating protein Grit activates the Rho GTPase cdc42, which promotes filopodial formation. The small GTPase RalA, which along with RalB promotes filopodial formation and neurite branching, activates the exocyst complex, which in turn activates protein kinase C (PKC), located in the center of the diagram. The exocyst also activates cdc42. RalA causes the actin-binding protein filamin to join the F-actin filament, which also aids in the extension of the filopodium. RalB, on the right, activates phospholipase D (PLD), which in turn cleaves the phospholipid phosphatidylcholine (PC) to phosphatidic acid (PA), which is converted to diacylglycerol (DAG). The DAG then stimulates protein kinase C. The activated protein kinase C phosphorylates GAP-43 at Ser-41, as indicated by the arrow, which releases phosphorylated GAP-43 from PIP2 and allows the GAP-43 to potentially act as a lateral stabilizer of the F-actin, shown to the left of GAP-43. The released PIP2 diffuses in the membrane, as shown by the arrow. It then promotes the adhesion of the F-actin with the membrane, and by so doing aids in the formation of the filopodium. This effect also involves the activation of the Wiskott-Aldrich syndrome protein (WASP) by cdc42 and PIP2 and by the recruitment of the Arp2/3 complex (not shown) to the site. WASP binds directly to PIP2, actin filaments, and cdc42. For simplicity, the binding of GAP-43 to calmodulin is not shown, but in the unstimulated cell it would occur similarly to what is shown for the binding of GAP-43 to PIP2. Upon phosphorylation by protein kinase C, GAP-43 would be released from calmodulin, as it is from PIP2, possibly for interaction with F-actin. Unphosphorylated GAP-43, if not bound to PIP2, would act as a capping protein for the actin filament and would oppose filopodial extension. The F-actin shown is part of the membrane skeleton and is also called cell cortex actin. It is possible that GAP-43 is palmitoylated while the above interactions are taking place.

with filopodia [34]. If a dominant negative form of cdc42 was introduced, the number of cells possessing filopodia was less than in those that contained the GAP-43 construct alone [34]. It was therefore suggested that cdc42 may act downstream of the effect produced by the GAP-43 construct [34]. Since the application of the palmitoylation inhibitor 2-bromopalmitate reduced the number of filopodia after they had formed, it was suggested that continuous protein palmitoylation is necessary for filopodia to be maintained [34]. When a constitutively active form of ADP-ribosylation factor 6 (ARF-6), a non-Rho GTPase, was expressed in COS-7 cells, the filopodial-inducing activity of the GAP-43 construct was reversed [34]. ARNO (ADP-ribosylation factor nucleotide-binding site opener) and ARF-6 have been shown to regulate axonal elongation and branching through the activation of phosphatidylinositol 4-phosphate 5-kinase [42]. ARNO inhibition was found to increase axonal extension [42].

All of these data suggest that different mechanisms exist by which filopodia may be formed. The addition of GAP-43 to a non-filopodial producing cell like the Rat-1 fibroblast is clearly sufficient to cause that cell to make filopodia [2]. However, some cells have sufficient alternate factors to form filopodia even in the absence of GAP-43. This occurs when COS-7 cells, which do not express GAP-43, are transfected with a constitutively active form of cdc42 [34]. Consistent with this idea, the effects of GAP-43 (1-14) and constitutively active cdc42 together are additive [34]. Since the first 14 residues of GAP-43 do not contain the protein kinase C phosphorylation site, it was suggested that interaction of only the first 14 residues with membranes is sufficient to

alter the membrane tension in such a way that filopodial extension can be promoted [34].

The exocyst complex, phospholipase D, and GAP-43 all appear to be downstream effectors of GTPases. If certain GTPases cause GAP-43 to be phosphorylated, the question still remains as to how phosphorylated GAP-43 itself functions.

GAP-43 MECHANISM OF ACTION: INTERACTIONS WITH CALMODULIN, ACTIN FILAMENTS, AND PI (4,5) P_2 AND MODULATION BY PROTEIN KINASE C

Protein kinase C-mediated phosphorylation of GAP-43 at Ser-41, which lies within the effector domain, introduces a negatively charged phosphate group that eliminates the binding of GAP-43 to PI (4,5) P_2 [51] and calmodulin [16] and changes the interaction of GAP-43 with actin filaments [40]. GAP-43 can interact with both Ca/calmodulin and with apocalmodulin [16]. In the resting neuron, the concentration of calmodulin exceeds that of free calcium, implying that most of the calmodulin is in the apocalmodulin form [44]. Most of this calmodulin is also found to be membrane-bound [44]. Calmodulin blocked the *in vitro* association of GAP-43 with phospholipid vesicles [39]. However, since the binding of GAP-43 to calmodulin is terminated upon the phosphorylation of GAP-43 by protein kinase C, it implies that the GAP-43-calmodulin interaction occurs mainly in the resting cell. Consistent with this idea, a crosslinking study showed that GAP-43 is bound to calmodulin *in vivo* under conditions of low calcium but is released when the calcium concentration is increased or when GAP-43 is phosphorylated by protein kinase C [33].

Two models have been proposed for the mechanism of action of GAP-43 that take into consideration the fact that GAP-43 is phosphorylated by protein kinase C in the stimulated cell. In the first model, GAP-43 binds directly to actin filaments [40]. GAP-43 has been shown to bind to actin filaments in its unphosphorylated state, at a ratio of one GAP-43 molecule per 77 actin molecules, or one GAP-43 molecule per actin filament of approximately 203 nm in length [40]. When it is phosphorylated by protein kinase C at Ser-41, GAP-43 was bound to actin filaments at a different ratio of one GAP-43 molecule per 27 actin molecules, or one GAP-43 molecule bound every 63 nm along an actin filament [40]. Unphosphorylated GAP-43 appears to function as a barbed end actin capping protein that does not sever actin filaments, thus making GAP-43 similar to the protein capZ rather than members of the gelsolin family [40]. The capping function would attenuate filopodial extension in growth cones. When phosphorylated at Ser-41, GAP-43 appears to be a lateral stabilizer of actin filaments, which would favor filopodial extension [40]. Phosphorylated GAP-43 bound to actin with a higher affinity (K_d = 161 nM) than did unphosphorylated GAP-43 (K_d = 1.2 μM) [40]. Interaction of GAP-43 with G-actin appears to occur but is of low affinity [40]. Phosphorylation of GAP-43 by protein kinase C was required for GAP-43 to be associated with the membrane skeleton, since association was inhibited when Ser-41 was mutated to alanine [62]. GAP-43 also interacts with another cytoskeletal protein, fodrin or brain spectrin [80].

In the second model proposing the mechanism of action of GAP-43, the protein binds to the acidic phospholipid phosphatidylinositol 4,5-bisphosphate, and this binding, as indicated above, clusters the phospholipid in rafts at the inner leaflet of the plasma membrane [51]. This model brings GAP-43 into contact with the phosphoinositides, which have been reviewed previously [92]. Phosphatidylinositol can be phosphorylated at its head group, which places it in an important position in signal transduction [92]. PI (4,5) P_2 can bind the actin-binding proteins profilin, cofilin, and gelsolin, thus concentrating them at the plasma membrane and inhibiting their action on the membrane skeleton [51]. In this model, GAP-43-induced sequestration of PI (4,5) P_2 removes this inhibitory effect [51]. As a result, cortical actin dynamics are enhanced and cell spreading occurs. This interaction of GAP-43 with PI (4,5) P_2 is blocked if GAP-43 binds calmodulin or is phosphorylated by protein kinase C, and PI (4,5) P_2 is thereby released. Other factors that are activated by protein kinase C and Ca/calmodulin now cause a stimulus-induced recruitment of actin, and local actin assembly and neurite extension occur [51]. For example, the neuronal form of the Wiskott-Aldrich syndrome protein (WASP), termed N-WASP, which is activated by the Rho GTPase cdc42, contibutes to filopodial extension *via* the Arp2/3 complex [37,81,102]. The released PI (4,5) P_2 may itself promote local interaction of the membrane skeleton with the plasma membrane [78], aiding filopodial extension. PI (4,5) P_2 has also been shown to activate N-WASP [81]. WASP binds directly to PI (4,5) P_2, actin, and cdc42 [92]. In addition, PI (4,5) P_2 is a cofactor for phospholipase D [92]. This mechanism of GAP-43 action also raises an important point regarding phospholipases C and D at the plasma membrane. The receptor-mediated hydrolysis of PI (4,5) P_2 *via* phospholipase C represents a mechanism whereby profilin, cofilin, and gelsolin could be released from the membrane to promote actin filament synthesis, with the accompanying production of the protein kinase C activator, diacylglycerol , and has been suggested as a signaling mechanism for growth cone guidance [64]. The distinction is that phospholipase C would *remove* PI (4,5) P_2 from the site of receptor activation while release of GAP-43 from PI (4,5) P_2, following its phosphorylation by protein kinase C, would *provide* PI (4,5) P_2. The advantage of phospholipase D is that it consumes phosphatidylcholine, not PI (4,5) P_2, in order to eventually yield diacylglycerol. According to the second model, the cell cortex must first be prepared for stimulus-induced actin recruitment by the removal or sequestering of PI (4,5) P_2 by GAP-43 [51]. In this model GAP-43 is predicted to have minimal direct interactions with actin. GAP-43 was found to codistribute with MARCKS and CAP-23, suggesting that the three proteins are functionally related [99]. All three proteins have positively charged effector domains, and all three bind calmodulin, PI (4,5) P_2, and actin, and are phosphorylated by protein kinase C within the effector domain [51,99].

The two models for the mechanism of action of GAP-43 are not mutually exclusive. Once GAP-43 has been phosphorylated by protein kinase C and has been released from PI (4,5) P_2, it would be competent to bind to F-actin and act as a lateral stabilizer. This is shown in Fig. (**1**). Similarly, phosphorylated GAP-43 would be released from calmodulin and would be available for interaction with F-actin.

CLEAVAGE OF GAP-43 BY THE PROTEASOME

GAP-43, synthesized in a reticulocyte lysate translation mixture *in vitro* and in a nonubiquitinated form, was purified by binding to calmodulin-Sepharose and was found to be cleaved by purified 20S proteasome [23]. This result provided additional evidence that the protein is primarily unfolded following its synthesis in the cytosol, since folded proteins are too large to enter the 20S proteasome and are therefore not attacked. The cleavage was nonprocessive, meaning that large fragments of GAP-43 were produced rather than small fragments of eight amino acids or less. This nonprocessive cleavage may be a reflection of the unusual amino acid composition of GAP-43. It is not clear the extent to which the 20S proteasome might attack GAP-43 in the neuronal cytosol. The binding of GAP-43 to the membrane may occur at a sufficient rate so that attack by the proteasome occurs only minimally. Most of the 20S proteasome may also be in the form of the 26S proteasome, which could require that GAP-43 be ubiquitinated [23]. The addition of calmodulin and actin blocked the attack of GAP-43 by the 20S proteasome [23]. This again lends support to the idea that GAP-43 is unfolded. Complexes of GAP-43 with either calmodulin or actin would be expected to be too large to enter the proteasome, and GAP-43 would therefore be protected from attack.

The addition of the proteasome inhibitors lactacystin and MG132 to GAP-43-transfected NIH 3T3 cells and neuronal cultures resulted in increased levels of GAP-43 and polyubiquitinated forms of GAP-43 [22]. These results give evidence that the *in vivo* turnover of GAP-43 is, at least in part, due to the action of the ubiquitin/proteasome system. The relevance of these results is clear, given that the ubiquitin/proteasome system has been found in growth cones [14].

GAP-43, NEUROTROPHINS, CELL ADHESION MOLECULES, DEVELOPMENT, AND REGENERATION

The question has been asked whether axons extend constitutively or in response to extracellular agents. It is now clear that they extend in response to extracellular agents [35]. These agents are typically neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), as well as the extracellular matrix molecules laminin, heparan sulfate proteoglycans, and the cell adhesion molecules L1 and N-cadherin [35]. NGF binds with high affinity to the trkA receptor, and this receptor has recently been shown to bind the Rho GTPase-activating protein Grit [66], as shown in Fig. (**1**). Guidance cue families, which provide directional information to growing axons, inlcude the semaphorins, ephrins, slits, and netrins [37]. NGF increases GAP-43 expression in PC12 cells by increasing the half-life of GAP-43 mRNA [74]. It was shown that phosphorylated GAP-43 is required when neural cell adhesion molecules interact with growth cones [63]. It has been proposed that when NCAM, L1, or N-cadherin on the outer surface of a growth cone bind to an appropriate substrate, FGF receptors are clustered and activated and this results in the phosphorylation of GAP-43 and the subsequent changes in the membrane skeleton, causing the growth cone to advance [26]. Gap-43 is required for retinal ganglion cells axons to progress from the optic chiasm into the optic tract [47]. The optic chiasm was suggested as a decision point for retinal axons [89]. Although GAP-43 was not required for axonal growth cone formation, it nevertheless was required for the correct path to be followed by growth cones [89]. GAP-43 mediates retinal axon interaction with the lateral diencephalon during optic tract formation [103]. Mice lacking GAP-43 failed to form the anterior commissure, the hippocampal commissure, and the corpus callosum *in vivo* [83]. GAP-43 is required for the normal development of the serotonergic innervation of the forebrain, and was found to be necessary for axonal outgrowth and terminal arborization of serotonergic axons from the raphe nuclei [24]. GAP-43-null mice showed a nearly complete lack of serotonergic innervation of the cortex and hippocampus [24]. Abnormalities in serotonin innervation have been implicated in schizophrenia, depression, anxiety, and autism [24]. Inhibition of nitric oxide synthase (NOS) has been shown to induce production of GAP-43 in the developing retina of the postnatal rat [68]. Much stronger GAP-43 immunoreactivity was seen in the inner plexiform layer of the retina at postnatal days 10, 14, and 21 in rats treated with the NOS inhibitor L-NAME (N(G)-nitro-L-arginine methyl ester) than in untreated rats [68].

In the peripheral nervous system, an axon transected by injury or disease will regrow to its original destination and restore motor and sensory function. The fact that this does not occur in the central nervous system has been attributed to an inhibitory glial environment as well as to a loss of both intrinsic and extrinsic factors [35]. In adult mammals, retinal ganglion cells cannot regenerate their axons after optic nerve injury, and they subsequently undergo apoptotic cell death [52]. When the lens is injured by a small puncture wound, the outgrowth of ganglion cell axons into the optic nerve was found to be stimulated, possibly through the action of macrophages [52]. With an intact optic nerve, lens puncture still allowed macrophage infiltration into the eye that caused an increase in GAP-43 expression in ganglion cells across the entire retina [52]. GAP-43 and L1 have been shown to act synergistically to promote the regenerative outgrowth of Purkinje cell axons *in vivo* [104]. Purkinje cells do not express GAP-43 or L1 in adult mammals or regenerate axons into peripheral nerve grafts [104]. Transgenic mice were generated which expressed either L1, GAP-43, or L1 and GAP-43 [104]. Either protein alone could not produce the increase in Purkinje cell axonal sprouting into peripheral nerve grafts, implanted in the cerebellum, that was seen when both proteins were expressed [104]. This suggests that signals must be transduced from particular extracellular molecules to GAP-43 in order for neurite outgrowth to occur. Overexpression of GAP-43 and CAP-23 in dorsal root ganglion neurons increased axon growth *in vitro* and in the injured spinal cord [10].

GAP-43 IN THE ADULT CENTRAL NERVOUS SYSTEM

The level of GAP-43 declines in most neurons when mature synapses are formed, but the protein continues to be expressed in synapses of the limbic system and associative regions of the neocortex at high levels throughout life, while

being present at low levels in primary sensory and motor areas [9]. This pattern suggests that GAP-43 allows changes leading to information storage [9]. In an early study in the adult rat brain, GAP-43 mRNA was detected at pronounced levels in the hippocampus and particularly the CA3 region, the granular layer of the cerebellar cortex, locus coeruleus, mitral cells, the vagal motor nucleus and other parasympathetic preganglionic neurons, inferior olivary nucleus, raphe nuclei, certain thalamic midline and intralaminar nuclei, dopaminergic nigral and ventral tegmental nuclei, the granular olfactory paleocortex, the infragranular neocortex, and several nuclei of the hypothalamus and basal forebrain [48]. Many of the neurons showing substantial levels of GAP-43 mRNA had both long axonal paths and extensive arborization of terminals [48]. The hippocampal CA3 pyramidal cells, for example, were seen to have a highly dispersed axonal territory [48]. An increase in the level of GAP-43 has been found in the visual association and frontal cortices of schizophrenic individuals [75]. This suggests that an abnormally high level of GAP-43 leads to synaptic dysfunction [75]. A reduced level of GAP-43 mRNA was found in the dorsolateral prefrontal cortex of post-mortem samples from patients with schizophrenia [97]. A disrupted cortical map and an absence of cortical barrels were seen in GAP-43 knockout mice [57].

GAP-43 IN THE ADULT RETINA AND CORNEA

GAP-43 occurs in the mature retina [28]. Most of the immunoreactivity was seen in the inner plexiform layer [28]. During development, essentially all cell bodies in the ganglion cell layer and the majority of amacrine cells displayed immunoreactivity for the proteins HuC and HuD [28]. GAP-43 is present in tyrosine hydroxylase-positive retinal amacrine cells, suggesting that the protein may be involved in the release of dopamine from the terminals of these cells located in the inner plexiform layer [46]. The release of dopamine in the retina has been implicated in light/dark adaptation [58]. The levels of released dopamine increase in almost all retinae with light adaptation, and dopamine mimics the effects of light in many instances [98]. In additon, retinoic acid was found to have light-adaptive effects on horizontal cells in the retina [98]. GAP-43 is also present in the sensory neurons of the cornea and may contribute to their remodeling [59]. In humans, the fibers originate from both the trigeminal ganglion and the superior cervical ganglion [59]. Although GAP-43 is not prominent in either the retina or cornea, its presence nevertheless suggests some role for GAP-43 at these sites. An involvement in neurotransmitter release is a possibility in the retina. The need for GAP-43 in the terminals of the sensory neurons of the cornea is less clear. GAP-43 may play a role in adhesion in corneal nerve terminals, since the protein was found to always colocalize with NCAM in double label studies [59].

GAP-43, NEUROTRANSMITTERS, AND PHARMACOLOGICAL AGENTS

GAP-43 is present in certain neurons that release catecholamines, but it is not restricted to catecholaminergic neurons. As will be discussed below, long-term potentiation in the hippocampus involves neurons that contain GAP-43 but release glutamate as their neurotransmitter. Early studies showed that addition of anti-GAP-43 antibodies to permeabilized rat cortical synaptosomes blocked the calcium-induced secretion of norepinephrine [21]. These antibodies blocked the phosphorylation of GAP-43 [21]. In agreement with these studies, the convulsant drug 4-aminopyridine induced the phosphorylation of GAP-43 in hippocampal slices and caused the concomitant release of norepinephrine [41]. Recent work has shown that norepinephrine increases the expression of the GAP-43 gene, as well as those for the cell adhesion molecule L1 and laminin, in human neuroblastoma SH-SY5Y cells [49]. Because of a suggested decrease in noradrenergic transmission in depression, and an antidepressant-induced increase in the level of norepinephrine, the effect of this neurotransmitter on the neuronal plasticity effects of GAP-43 was studied [49]. Neuroblastoma cells treated with norepinephrine showed an elongated, granule-rich cell body and an increased number of neurites, when compared with untreated cells [49]. Cell survival was enhanced and proliferation was inhibited. Norepinephrine-treated cells showed alterations in the expression of 44 genes, as determined in a neurobiology cDNA microarray [49]. These effects suggest a role for norepinephrine in cell differentiation and in inducing the formation of synaptic connections. This increase in synaptic plasticity may play a role in the positive effects of antidepressants in the treatment of depression [49]. The level of GAP-43 gene expression was also studied in primary cultured rat hippocampal neurons treated chronically with 1 mM desipramine, 2 mM tranylcypromine, 1 mM lithium, and 10 mM haloperidol [18]. Desipramine was found to affect the expression of eight genes, and in particular to increase the expression of the GAP-43 gene. The increase in GAP-43 mRNA also occurred in the dentate gyrus of the hippocampus, as detected by *in situ* hybridization in brain slices, but not in other brain regions. GAP-43 expression was also increased by another antidepressant, tranylcypromine, but not by lithium or haloperidol [18]. Although the acute effect of desipramine is to inhibit the reuptake of norepinephrine in nerve terminals, these results dealing with GAP-43 suggest that desipramine may also function by altering synaptic plasticity [18]. Thus, desipramine may increase the level of norepinephrine not only by inhibiting reuptake but also by increasing the level of GAP-43-mediated norepinephrine release. The hippocampus receives noradrenergic innervation soley from the locus coeruleus, which contains GAP-43 mRNA in the adult [48]. When status epilepticus was induced in mice following treatment with pilocarpine, the level of GAP-43 was shown to decrease in the inner molecular layer of the dentate gyrus [12]. GAP-43 immunoreactivity was decreased in the inner molecular layer at 3-10 days following the induction of status epilepticus [12]. In rats, GAP-43 has been implicated in mossy fiber sprouting, which contributes to hippocampal hyperexcitability [12]. Treatment of rats with amphetamine followed by a withdrawal period caused an enhancement of amphetamine-induced dopamine release [43]. The phosphorylation of GAP-43 at Ser-41 in striatal synaptosomes was increased following amphetamine treatment [43]. Inhibition of protein kinase C blocked the amphetamine-induced dopamine release in rat striatal slices [45]. As indicated above, GAP-43 may assist in the release of dopamine from amacrine cells in the retina [46].

GAP-43 promotes peptide hormone secretion in mouse anterior pituitary AtT-20 cells [32]. The cells were transfected with GAP-43 cDNA. Potassium-stimulated release of β-endorphin was greatly enhanced in those cells expressing GAP-43, without a significant change in calcium influx, while corticotropin-releasing factor-evoked hormone secretion was not affected [32]. The transfected cells also showed extended processes when plated on laminin-coated substrates [32]. Angiotensin II, when binding to the angiotensin type 1 receptor, increased GAP-43 expression and neurite extension [101]. Angiotensin II stimulated the PI3K (phosphatidylinositol 3-kinase)-protein kinase B signal transduction pathway [100,101]. PI3K can phosphorylate PI (4,5) P_2 [92].

Long-term potentiation (LTP) is thought to be associated with the development of certain forms of learning and memory, such as spatial memory [82]. If the action of protein kinase C is blocked, LTP does not occur [56,82]. If GAP-43 is involved in learning, it was hypothesized that an overexpression of GAP-43 should lead to an increase in learning. The overexpression of GAP-43 in transgenic mice did lead to an enhancement of learning, as measured using an Olton maze [82]. This did not occur if Ser-41 of GAP-43 was mutated to alanine [82]. For LTP studies, the perforant path-granule cell synapse of the hippocampus was studied in the intact mouse. Transgenic mice, termed G-Perm, containing GAP-43 with Ser-41 mutated to Asp, showed enhanced LTP when compared to wild type mice, as did mice termed G-Phos, which contained the phosphorylatable form of GAP-43 [82]. In another study, the formation of spatial memories resulted in an increase in the level of the mRNA-binding protein HuD and posttranscriptional up-regulation of the GAP-43 gene in the rat hippocampus [71]. The Morris water maze was used as a learning paradigm, in which the spatial memory of rats was assessed by the time required to find a platform [71]. Studies involving alcohol and the hippocampus are relevant to the LTP studies. Fetal alcohol exposure (FAE) was found to alter the phosphorylation of GAP-43 by protein kinase C [91]. FAE caused a decrease in the amount of beta2 and epsilon isoforms of protein kinase C that were bound to the membrane [91]. Prenatal ethanol exposure caused a decrease in hippocampal protein kinase C activity in adult rat offspring [73]. In a study evaluating the effects of retinoic acid, the expression of GAP-43 mRNA was found to be increased following the application of both retinol and retinoic acid [38]. Ethanol was found to decrease GAP-43 mRNA expression in the presence or absence of retinol [38].

CONCLUDING REMARKS

GAP-43 appears to represent the union of two different proteins, one a palmitoylation substrate and one a phosphorylation substrate. The first 10 amino acids are encoded by one exon of the GAP-43 gene, and contain the palmitoylation sites and basic residues involved in membrane binding. The addition of a second exon allows GAP-43 interactions to be controlled by protein kinase C. In some ways GAP-43 appears to be similar to filamin. Both are intracellular and can interact with actin, both are phosphorylated by protein kinase C, both are activated following the action of small GTPases, and both contribute to filopodial extension. The major difference is that GAP-43 can be palmitoylated but filamin is not. Therefore, it is correct to say that GAP-43 is an actin-binding protein, but it is more than that as well.

The fact that the exocyst complex controls exocytosis, filopodial extension, and neurite branching illustrates how different processes at the plasma membrane are interrelated and how a single complex can participate in all of them. Thus, GAP-43 is associated with filopodial extension and neurite branching, which clearly allow a developing or regenerating neuron to grow. It is remarkable that these basic effects seem to have found special applications in the adult nervous system. In the normal adult brain, development and regeneration should not need to take place. But the neurons may still need to grow in the sense of adapting. Thus, long-term potentiation, learning, and memory occur as a means of adapting to new inputs, and this adaptation may require the release of different amounts of neurotransmitter and the release may need to occur in new places. Changes in neurotransmitter release and the formation of new branches, new synaptic connections, and new points of adhesion would be required continually and would require the continued expression of GAP-43. GAP-43 is not restricted to the release of a particular neurotransmitter. What appears to be more important is the manner in which a neurotransmitter needs to be released in a particular setting. The need for GAP-43 in the normal adult cerebral cortex, hippocampus, cerebellum, retina, cornea and many other areas can be rationalized based on these points.

The effects of various extracellular agents on GAP-43 expression and function show that pharmacological intervention in the actions of GAP-43 is possible. The fact that norepinephrine increases the expression of GAP-43 illustrates how this neurotransmitter may be capable of extending the range of its own activity, by up-regulating a protein that may facilitate its release. Pharmacological intervention would also include blocking the ubiquitin/proteasome system and thereby potentially increasing the expression of GAP-43. GAP-43 function appears to be interwoven not only with the function of actin and protein kinase C, but also with that of the phosphoinositides, which is reasonable since the phosphoinositides represent a very extensive signaling system. This system has many points of possible pharmacological intervention that could affect GAP-43.

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