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GTP-Binding Proteins and Potassium Channels Involved in Synaptic Plasticity and Learning

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Contents

Abstract

Inhibition of potassium channels is possibly the first step in the sequence of biochemical events leading to memory formation. These channels appear to be regulated directly or indirectly by GTP-binding proteins (G proteins), which may themselves be affected by phosphorylation and dephosphorylation in response to elevated calcium levels or other phenomena resulting from the blockage of the potassium channels. A wide variety of cellular phenomena, from transcriptional changes to axonal transport, are thus capable of being initiated by these events.

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Introduction

One of the most interesting questions in neurobiology is the question of what cellular changes are associated with learning and memory. These changes might consist of structural changes, ion current changes, messenger ribonucleic acid (mRNA) increases or other nuclear changes, or protein changes. Each of these categories overlaps with other categories. For instance, changes in proteins must underlie dendritic structural changes, and ion current changes may produce protein changes. Of the protein changes that have been found by various researchers, those involving biochemical information transduction or control pathways are most closely analogous to the information transmitting process in neurons themselves and have attracted the most attention. This is particularly true for G proteins, about which considerable new information has emerged in recent years. This article will discuss new findings concerning the role of G proteins in memory storage and their effect on other cellular phenomena involved in memory, including potassium channels and protein kinases.

G Proteins and Potassium Currents

Although comparatively little is known about the mechanisms of action of low-molecular-weight G proteins on potassium channels, activation and inhibition of potassium and calcium conductances by heterotrimeric G proteins are a comparatively well-studied phenomenon (Brown, 1990). In the most well-studied prototype mechanism, stimulation of β-adrenergic receptors results in activation of the stimulatory G protein G_s, which in turn activates adenylyl cyclase. This leads to higher levels of cyclic adenosine 5'-monophosphate (cAMP), which activates cAMP-dependent protein kinase, which then phosphorylates ion channels (Reuter, 1983; Trautwein and Hescheler, 1990).

In an alternative pathway, the receptor-associated G protein G_p activates phospholipase C, which hydrolyzes membrane lipids to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). The IP_3 acts to release Ca^{2+} from endoplasmic reticulum or (in muscles) sarcoplasmic reticulum (Berridge, 1984), whereas the DAG can activate protein kinase C (PKC) (Nishizuka, 1984). The PKC might possibly in turn phosphorylate the ion channels (Brown, 1990).

It has also been suggested that small G proteins can regulate K^+ channels indirectly by regulating the activity of the trimeric G proteins, since an endogenous *ras*, after binding to *ras*-GTPase-activating protein (*ras* GAP), inactivates cardiac muscarinic K^+[ACh] channels (Brown, 1990; Yatani et al., 1990). In contrast to the second messenger-mediated effects of G_p, the G_k trimeric G protein directly activates the atrial K^+[ACh] channel (Breitwieser and Szabo, 1985). Pertussis toxin-sensitive, inhibitory trimeric G proteins (G_i) are a component of the pathway for inhibition of muscarinic Ca^{2+}-activated K^+ channels in colonic myocytes (Cole and Sanders, 1989). The "other" G protein, G_o, activates K^+ channels in hippocampal pyramidal cells (Van Dongen et al., 1988). ATP-dependent K^+ channels are also directly gated by G proteins (Eddlestone et al., 1989; Parent and Coronado, 1989). In mast cells, activators of G proteins (including GTPγS, GppNHp, and AlF_4^-) inhibit an inward rectifier K^+ current and activate a different K^+ channel in excised patches (McCloskey and Cahalan, 1990). GTPγS also activates the S-potassium channel in Aplysia neurons (Brezina, 1988). The G protein(s) involved in these latter two experiments have not yet been characterized.

Thus, most of the K^+-channel effects of trimeric G proteins that have been studied consist of an activation or inhibition of agonist-linked K^+ channels. In contrast to the usually excitatory effects of the larger trimeric G proteins, the two low-mol-wt G proteins thus far found to affect K^+ channels (*ras* and cp20) are both inhibitory (*see below*).

Potassium Currents and Learning

Associative conditioning of *Hermissenda* by repeatedly pairing short flashes of light with mechanical vibration, e.g., from a rapidly accelerating turntable or a platform shaker, causes a sequence of well-defined ion current changes in the photosensitive cells of the *Hermissenda* eye, a five-neuron structure attached to the circumesophageal ganglion (Lederhendler et al., 1986; Crow and Alkon, 1978; Goh and Alkon, 1984). These current changes consist of a reduction of the voltage-dependent K^+ current (I_A) in the B cell soma membrane, in the cell's input resistance, and in the calcium-dependent potassium currents ($I_{K^+-CA^{2+}}$) (Farley et al., 1983; Alkon, 1984; Alkon et al., 1985). Reduction of these outward currents causes increased neuronal excitability, as well as a greater total influx of calcium during an action potential (Connor and Alkon, 1984; Alkon et al, 1982b). Artificially simulating these learning-induced changes in naive animals by injecting current into the B cells and pairing these injections with light stimuli, can mimic the behavioral effects of conditioning (Farley et al., 1983). Thus, decreases in K^+ currents, which lead to increased cellular excitability, have been shown to be necessary and sufficient for memory in *Hermissenda* (Alkon et al., 1982a).

In rabbit, the most widely used conditioning protocol is tone-eyeblink conditioning, in which an animal is subjected to a puff of air or mild periorbital electrical stimulus coincident with an audible tone. The primary change that has been observed following conditioning in rabbit hippocampal pyramidal cells is a reduction in the slow afterhyperpolarization (AHP) (Disterhoft et al., 1986, Coulter et al., 1989), which has been attributed to a reduction in the Ca^{2+}-dependent potassium current (Gustafsson and Wigstrom, 1981; Brown and Griffith, 1983). Reduction of this current permits the neuron to depolarize to its resting potential more rapidly, shortening its refractory period, thereby allowing it to fire more action potentials. Such an increase in excitability was observed in conditioned rabbits (Disterhoft et al., 1988). Decreases in K^+ currents have been found in approx 62% of the pyramidal cells (Disterhoft et al., 1986). Roughly the same proportion of cells (50%) show an increase in firing frequency after conditioning (Berger et al., 1983).

Thus, in both invertebrates and vertebrates, changes in potassium currents are an early important step in memory acquisition. Since G proteins are intimately involved in potassium currents, it is reasonable to ask whether G proteins are also involved in learning.

Learning and G Proteins

In an experiment from our laboratory designed to answer this question, *Hermissenda* were subjected to associative conditioning using the light-rotation paradigm of Alkon et al. (1985). Proteins were separated on a high-pressure liquid chromatography (HPLC) column under conditions designed to observe phosphoproteins. Associative conditioning increased the HPLC peak area of the phosphorylated form of a 20-kDa G protein designated cp20 by approx threefold (Figs. 1 and 2). Since the rate of ^3H-amino acid incorporation into this protein was not increased, but actually decreased by approx 30%, it was concluded that the effect was most likely posttranslational, i.e., an increase in its state of phosphorylation (Nelson et al., 1990).

Several additional experiments confirmed the fact that cp20 was a low-mol-wt G protein (Nelson et al., 1990):

1. Each fraction of cp20 from *Hermissenda* CNS eluting from the AX-300 ion-exchange column was assayed for GTPase and GTP-binding activity. A plot of the results showed a sharp peak of activity at the same position as cp20.
2. The cp20 peak from the AX-300 column was concentrated and injected into a size-exclusion HPLC column (GPC-100), and each fraction was analyzed for GTPase and GTPγS binding.

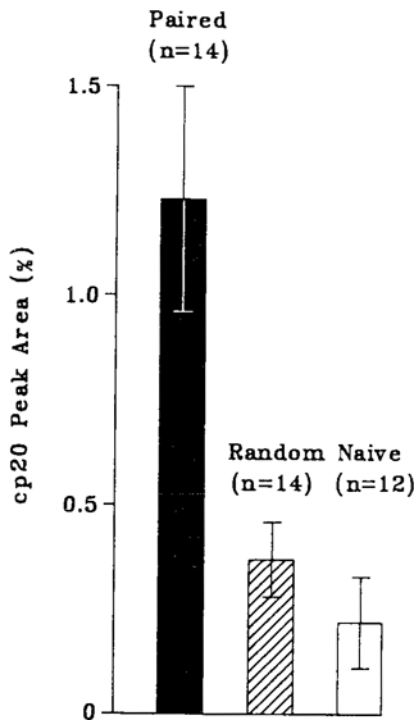

Fig. 1. Effect of associative conditioning on peak areas of phosphorylated cp20 in *Hermissenda* eye. *Hermissenda* were individually trained, and proteins from single eyes were separated by ion-exchange HPLC. The increase in cp20 phosphorylation, evident as an increase in the A_{280} peak areas as a percentage of total area of the retained peaks, was significant at $p < .01$ (Nelson et al., 1990).

GTPase and GTPγS binding were consistently found in the 20-kDa region. The stoichiometry of ^{35}S-GTPγS binding was at least 0.3–0.4 mol/mol protein. More recent measurements give values near 0.93 mol/mol.

3. Purified cp20 reacted with ^{32}P-labeled azidoanilido-GTP, which is a photoaffinity label for G proteins, to form a covalently-labeled derivative. This derivative was observed as a single spot on a one-dimensional SDS gel (Nelson, unpublished data).
4. Analyzing the cp20 peak from the ion-exchange column on an SDS acrylamide gel and then probing a nitrocellulose blot of the gel with ^{32}P-GTP revealed a single ^{32}P-band on autoradiography, at 20 kDa. Binding of blotted proteins to GTP is a common property of the low-mol-wt G proteins.
5. Cp20 was retained on GTP-agarose, an affinity chromatography medium for G proteins (Nelson, unpublished data).

Low-Mol-Wt G Proteins in Rabbit

In a similar experiment, the photoaffinity label azidoanilido-GTP-^{32}P was used to label all G proteins from the rabbit CA1 hippocampal region at a period 24 h after the end of 3 d of tone-eyepuff conditioning. Labeling of a 20-kDa G-protein was decreased after conditioning by about 35% in the paired group in the cytosol fraction and 25% in the particulate fraction (Nelson et al., 1991). One possible explanation for this was that the protein was bound to more endogenous GTP in the paired group, and this prevented labeling by the photoaffinity label. Alternatively, the rate of synthesis could have been decreased. Additionally, there were decreases of a small G protein (16 kDa) and a G protein between 32 and 36 kDa in the cytosol. These experiments suggest that there are also changes in G proteins following conditioning in higher animals. At present, it has not been established whether the 20 kDa protein, which is changed in rabbit, is rabbit cp20 or some other 20-kDa G protein.

Biological Effects of Cp20

The most obvious effect of injecting cp20 into the photoreceptor neurons from which it was isolated is an immediate increase in the response to light. Exposure of isolated dark-adapted *Hermissenda* circumesophageal ganglia to very low-intensity light flashes of light elicits a slow transient depolarization accompanied by a few action potentials. In contrast, in the same cell after an iontophoretic injection of cp20, a prolonged depolarization accompanied by numerous action potentials was observed (Alkon and Nelson, 1990) (Fig. 3). Iontophoretic injection of salt solution (K acetate) alone had no effect. This effect is

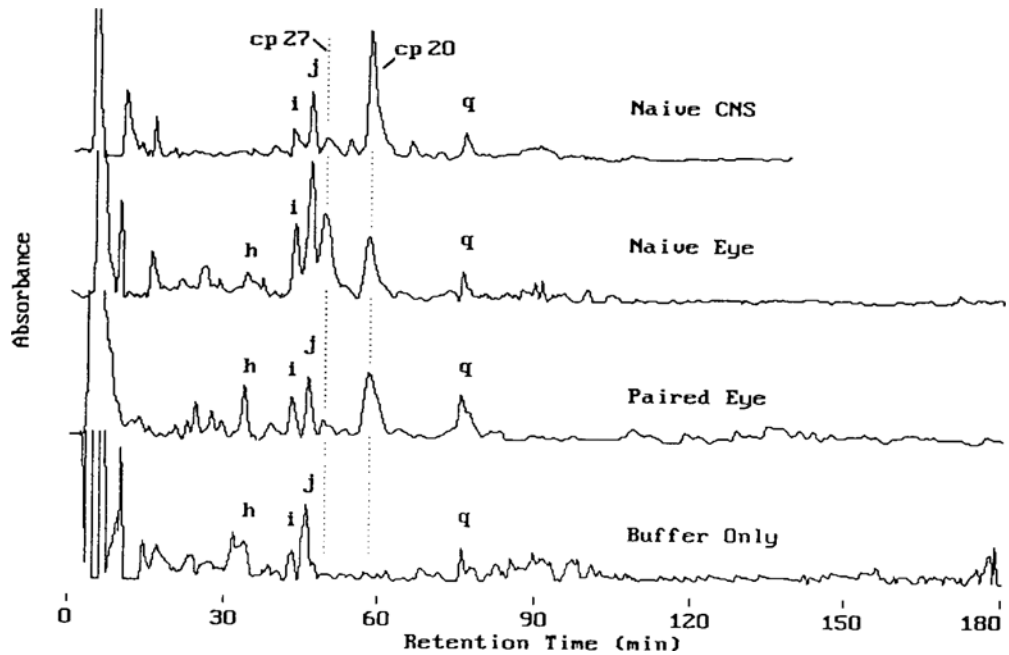

Fig. 2. Reversed-phase (C18) HPLC rechromatography of the phosphorylated peak of conditioning-associated GTP-binding protein cp20 isolated by ion-exchange (AX-300) HPLC. Samples equivalent to 1.14 naive central nervous systems, 18 naive eyes, or 6 paired eyes were chromatographed on AX300 under conditions designed to maximize separation of phosphoproteins. The cp20 peak was collected, concentrated, and reinjected onto the C18 column.

This experiment demonstrated that cp20 from paired eyes was the same as cp20 from naive eyes. This eliminated the possibility that the increased peak size was the result of a new protein that cochromatographed with an old peak, and supported the idea that the increased peak size was the result of a change in a preexisting protein. In other experiments (not shown), analysis of the cp20 peak on size-exclusion HPLC and SDS gel electrophoresis showed that this peak consisted of a single 20-kDa protein. Since the amount of material injected from the paired sample was only about 1/3 as much as the amount injected from the naive eye sample, this RP-HPLC experiment was also the clearest demonstration thus far of an increase in the phosphorylation of cp20 in *Hermissenda* after conditioning. The curve marked "buffer only" is a blank containing a sample of buffer from the ion-exchange column. Peaks h, i, j, and q are trace buffer contaminants. Absorbance scale: 0.0008 AUFS, except top curve, 0.0016 AUFS. (Nelson et al., 1990).

similar to that observed after conditioning. No other biomolecules, including *ras*, various protein kinases, or proteases, could produce this effect at the low concentrations at which cp20 affects these cells.

The biophysical basis for this excitatory effect is a blockage of the same two potassium channels that are partially blocked after associative conditioning, i.e., I_A and $I_{K^+ - Ca^{2+}}$ (Alkon et al., 1985). Voltage-clamp analysis of these cells indicated that I_A and $I_{K^+ - Ca^{2+}}$ were reduced by 50 and 70%, respectively. The inhibition of the currents was maximal 5 min after injection and remained constant for at least 15 min (Nelson et al., 1990). Cp20 has similar effects on other *Hermissenda* central neurons, such as LP1, a large motoneuron in the circumesophageal ganglion (C. Collin, unpublished results).

Recent data suggest that G proteins are associated with axonal transport. Application of 1μ*M* cp20 to isolated crab walking leg neurons reduced the number of particles moving in the retrograde direction, as observed by contrast-enhanced video microscopy (Moshiach et al., 1991). Other researchers have also found low-mol-wt G proteins associated with small axonal vesicles

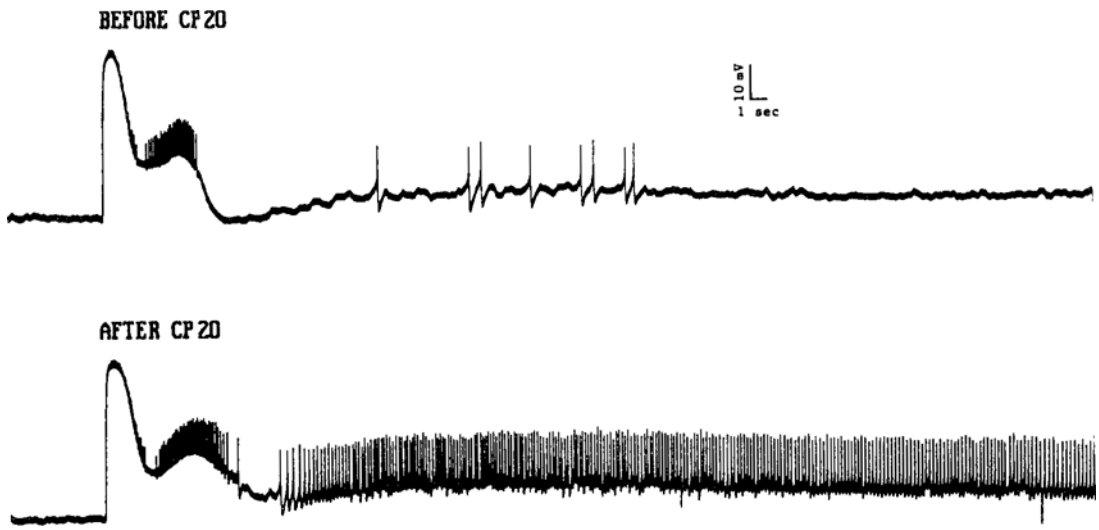

Fig. 3. Intracellular recordings of the effect of cp20 on the light response of *Hermissenda* type B photoreceptor neurons. Cells were maintained in darkness for 20 min with periodic 5-ms flashes of extremely faint white light. After several control light responses were recorded, cp20 was injected iontophoretically into the cell, and the responses to identical flashes of light were recorded. Approximately 90 ng of cp20 were present in 15 μL of 1*M* KAc in the iontophoresis electrode. It was estimated, based on the amount of current injected, that less than 3×10^{-14} g of protein was injected. Cp20 caused a marked increase in excitability, which was traced to inhibition of outward K^+ currents, whereas the sodium currents (the initial broad peak) and resting potential were unaffected. Injection of other proteins had no effect (Alkon and Nelson, 1990).

(Bielinski et al., 1989). The effect of cp20 on active particle transport is similar to the effects of other low-mol-wt G proteins on particle transport through the Golgi apparatus (Segev et al., 1988). For instance, it has been shown that mutant yeast defective in Ypt1 are unable to transport proteins into the vacuoles (Bacon et al., 1989). The *ras*-like G protein sec4, which cycles between the plasma membrane and secretory vesicles (Salminen and Novick, 1987; Goud et al., 1988), also regulates vesicular transport (Salminen and Novick, 1989). Other small G proteins, such as rab3a, which is only found in synaptic vesicles, may regulate synaptic vesicle fusion with the membrane in depolarization-induced exocytosis (von Mollard et al., 1990, 1991). Rab proteins may function in vesicular transport in higher animals in an analogous manner to YPT in yeast (Zahraoui et al., 1989).

Modification of axonal transport could be a mechanism by which a G protein could affect cellular morphology. Dendritic branching is known to be affected by associative conditioning (Alkon et al., 1990). Other small G proteins, such as rab2, can induce neurite outgrowth in vitro. Aggregates of cultured mouse mesencephalic neurons incubated with rab2 became dissociated, forming highly branched neurites with growth cones (Ayala et al., 1990).

Effects of *Ras* on Potassium Channels

The effects of *ras* on K^+ channels are similar to those of cp20, although *ras* is less potent than cp20. Iontophoretic injections of Ha-*Ras* into *Hermissenda* LP1 cells progressively reduced the amplitudes of the potassium current I_A by 30–40% and that of $I_{K+\ Ca2+}$ by 40–60% after a delay of 20 min, possibly representing the time required for posttranslational modification or membrane association of the *ras* (Collin et al., 1990a). The effect on I_A may have been caused by a reduc-

tion in the current inactivation kinetic rate. Virally transformed ras^{Val12} (v-*ras*) was much more effective than the nontransforming form (c-*ras*) at reducing the currents, which showed no sign of returning to normal after 60 min. In contrast, currents completely returned to normal levels by 50 min after injection of the nontransforming c-*ras* (Collin et al., 1990b).

Ras also activates outward Ca^{2+} currents in the same cells. A similar difference between c-*ras* and v-*ras* was observed, with the effects of v-*ras* being at least twice as large as c-*ras*, and showing no signs of reversal at 60 min. Moderately high electrode concentrations of *ras* (150 μg/mL) were required to observe both of these effects. In contrast, the cp20 effects are typically observed with concentrations of 0.1–1 μg/mL in the iontophoresis electrode.

Interestingly, the effects of cp20 on K^+ currents more closely resembled those of the virally transformed *ras* than normal c-*ras* in their intensity as well as the time duration. Since some of the effects of *ras* may involve *fos* and *jun* , other protooncogenes that affect DNA transcription (Sassone-Corsi et al., 1989; Doucet et al., 1990), it will be interesting to see what if any effects cp20 may have on this pathway. There is some evidence that learning can induce mRNA synthesis in *Hermissenda* and other species (Flexner et al., 1962; Zemp et al., 1966; Shashoua, 1968,1974; Rainbow, 1979; Mizumori et al., 1985; Nelson and Alkon, 1989, 1990). It is not yet known whether a G protein mediates this effect.

Long-Term Potentiation

Since long-term potentiation (LTP) in mammalian hippocampus has been used as a model for learning (Berger, 1984; Byrne, 1987), it would be of interest to know whether G proteins are also involved in this form of synaptic plasticity. Tetanus-induced LTP could not be induced in hippocampal slices from rats that had been intracerebrally injected 3–4 d earlier with pertussis toxin (Goh and Pennefather, 1989). Since pertussis toxin covalently labels and inactivates some G proteins (predominantly 30–40-kDa trimeric G proteins), this suggests that a functional G protein may be required for LTP. Pertussis toxin injected 2 d or less before the slices were prepared had no effect. The long time delay was presumed to be necessary for the toxin to cross the membrane.

These results suggest that a G protein could be involved at some point in the induction of LTP. Three days after injection of pertussis toxin into rat hippocampus, the late IPSP was reduced or absent, whereas the early IPSP was normal (Thalmann,1988). It was suggested that this could be owing to attenuation of the K^+ conductance. Surprisingly, injection of GTPγS, a nonhydrolyzable G protein agonist, into postsynaptic neurons, caused hyperpolarization and reduced the late IPSP current in a manner similar to pertussis toxin.

LTP is initiated by influx of calcium through *N*-methyl-D-aspartate (NMDA) receptor channels and is maintained by calcium-dependent intracellular messengers (Lynch et al., 1990). Although the main LTP effect involves calcium rather than potassium, there are also recent reports of an NMDA-independent form of LTP induced by high concentrations of the potassium channel blocker tetraethylammonium (Aniksztejn and Ben-Ari, 1991). Application of 50–100 μ*M* 4-aminopyridine, a K^+ channel blocker, caused effects resembling afferent tetanization, a plastic change in the hippocampus observed after high-strength stimulation (Hess and Gustafsson, 1990). However, evidence implicating G proteins and potassium currents acting together in either posttetanic potentiation or LTP is still lacking.

Possible Involvement of PKC

Although cp20 is phosphorylated in vivo following conditioning of *Hermissenda*, the active kinase for cp20 is not yet known. However, substantial evidence points to the involvement of PKC in learning and learning-related phenom-

ena in several species, including *Hermissenda* (Neary et al., 1986). Under conditions of intracellular Ca^{2+} elevation, introduction of 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) or phorbol esters into type B photoreceptor neurons reduces the conductance of the K+ channels involved in memory acquisition (Alkon et al., 1988). The ATP analog H-7 also prevents the changes in these two K^+ channels when bath-applied to *Hermissenda* circumesophageal ganglia during in vitro associative conditioning (Matzel et al., 1990).

Bank et al. (1988a) first observed a decrease in enzymatically measured PKC activity in the cytosolic fraction of rabbit CA1 hippocampus following tone-eyepuff conditioning. This was accompanied by a corresponding increase in membrane-associated PKC activity, suggesting translocation. Binding of phorbol esters, which are structural analogs of DAG, is also increased in rabbit hippocampus after conditioning, as well as in the specific *Hermissenda* neurons known to be involved in storage of learned light-rotation associative memories (Olds et al., 1989; McPhie et al., 1991). This suggests a possible membrane translocation or some other change in PKC that could lead to increased binding of DAG. Based on this involvement of PKC in other aspects of conditioning in *Hermissenda* and in rabbit, it is likely that PKC is also involved at some point in the actions of cp20. In this context, the analogy with the interaction between *ras* and PKC may be instructive. Inhibition of potassium channels in *Hermissenda* LP1 cells by v-*ras* was blocked by the protein kinase C (PKC) inhibitor staurosporine (Collin et al., 1990a). Inection of *ras* can cause an increase in the level of PKC's cofactor, DAG (Lacal et al., 1987a), as well as arachidonic acid (AA), both of which can activate PKC (Nishizuka, 1984; Price et al., 1989 Shearman et al., 1989). Rat thyroid epithelial cells transfected with v-*ras* have increased PKC activity (Spina et al., 1988). However, some of the effects induced by *ras*, such as morphological transformation, increases in c-*myc* expression, and the serum-response element of c-*fos*, may not be mediated by PKC (Fukumoto et al., 1990).

Phosphorylation of G Proteins

Of the various forms of *ras*, a variety of different kinase specificities have been reported. The c-Ki-*ras* p21 can be phosphorylated by cyclic cAMP-dependent protein kinase (A-kinase) as well as PKC (Ballester et al., 1987). Also, v-Ha-*ras* is phosphorylated exclusively by PKC (Jeng et al., 1987), whereas c-Ha-*ras* reportedly may be phosphorylated by both PKC and A-kinase (Saikumar et al., 1988). Platelet smg-21 and yeast RAS2 are phosphorylated only by A-kinase (Hoshijima et al., 1988; Resnick and Racker, 1988; Kawata et al., 1989). The *ras*-related protein rap-1b can be phosphorylated by calmodulin-dependent kinase Gr, as well as A-kinase (Sahyoun et al., 1991). A side reaction of the GTPase reaction can also result in autophosphorylation of *ras* independent of any kinase (John et al., 1988). *Ras* may also affect PK C activity. The mitogenic response to injected *ras* could be blocked in Swiss 3T3 cells by downregulating endogenous PKC by prolonged phorbol ester treatment. Conversely, microinjection of PKC could overcome this blockage (Lacal et al., 1987b). An additional complication is that phosphorylation may translocate *ras* proteins (Lapetina et al., 1989), and translocation may affect the susceptibility of low-mol-wt G proteins to phosphorylation (Nagata et al., 1989), or the activities of A-kinase (Scott et al., 1990) and PKC (Nishizuka, 1986). Phosphorylation of *ras* GTPase-activating protein (*ras*-GAP) by tyrosine kinases may inactivate GAP (Ellis et al., 1990; Molloy et al., 1989). Clearly, if all of these reactions occur under physiological conditions, there is a potential for complex regulatory interactions among the various G proteins and kinases.

Phospholipases

Involvement of phospholipase C or phospholipase A_2 could also be a possible site of action for cp20 or *ras*. Evidence for direct effect of *ras* on PKC via activation of phospholipases C or A_2 has been conflicting (Santos and Nebreda,1989). However, *ras*-GAP can be inactivated by the intra-

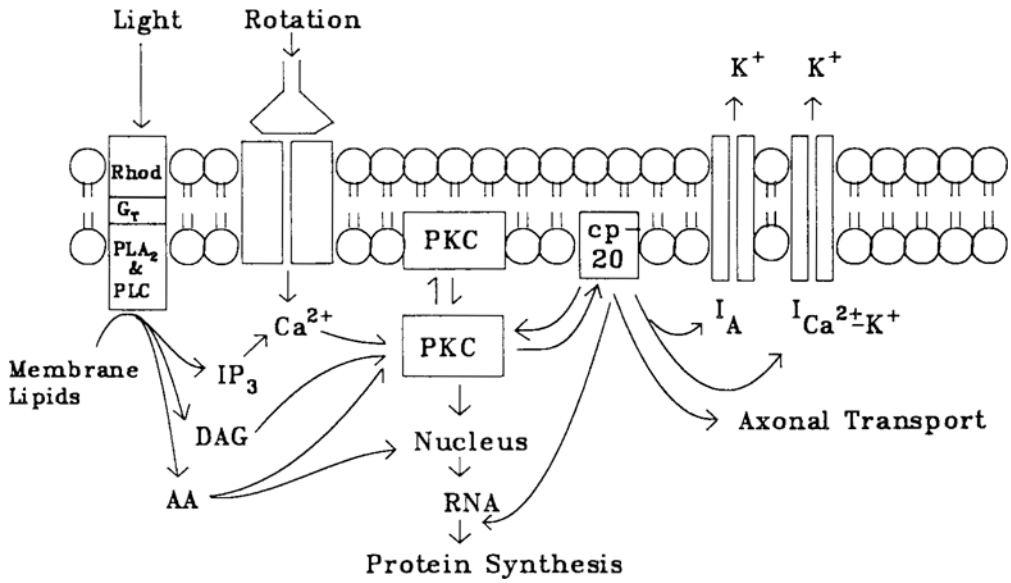

Fig. 4. Model describing the biochemical phenomena believed to occur during associative learning in *Hermissenda*. In this scheme, light or, in nonphotosensitive neurons, a neurotransmitter receptor, is hypothesized to act via rhodopsin (Rhod) and transducin (G_T) to activate phospholipases (PLA_2 and PLC) to produce IP_3, DAG, and AA. Synaptic input from the unconditioned stimulus (Rotation) simultaneously impinges on the neuron, resulting in the influx of calcium. Calcium is also released by IP_3 from intracellular stores. These three components (Ca^{2+}, AA, and DAG) converge to activate PKC synergistically, translating PKC to the membrane, where it phosphorylates the G protein cp20 and other proteins, possibly including the potassium channel. Cp20 then acts either directly or indirectly to block the two K^+ channels (I_A and $I_{Ca2+-K+}$) resulting in decreased K^+ efflux and a prolongation of the depolarization. Cp20 may also act on PKC and axonal transport or affect protein synthesis at the level of RNA translation. PKC and AA may also affect protein synthesis at the DNA level.

cellular messengers arachidonic acid and phosphatidic acid (Trahey and McCormick, 1987; Tsai et al., 1989; Yu et al., 1990). Conversely, AA metabolites, including prostaglandins $PGF_{2\alpha}$, PGE_2, and PGA_2, stimulate *ras*-GAP (Han et al., 1991). Since GAP normally acts to deactivate *ras* (by facilitating *ras*'s GTPase activity), these prostaglandins would act to increase the deactivation rate. Other G proteins, including transducin, are known to affect phospholipase A_2, which produces AA (Burch, 1989). A lipid-mediated effect is particularly appealing in view of the recent finding in our laboratory that AA and DAG together are substantially more effective at activating PKC than either alone (Bramham et al., 1991; Lester et al., 1991). It has been suggested that arachidonic acid from phospholipase A_2 and diacylglycerol from phospholipase C could act synergistically on PKC and serve as a convergence point for two associated stimuli, such as a light stimulus and a neural stimulus from the motion-detecting statocysts (Bank et al., 1988b; Lester and Alkon, 1991). AA metabolites have been implicated as possible modulators of the Aplysia S potassium channel involved in long-term facilitation (Buttner et al., 1989) as well as of the G protein-gated cardiac K^+ channel (Kurachi et al., 1989, Scherer and Breitwieser, 1990). Thus, membrane lipids and their metabolites are intimately involved with both the large trimeric G proteins and the low-mol-wt G proteins.

Summary

Figure 4 shows a model summarizing the current state of research on the *Hermissenda* photo-

receptor neuron, combining elements from research in rabbit and other species. In invertebrates, a light stimulus acts via rhodopsin and transducin to activate phospholipase C and possibly phospholipase A2 (Vandenberg and Montal, 1984; Rayer et al., 1990), producing inositol triphosphate (IP_3), DAG, and AA by hydrolysis of membrane lipids. In nonphotosensitive neurons, a neurotransmitter receptor could provide the same function. Synaptic input from other neurons detecting the unconditioned stimulus, in this case the motion-sensitive statocyst cells, simultaneously impinges on the neuron, resulting in the influx of calcium. Calcium can also be released by IP_3 from intracellular stores. Calcium, AA, and DAG would converge to activate PKC, translating it to the membrane, where it phosphorylates the G protein, cp20, which is loosely associated with the membrane (Alkon et al., 1988), and other proteins, possibly including the potassium channel. Cp20 then would act to block the two K^+ channels (I_A and $I_{Ca^{2+}} - {}_{K^+}$) resulting in decreased K^+ efflux and increased responsiveness of the cell to additional stimuli. Cp20 may also act on PKC, axonal transport, or protein synthesis at the level of ribonucleic acid (RNA) translation. PKC and AA can also affect protein synthesis at the deoxyribonucleic acid (DNA) level.

Unanswered questions still remain about many details of this model. For instance, is potassium conductance regulated by phosphorylation, by direct interaction with the low-mol-wt G protein, or by an indirect interaction involving additional components? The basis for long-term regulation of the K^+ channels is also still unknown. Although some of the observed long-term changes in K^+ conductance may result from local structural or biochemical interactions, a large body of evidence indicates that protein synthesis is also required. The role of the trimeric G proteins in learning is almost completely unexplored, as is the possible involvement of *ras* and other protooncogenes.

References

Alkon D. L. (1984) Calcium-inactivated potassium currents: a biophysical memory trace. *Science* **276,** 1037–1045.

Alkon D. L. and Nelson T. J. (1990) Specificity of molecular changes in neurons involved in memory storage. *FASEB J.* **4,** 1567–1576.

Alkon D. L., Lederhendler I., and Shoukimas J. L. (1982) Primary changes of membrane currents during retention of associative learning. *Science* **215,** 693–695.

Alkon D. L., Shoukimas J., and Heldman E. (1982b) Calcium-mediated decrease of a voltage-dependent potassium current. *Biophys. J.* **40,** 245–250.

Alkon D. L., Sakakibara N., Forman R., Harrigan J., Lederhendler I., and Farley J. (1985) Reduction of two voltage-dependent K^+ currents mediates retention of a learned association. *Behav. Neural Biol.* **44,** 278–300

Alkon D. L., Ikeno H., Dworkin J., McPhie D. L., Olds J., Lederhendler I., Matzel L., Schreurs B., Kuzirian A., Collin A., and Yamoah E. (1990) Contraction of neuronal branching volume: an anatomic correlate of Pavlovian conditioning. *Proc. Nat. Acad. Sci. USA* **87,** 1611–1614.

Alkon D. L., Naito S., Kubota M., Chen C., Bank B., Smallwood J., Gallant P., and Rasmussen H. (1988) Regulation of Hermissenda K^+ channels by cytoplasmic and membrane-associated C-kinase. *J. Neurochem.* **51,** 903–917.

Aniksztejn L. and Ben-Ari Y. (1991) Novel form of long-term potentiation produced by a K^+ channel blocker in the hippocampus. *Nature* **349,** 67–69.

Ayala J., Touchot N., Zahraoui A., Tavitian A., and Prochiantz A. (1990)The product of rab2, a small GTP binding protein, increases neuronal adhesion, and neurite growth in vitro. *Neuron* **4,** 797–805.

Bacon R. A., Salminen A., Ruohola H., Novick P., and Ferro-Novick S. (1989) The GTP-binding protein Ypt2 is required for transport in vitro: the Golgi apparatus is defective in ypt1 mutants. *J. Cell. Biol.* **109,** 1015–1022.

Ballester R., Furth M. E., and Rosen O. M. (1987) Phorbol ester and protein kinase C-mediated phosphorylation of the cellular Kirsten *ras* gene product. *J. Biol. Chem.* **262,** 2688–2695.

Bank B., Nelson T., and Alkon D. L. (1988a) Molecular mechanisms of associative learning in mammal and mollusc. *J. Physiol. Paris* **83,** 119–125.

Bank B., DeWeer A., Kuzirian A., Rasmussen H., and Alkon D. L. (1988b) Classical conditioning induces long-term translocation of protein kinase C in rabbit hippocampal CA1 cells. *Proc. Nat. Acad. Sci. USA* **85,** 1988–1992.

Berger T. W. (1984) Long-term potentiation of hippocampal synaptic transmission affects rate of behavioral learning. *Science* **224,** 627–630.

Berger T. W., Rinaldi P. C., Weisz D. J., and Thompson R. F. (1983) Single unit analysis of different hippocampal cell types during classical conditioning of rabbit nictitating membrane response. *J. Neurophys.* **50,** 1197–1219.

Berridge M. J. (1984) Inositol triphosphate and diacylglycerol as second messengers. *Biochem. J.* **220,** 345–360.

Bielinski D. F., Morin P. J., Dickey B. F., and Fine R. E. (1989) Low molecular weight GTP-binding proteins are associated with neuronal organelles involved in rapid axonal transport and exocytosis. *J. Biol. Chem.* **254,** 18,363–18,367.

Bramham C. R., Lester D. S., and Alkon D. L. (1991) Low concentrations of arachidonic acid produce an activity-dependent enhancement of synaptic transmission in hippocampal slices preloaded with a diacylglycerol analog. *Soc. Neurosci Abstr.* **20,** 1991.

Breitwieser G. E., and Szabo G. (1985) Uncoupling of cardiac muscarinic and β-adrenergic receptors from ion channels by a guanine nucleotide analogue. *Nature (London)* **317,** 538–540.

Brezina V. (1988) Guanosine-5'-triphosphate analogue activates potassium current modulated by neurotransmitters in *Aplysia* neurones. *J. Physiol.* **407,** 15–40.

Brown A. M. (1990) Regulation of heartbeat by G protein-coupled ion channels. *Am. J. Physiol.* **259,** H1621–H1628.

Brown D. A. (1990) G proteins and potassium currents in neurons. *Ann. Rev. Physiol.* **52,** 215–242.

Brown D. A. and Griffith W. H. (1983) Calcium-activated outward current in voltage-clamped hippocampal neurones of the guinea-pig. *J. Physiol.(London)* **337,** 287–301.

Burch R. M (1989) G protein regulation of phospholipase A2. *Molecular Neurobiology* **3,** 155–171.

Buttner N., Siegelbaum S. A., and Volterra A. (1989) Direct modulation of *Aplysia* S-K^+ channels by a 12-lipoxygenase metabolite of arachidonic acid. *Nature* **342,** 553–555.

Byrne, J. (1987) Cellular analysis of associative learning. *Physiol. Rev.* **67,** 329–439.

Cole W. C. and Sanders K. M. (1989) G proteins mediate suppression of Ca^{2+}-activated K current by acetylcholine in smooth muscle cells. *Am. J. Physiol.* **257,** C596–C600.

Collin C., Papageorge A. G., Sakakibara M., Huddie P., Lowy D. R., and Alkon D. L. (1990a) Early regulation of membrane excitability by *ras* oncogene proteins. *Biophys. J.* **58,** 785–790.

Collin C., Papageorge A. G., Lowy D. R., and Alkon D. L. (1990b) Early enhancement of calcium currents by H-*ras* oncoproteins injected into *Hermissenda* neurons. *Science* **250,** 1743–1745.

Connor J. A. and Alkon D. L. (1984) Light- and voltage-dependent increases of calcium ion concentration in molluscan photoreceptors. *J. Neurophysiol.* **51,** 745–752.

Coulter D. A., LoTurco J. J., Kubota M., Disterhoft J. F., Moore J. W., and Alkon D. L. (1989) Classical conditioning reduces amplitude and duration of calcium-dependent afterhyperpolarization in rabbit hippocampal pyramidal cells. *J. Neurophysiol.* **61,** 971–981.

Crow T. J. and Alkon D. L. (1978) Retention of an associative behavioral change in *Hermissenda*. *Science* **201,** 1239–1241.

Disterhoft J. F., Coulter D. A., and Alkon D. L. (1986) Conditioning-specific membrane changes of rabbit hippocampal neurons measured in vitro. *Proc. Nat. Acad. Sci. USA* **83,** 2733–2737.

Disterhoft J. F., Coulter D. A., and Alkon D. L.(1988) Conditioning—specific biophysical alterations in rabbit hippocampus, *Cellular Mechanisms of Conditioning and Behavioral Plasticity*. Woody C. D., Alkon D. L., and McGaugh J. L., eds., Plenum.

Doucet J. P., Squinto S. P., and Bazan N. G. (1990) *Fos-jun* and the primary genomic response in the nervous system: possible physiological role and pathophysiological significance. *Molec. Neurobiol.***4,** 27–55.

Eddlestone G. T., Ribalet B., and Ciani S. (1989) Differences in K channel behavior in glucose-responsive and glucose-unresponsive insulin secreting tumor cell lines. *Biophys. J.* **55,** 541a.

Ellis C., Moran M., McCormick F., and Pawson T. (1990) Phosphorylation of GAP and GAP-associated proteins by transforming and mitogenic tyrosine kinases. *Nature* **343,** 377–381 .

Farley J., Richards W., Ling L., Liman E., and Alkon D. L. (1983) Membrane changes in a single photoreceptor cause associative learning in *Hermissenda*. *Science* **221,** 1201–1203.

Flexner J., Flexner J., Stellar E., De LaHaba G., and Roberts R. (1962) Inhibition of protein synthesis in brain and learning and memory following puromycin. *J. Neurochem.* **9,** 595–605 .

Fukumoto Y., Kaibuchi K., Oku M., Hori Y., and Takai Y. (1990) Activation of the c-*fos* serum-response element by the activated c-Ha-*ras* protein in a manner independent of protein kinase C and cAMP-dependent protein kinase. *J. Biol. Chem.* **265,** 774–780.

Goh J. W. and Pennefather P. S. (1989) A pertussis toxin-sensitive G protein in hippocampal long-term potentiation. *Science* **244,** 980–983.

Goh Y. and Alkon D. L. (1984) Sensory, interneuronal, and motor interactions within the *Hermissenda* neural pathway. *J. Neurophysiol.* **43,** 669–685.

Goud B., Salminen A., Walworth N. C., and Novick P. J.(1988) A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast. *Cell* **53,** 753–768 .

Gustafsson B. and Wigstrom H. (1981) Evidence for two types of afterhyperpolarization in CA1 pyramidal cells in the hippocampus. *Brain Res.* **206,** 462–468.

Han J.-W., McCormick F., and Macara I. G. (1991) Regulation of *ras*-GAP and the neurofibromatosis-1 gene product by eicosanoids. *Science* **252,** 576–579.

Hess G. and Gustafsson B. (1990) Changes in field excitatory postsynaptic potential shape induced by tetanizaton in the CA1 region of the guinea-pig hippocampal slice. *Neuroscience* **37,** 67–69 .

Hoshijima M., Kikuchi A., Kawata M., Ohmori T., Hashimoto E., Yamamura H. and Takai Y. (1988) Phosphorylation by cyclic AMP-dependent protein kinase of a human platelet M_r 22,000 GTP-binding protein (smg p21) having the same putative effector domain as the *ras* gene products. *Biochem. Biophys. Res. Commun.* **157,** 851–860.

Jeng A. Y., Srivastava S. K., Lacal J. C., and Blumberg P. M. (1987) Phosphorylation of *ras* oncogene product by protein kinase C. *Biochem. Biophys. Res. Commun.* **145,** 782–788.

John J., Frech M., and Wittinghofer A. (1988) Biochemical properties of Ha-*ras* encoded p21 mutants and mechanism of the autophos-phorylation reaction. *J. Biol. Chem.* **263,** 11,792–11,799.

Kawata M., Kikuchi A.,Hoshijima M., Yamamoto J., Hashimoto E., Yamamura H., and Takai Y. (1989) Phosphorylation of smg p21, a *ras* p21-1ike GTP-binding protein, by cyclic AMP-dependent protein kinase in a cell-free system and in response to prostaglandin E1 in intact human platelets. *J. Biol. Chem.* **264,** 15,688–15,695.

Kurachi Y., Ito H., Sugimoto T., Shimizu T., Miki I., and Ui M. (1989) Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K^+ channel. *Nature* **337,** 555–560.

Lacal J. C., De la Pena P., Moscat J., Gracia-Barreno P., Anderson P. S., and Aaronson S. A. (1987a) Rapid stimulation of diacylglycerol production in Xenopus oocytes by microinjection of H-*ras* p21. *Science* **238,** 533–536.

Lacal J. C., Fleming T. P., Warren B. S., Blumberg P. M., and Aaronson S. A. (1987b) Involvement of functional protein kinase C in the mitogenic response to the H-*ras* oncogene product. *Mol. Cell. Biol.* **7,** 4146–4149.

Lapetina E. G., Lacal J. C., Reep B. R., Molina Y., and Vedia L. (1989) A *ras*-related protein is phosphorylated and translocated by agonists that increase cAMP levels in human platelets. *Proc. Nat. Acad. Sci. USA,* **86,** 3131–3134.

Lederhendler I., Gart S., Alkon D. L. (1986) Classical conditioning of *Hermissenda:* origin of a new response. *J. Neurosci.* **6,** 1325–1331.

Lester D. S. and Alkon D. L. (1991) Activation of protein kinase C phosphorylation pathways: a role for storage of associative memory. *Prog. Brain Res.* **89,** 235–248.

Lester D. S. , Collin C., Etcheberrigaray R., Alkon D. L. (1991) Arachidonic 2cid and diacylglycerol act synergistically to activate protein kinase C in vitro and in vivo. *Biochem. Biophys. Res. Commun.* **179,** 1522–1528.

Lynch G., Kessler M., Arai A., and Larson J. (1990) The nature and causes of hippocampal long-term potentiation. *Prog. Brain Res.* **83,** 233–250.

Matzel L. D., Lederhendler I., and Alkon D. L. (1990) Regulation of short-term associative memory by calcium-dependent protein kinase. *J. Neurosci.* **10,** 2300–2307.

McCloskey M. A. and Cahalan M. D. (1990) G protein control of potassium channel activity in a mast cell line. *J. Gen. Physiol.* **95,** 205–227.

McPhie D. L., Matzel L. D., Olds J. Lester D. S., Kuzirian A., and Alkon D. L. (1991) Cell-specificity

of molecular changes during memory storage. Submitted.

Mizumori S. J., Rosenzweig M. R., and Bennett E. L. (1985) Long-term working memory in the rat: effects of hippocampally applied anisomycin. *Behav. Neurosci.* **99,** 220–232.

Molloy C. J., Bottaro D. P., Fleming T. P., Marshall M. S., Gibbs J. B., and Aaronson S. A. (1989) PDGF induction of tyrosine phosphorylation of GTPase activating protein. *Nature* **342,** 711–714.

Moshiach S., Nelson T. J., Sanchez-Andres J.-V., and Alkon D. L. (1991) A G-protein regulates intra-axonal particle movement. Submitted.

Nagata K.-I., Nagao S., and Nozawa Y. (1989) Low M_r GTP-binding proteins in human platelets: cyclic AMP-dependent protein kinase phosphorylates m22KG(I) in membrane but not c21KG in cytosol. *Biochem. Biophys. Res. Commun.* **160,** 235–242.

Neary J. T., Naito S., DeWeer A., and Alkon D. L. (1986) Ca^{2+}/diacylglycerol-activated, phospholipid-dependent protein kinase in the *Hermissenda* CNS. *J. Neurochemistry* **47,** 1405–1411.

Nelson T. J. and Alkon D. L. (1989) Prolonged RNA changes in the *Hermissenda* eye induced by classical conditioning. *Proc. Nat. Acad. Sci. USA* **85,** 7800–7804.

Nelson T. J. and Alkon D. L. (1990) Specific high molecular weight mRNAs induced by associative learning in *Hermissenda*. *Proc. Nat. Acad. Sci. USA* **87,** 269–273.

Nelson T. J., Collin C., and Alkon D. L. (1990) Isolation of a G protein that is modified by learning and reduces potassium currents in *Hermissenda*. *Science* **247,** 1479–1483.

Nelson T. J., Sanchez-Andres J.-V., Schreurs B. G., and Alkon D. L. (1991) Classical conditioning-induced changes in low molecular weight GTP-binding proteins in rabbit hippocampus. *J. Neurochem.* **57,** 2065–2069.

Nishizuka, Y. (1984) The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* **308,** 693–698.

Nishizuka, Y. (1986) Studies and perspectives of protein kinase C. *Science* **233,** 305–312.

Olds J., Anderson M., McPhie D., Staten L., and Alkon D. L. (1989) Imaging of memory-specific changes in the distribution of protein kinase C in the hippocampus. *Science* **245,** 866–869.

Parent L. and Coronado R. (1989) Reconstitution of the ATP-sensitive potassium channel of skeletal muscle: activation by a G protein-dependent process. *J. Gen. Physiol.* **94,** 445–463.

Price B., Morris J. D. H., Marshall C. J., and Hall A. (1989) Stimulation of phosphatidylcholine hydrolysis, diacylglycerol release, and arachidonic acid production by oncogenic *ras* is a consequence of protein kinase C activation. *J. Biol. Chem.* **264,** 16,638–16,643 .

Rainbow T. (1979) Role of RNA and protein synthesis in memory formation. *Neurochem. Res.* **4,** 297–312.

Rayer B., Naynert M., and Stieve H. (1990) Phototransduction: different mechanisms in vertebrates and invertebrates. *J. Photochem. Photobiol. B,* **7,** 107–148.

Resnick R. J., Racker E. (1988) Phosphorylation of RAS2 gene product by protein kinase A inhibitis the activation of yeast adenylate cyclase. *Proc. Nat. Acad. Sci. USA* **85,** 2474–2478.

Reuter, H. (1983) Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature London* **301,** 569–573.

Sahyoun N., McDonald, O. B., Farrell F., and Lapetina E. G. (1991) Phosphorylation of a *ras*-related GTP-binding protein, rap1b, by a neuronal Ca^{2+}-calmodulin-dependent protein kinase, CaM kinase Gr. *Proc. Nat. Acad. Sci. USA* **88,** 2643–2647.

Saikumar P., Ulsh L. Clanton M., Huang K.-P., and Shih T. Y. (1988) Novel phosphorylation of c-*ras* by protein kinases. *Oncogene Res.* **3,** 213–222.

Salminen A. and Novick P. J. (1987) A *ras*-like protein is required for a post-Golgi event in yeast secretion. *Cell* **49,** 527–538.

Salminen A. and Novick P. J. (1989) The sec15 protein responds to the function of the GTP binding protein, sec4, to control vesicular traffic in yeast. *J. Cell. Biol.* **109,** 1023–1036.

Santos E. and Nebreda A. (1989) Structural and functional properties of *ras* proteins. *FASEB J.* **3,** 2151–2163.

Sassone-Corsi P., Der C. J., and Verma I. M. (1989) *ras*-Induced neuronal differentiation of PC-12 cells: possible involvement of *fos* and *jun*. *Mol. Cell. Biol.* **9,** 3174–3183.

Scherer R. W. and Breitwieser G. E. (1990) Arachidonic acid metabolites alter G protein-mediated signal transduction in heart. *J. Gen. Physiol.* **96,** 735–755.

Scott J. D., Stofko R. E., McDonald J. R., Comer J. D., Vitalis E. A., and Mangili J. A. (1990) Type II regulatory subunit dimerization determines the subcellular localization of the cAM—dependent protein kinase. *J. Biol. Chem.* **255,** 21,561–21,566.

Segev N., Mulholland J., and Botstein D. (1988) The

yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. *Cell* **52,** 915–924.

Shashoua V. (1968) RNA changes in goldfish brain during learning. *Nature (London)* **217,** 238–240.

Shashoua V. (1974) RNA metabolism in goldfish brain during acquisition of new behavioral patterns. *Proc. Nat. Acad. Sci. USA* **65,** 160–167.

Shearman M. S., Naor Z., Sekiguchi K., Kishimoto A., and Nishizuka Y. (1989) Selective activation of the gamma-subspecies of protein kinase C from bovine cerebellum by arachidonic acid and its lipoxygenase metabolites. *FEBS Lett.* **243,** 177–182.

Spina A., Chiosi E., Illiano G., Berlingieri M. T., Fusco A., and Grieco M. (1988) Protein kinase C activities are increased in rat thyroid epithelial cells expressing v-*ras* genes. *Biochem. Biophys. Res. Comm.* **157,** 1093–1103.

Thalmann R. H. (1988) Evidence that guanosine triphosphate (GTP)-binding proteins control a synaptic response in brain: effect of pertussis toxin and GTPγS on the late inhibitory postsynaptic potential of hippocampal neurons. *J. Neurosci.* **8,** 4589–4602.

Trahey M. and McCormick F. (1987) A cytoplasmic protein stimulates normal N-*ras* p21 GTPase, but does not affect oncogenic mutants. *Science* **238,** 542–545.

Trautwein W. and Hescheler J. (1990) Regulation of cardiac L-type calcium current by phosphorylation and G proteins. *Ann. Rev. Physiol.* **52,** 257–274.

Tsai M.-H., Yu C.-L., Wei F.-S., and Stacey D. W. (1989) The effect of GTPase activating protein upon *ras* is inhibited by mitogenically responsive lipids. *Science* **243,** 522–526.

Vandenberg C. A. and Montal M. (1984) Light-regulated biochemical events in invertebrate photoreceptors. 2. Light-regulated phosphorylation of rhodopsin and phosphoinositides in squid photoreceptor membranes. *Biochemistry* **23,** 2347–2352.

Van Dongen A. M., Codina J., Olate J., Mattera R., Joho R., Birnbaumer L., and Brown A. M. (1988) Newly identified brain potassium channels gated by the guanine nucleotide binding protein Go. *Science* **242,** 1433–1437.

von Mollard G. F., Südhof T. C., and Jahn R. (1991) A small GTP-binding protein dissociates from synaptic vesicles during exocytosis. *Nature* **349,** 79–81.

von Mollard G. F., Mignery G. A., Baumert M., Perin M. S., Hanson T. J., Burger P. M., Jahn R., and Südhof T. C. (1990) rab3 is a small GTP-binding protein exclusively localized to synaptic vesicles. *Proc. Nat. Acad. Sci. USA* **87,** 1988–1992.

Yatani A., Okabe K., Polakis P., Halenbeck R., and McCormick F., Brown A. M. (1990) *Ras* p21 and GAP inhibit coupling of muscarinic receptors to atrial K^+ channels. *Cell* **61,** 769–776.

Yu C.-L., Tsai M.-H., and Stacey D. W. (1990) Serum stimulation of NIH 3T3 cells induces the production of lipids able to inhibit GTPase-activating protein activity. *Mol. Cell. Biol.* **10,** 6683–6689.

Zahraoui A., Touchot N., Chardin P., and Tavitian A. (1989) The human rab genes encode a family of GTP-binding proteins related to yeast YPT1 and SEC4 products involved in secretion. *J. Biol. Chem.* **264,** 12,394–12,401.

Zemp J., Wilson J., Schlesinger K., Boggan W., and Glassman E. (1966) Brain function and macromolecules. I. Incorporation of uridine into RNA of mouse brain during short-term training experience. *Proc. Nat. Acad. Sci. USA* **55,** 1423–1431.