

Neuronal Circuits: An Evolutionary Perspective

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To understand neural circuits completely, it is necessary to know not only how they work, but also why they work that way. Answers to the latter question have been almost teleological in their assumption of optimal design. However, close examination of certain systems has revealed features that apparently lack adaptive value. Their existence can be understood only if the evolution of these circuits is considered and, in particular, how nonadaptive determinants have guided that evolution.

ALTHOUGH INVERTEBRATE NERVOUS SYSTEMS ARE INTRINSICALLY interesting, a major reason for studying them has been to understand more complex systems. It has long been held that the study of such experimentally accessible systems will yield organizational principles linking the properties of neural circuits to the behaviors they control (1). Confidence in the reliability of such a relation even led to the assertion that the general design of a neural circuit may be deduced solely from an examination of its output (2). However, with a few notable exceptions such as the study of lateral inhibition in the horseshoe crab *Limulus* (3), such organizational principles have been slow to emerge. Instead, even circuits that control simple behaviors have turned out to be exceedingly complex (4), and circuits that control apparently similar behaviors may be constructed differently (5). Recently, even the existence of any such underlying principles has been questioned (6).

We suggest that an alternative approach is required if we are to understand this apparent lack of functional principles. It may not be possible to explain many features of nervous systems in terms of adaptive significance. Rather, it may be more appropriate to consider how a neural circuit, or any other feature, is shaped during evolution. The effects of evolution can be considered to be influenced by four types of determinants: (i) adaptive influences, which are directly related to optimization of the effect of the behavior; (ii) developmental constraints, which pose restrictions on the final form of the nervous system; (iii) historical influences, by which the form of the present-day nervous system reflects the ancestral form; and (iv) certain architectural features, which are imposed by the materials and design of the organism.

Although this classification of determinants is by no means perfect, adaptation clearly does not act alone to shape a circuit during evolution. In particular, developmental, historical, and architectural determinants will produce features of the nervous system unrelated to its function, but perhaps idiosyncratic to a group of animals sharing a common evolutionary past or developmental constraints, that is, a phyletic group. Recently, evolutionary biologists have put forward convincing arguments for the importance of such nonadaptive processes in evolution (7, 8). Here we discuss neural circuits that demonstrate the effects of these processes.

One way to study the evolution of neural circuits is by comparing homologous systems, either between related species or between different ganglia within the same species (9–11). Such systems share

a common ancestry and hence common developmental constraints and evolutionary history. In the absence of functional specializations, they tend to be precisely conserved. For example, when 30 identified neurons from the unspecialized ganglia (that is, excluding those in the head, tail, or sex segments) were compared among three different species in two subfamilies of leech, no differences in form or function were found (12). When functional specialization does occur, the differences in neural circuitry may be related to the behavioral adaptation. For example, in the locust, the motor neurons controlling the jumping leg are homologous to those controlling the walking legs. Both neural groups are similar, but those controlling jumping are linked by a network of mutual excitation that is necessary for the tension-building phase of the jump (13).

The success of this and other studies (14) in indicating the functional value of specific differences in circuitry should not lead us to assume that such explanations may always be applied. For example, in the cockroach *Gromphadorhina* one pair of spiracles is specialized for sound production and is inactive during respiration. Yet the motor neurons innervating these spiracles fire in phase with the respiratory rhythm, but at frequencies too low to produce movement (15). Arbas (16) compared the flight motor system in the locust with the homologous system in a flightless grasshopper. Although some flight muscles are missing in the flightless species, the motor neurons responsible for their control remain, and, although reduced in size, they still send axons to the former location of the muscles. Thus, the vestigial neural elements and connections are conserved although they no longer have adaptive value. Although the absence of functional significance may be impossible to prove, this conservation seems the most plausible explanation for certain aspects of the flight system of the locust and the escape tailflip of the crayfish.

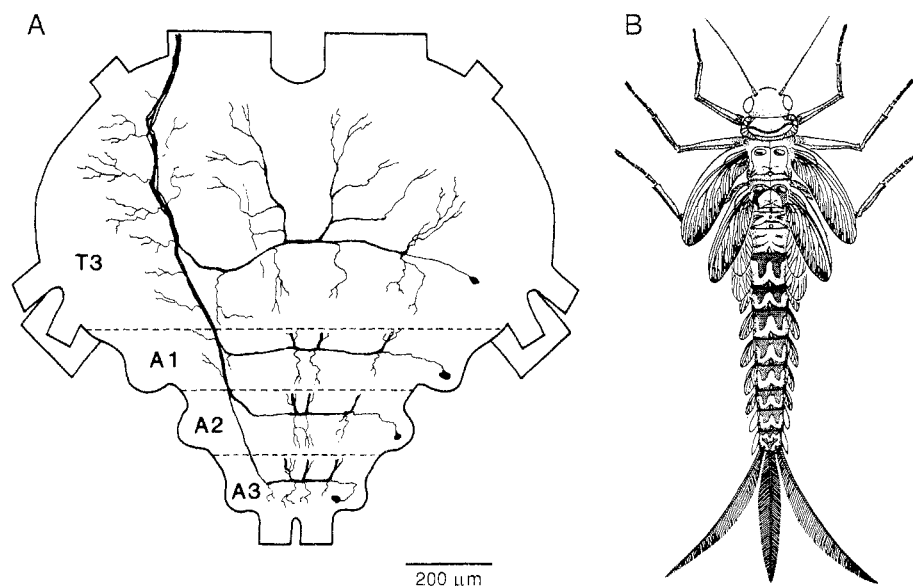
The Locust Flight System

Locust flight is accomplished by the alternating elevation and depression of each of two pairs of thoracic wings, with hindwing movements preceding equivalent movements of the forewings by between 5 and 10 msec (17). The peripheral structures (wings, sclerites, and wing muscles) and their innervation (motor and sensory neurons) are duplicated in the mesothoracic and metathoracic segments with little difference between segments (18). In contrast, most interneurons controlling flight are not organized as meso- and metathoracic duplicates, although isolated examples of this sort of organization can be found (19). A particularly interesting departure from simple duplication is the occurrence of sets of up to four serially homologous interneurons, all having nearly identical

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Fig. 1. Segmental iteration of flight interneurons in the locust mirrors segmental repetition of articulated wing appendages on a fossil mayfly nymph. (A) Drawing of apparently homologous interneurons in the metathoracic ganglion of an adult locust, *Locusta migratoria*. The adult metathoracic ganglion is a fusion of four embryologically distinct ganglia (dashed lines) (43). Only one neuron of each bilateral pair is drawn; the dendritic branching of each is shown only in the neuromere of origin, whereas the axon is shown in full. The physiology of each of these interneurons is identical. They have direct excitatory connections with wing elevator motor neurons; stimulation of any one of these neurons can reset the timing of the flight motor rhythm, which indicates their importance in the flight motor pattern generator. Three other similar sets of homologous interneurons with important functions in the flight system are known (19). [From figure 2b in (22)] (B) Reconstruction of a Paleozoic late-instar mayfly nymph, *Kukalovia americana*, to show the homologous wing appendages, which are repeated on all thoracic and nine abdominal segments. [Drawn by J. Kukalová-Peck, from (23)]



physiology and basic morphology (Fig. 1A). A startling feature of these sets of interneurons is that three out of four of the cells are abdominal in origin, yet they play important roles in the generation and expression of the flight motor pattern (19, 20). None of them is involved in controlling abdominal structures or in coordinating the abdomen with the thorax during flight.

It might be possible to argue for some adaptive value in this unexpected arrangement. However, by adopting an evolutionary perspective we can propose other, more reasonable, explanations. For instance, one step in the evolution of the neuronal circuit for flight may have been a change in the development of the nervous system that affected the development of all segmental ganglia in the same way. This would explain the presence of available abdominal interneurons, but does not account for their incorporation into a thoracic behavior. An alternative, more plausible explanation compares this system with other rhythmical motor systems.

In general, when a rhythmic behavior involves segmentally repeated structures, the rhythm-generating neurons are similarly distributed in the corresponding segmental ganglia. This organization is exemplified in the control of ventilation of locusts, swimmeret beating in Crustacea, swimming in the leech, and heartbeat in the leech (21). Thus the distribution of the locust flight motor in at least six embryologically distinct ganglia, of which three are abdominal, might indicate that flight itself originated as behavior involving segmentally repeated structures (22). Genetic, embryological, and fossil evidence (Fig. 1B) supports the pleural appendage theory for the evolutionary origin of the insect wing (23, 24). This theory posits that wings evolved from articulated, movable appendages of legs that were serially repeated along the thorax and the abdomen and that had been previously adapted for a motor function other than flight (for example, ventilatory movements). Thus, the serial repetition of flight interneurons and the abdominal origin of some of them results from the evolutionary history of the animal and its earlier behavior patterns, but has no current functional significance.

Crayfish Escape Tailflip

Crayfish normally swim backward by flexing all six segments of the abdomen. But, when startled by an abrupt stimulus from behind, they produce a modified movement in which the anterior three segments are flexed and the posterior three remain extended.

As a result the animal is flipped forward in a somersault, reorienting it away from danger. This movement is controlled by a pair of specialized interneurons, the lateral giants (LG's), which have axons that run the length of the animal (25). These cells produce different effects in the anterior and posterior segments of the abdomen, even though they act on homologous neurons in all six segmental ganglia (26).

From observations of this behavior, one might predict that the LG's would have a powerful input to the anterior fast flexor motor neurons (FF's) and little or no effect on the posterior ones (Fig. 2A). Instead, a smooth decline in the effectiveness of the input was found from anterior to posterior (Fig. 2A) (27, 28). Although the probability of firing individual motor neurons in the sixth and most posterior ganglion was small, the overall probability of producing contraction was over 50% in this segment because the flexor muscles are multiply innervated; it was even higher in segments 4 and 5 (28). Activation of these muscles would disrupt the movement, propelling the animal back toward the source of the danger. This would presumably be maladaptive and does not occur (28). Apparently the expression of the LG to FF connections is blocked in the posterior segments.

The blockade of this input to the posterior FF's depends on the effects of the original sensory stimulus. Activation of the sensory neurons not only excites the LG's, but also independently excites the anterior FF's and inhibits the posterior FF's (Fig. 2B). In addition, sensory stimulation, together with LG activation, causes recruitment of the inhibitory motor neuron to the flexor muscles (FI). These effects thus tend to increase the probability of flexion in the anterior segments and decrease it in posterior segments during a naturally elicited LG tailflip, thereby more closely modeling the observed behavior (dotted line in Fig. 2A) (28).

Although the final result of this circuitry is an efficient escape behavior, the process by which it is generated is unexpected. The posterior ganglia contain parallel excitatory and inhibitory pathways converging on the FF's and the muscles (Fig. 2B). This is a common feature in nervous systems, and it is usually interpreted as a means of allowing control flexibility. But in this case, the inhibitory pathways prevent the excitatory ones from having any effect. Rather than a flexible behavior, this organization produces a highly stereotyped one. Moreover, if the posterior FF's were activated during an escape tailflip, the escape would be disrupted, the animal being propelled backward toward the source of danger.

As with interneurons in the flight system of the locust, there is no apparent satisfactory explanation for the conflicting inputs to the FF's in terms of adaptive significance. We can, however, suggest a reasonable evolutionary sequence for their emergence. First, we must assume that the crayfish evolved from a primitive crustacean with abdominal segments that were much more uniform than they are now, perhaps even identical. This assumption is supported by comparative, embryological, and paleontological evidence (29). Second, we must assume that the ancestral LG's would produce complete abdominal flexion, similar to that seen in other forms today, through excitatory connections with all the flexor motor neurons. An animal with exactly these characteristics, *Anaspides tasmaniae*, exists today (30). It is a primitive crustacean that diverged from the mainstream of decapod evolution more than 300 million years ago. Although we cannot be sure that its neural circuitry truly reflects that of the primitive ancestor of the crayfish, its LG's are strikingly similar to those of the crayfish in both form and function.

As these early, chiefly benthic, forms became better adapted for swimming, selection would favor the development of a movement like the present-day LG tailflip that would help the animal escape attacks from the rear. The development of such a behavior depended on the interruption of the LG to FF connection in posterior ganglia. This could be achieved in two ways. Either the connection could be weakened, or its effects could be blocked by inhibition. It is not surprising that both occurred. As a result, the LG to FF connection became ineffective. Once it was no longer expressed in the behavior, it was no longer subject to selection, and so the paradoxical excitatory effects of the LG on the posterior FF's remain embedded in the system we see today.

Discussion

We have considered features of neuronal circuits that are unlikely to have any adaptive significance but whose existence can be explained from an evolutionary perspective. In particular, the locust flight system seems to be a clear-cut example of a preadaptation, a characteristic that has evolved as an adaptation to one set of conditions and has subsequently been co-opted to perform a new

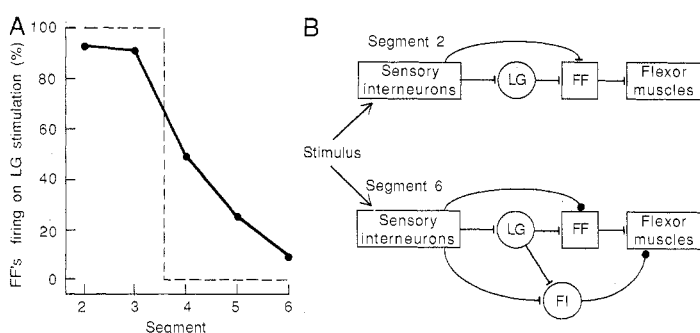


Fig. 2. The neural circuits controlling flexion during an LG-mediated tailflip. (A) The frequency of firing of the FF's by the LG's. The dashed line is predicted from observation of movements; such firing would cause flexion of the anterior segments (1, 2, and 3), but not of the posterior segments (4, 5, and 6). The solid line shows actual frequencies observed when recording from FF's and directly stimulating LG's (27, 28). (B) Diagrams of LG flexion circuits in anterior segment 2 and posterior segment 6 to show the effects of the sensory input in modifying the effects of the LG. Filled circles represent inhibitory inputs, and T bars, excitatory inputs. Connections are not necessarily monosynaptic. The sensory input to the FF's is excitatory in anterior segments, but inhibitory in posterior segments. This pattern tends to bring the activity of the FF's closer to the dashed line in (A). This effect is complemented by the activation of the FI in segment 6.

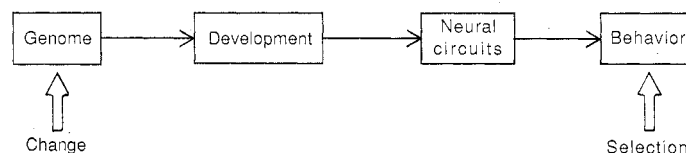


Fig. 3. A model to describe the action of natural selection on neural circuits. Selection operates on behavior to produce a change in the genome. This can alter the circuits only by developmental processes and not directly by selection.

and different function under different conditions (11, 31). Other examples of preadaptations include feathers, which were originally used for thermoregulation and subsequently became used for flight, and the swim bladder of teleosts, which originated as a lung in lung-fish. An important result of preadaptation is that certain features of the resulting system were determined not by their present function, but by the function for which they were originally adapted. Hence, abdominal interneurons are involved in the flight system of the locust because they were part of an original motor system from which flight evolved.

The continued presence of elements of neural circuitry that conflict with parsimony of design is a feature of all the systems we have mentioned. This is the result of the indirect way in which selection can change neural circuits (Fig. 3). Behavior, rather than the neural circuit itself, is the phenotypic feature exposed to selection. Selection produces a change in the genotype, but this change is only indirectly related to the final form of the circuit by its influence on developmental processes. Therefore, selection cannot operate directly on the circuits themselves. If the behavior produced is efficient and the energetic costs of anomalies such as extra neurons or inappropriate connections are negligible, they cannot be removed.

It should not surprise us to find such evolutionary anomalies in neural circuits; such anomalies are found at all other stages of the sequence shown in Fig. 3. For example, head scratching in birds resembles that in both reptiles and most mammals; the hindlimb is lifted over the forelimb. However, unlike mammals and reptiles, birds should find this unnecessary because the forelimb is the wing, which does not obstruct hindlimb movement. Use of this motion in birds is presumably the result of the retention of the ancestral neural apparatus underlying the behavior (32). The development of the bilaterally symmetrical adult nervous system of the nematode *Caenorhabditis elegans* is itself an asymmetrical process, involving different neuronal lineages on the two sides. This phenomenon is termed a developmental fossil by Sulston *et al.* (33). Finally, the genome of eukaryotes contains a large amount of apparently functionless DNA, the presence of which seems to depend on its neutrality and the consequent inability of natural selection to remove it (34).

This perspective has implications for the way we interpret neural circuits.

1) Certain features of nervous systems may not have functional significance. It is, of course, theoretically impossible to prove that such features are unnecessary, since an untried set of conditions may always occur in which a meaningful function may emerge. For example, the apparently unexpressed respiratory rhythm in the sound-producing spiracles of the cockroach *Gromphadorhina* may become functional in heavy respiration after exertion. However, in other cases (the locust flight system, the motor neurons in the flightless cricket, and the crayfish tailflip), the burden of proof must lie with those who argue that these neural arrangements do have adaptive significance.

It is also difficult to claim functional significance for the outputs of a large visual interneuron, the descending contralateral movement

detector, in the locust (35). These cells have strong disynaptic connections to motor neurons, but their monosynaptic connections to the same cells are weak, variable, and can even be absent without having deleterious effects on the animal's behavior (35, 36). This variability of an apparently insignificant connection is also seen in the direct connection of the LG interneuron to the FF motor neuron in the crayfish (28) and may indicate an absence of selective pressure on a neuronal connection. This may be analogous to the high incidence of nucleotide substitution in the third position in the triplet codon, as these substitutions often do not change the amino acid sequence (37).

2) Evolution does not work logically, or with a long-term perspective, on the design of the neural circuits, but rather selects the most successful behavior from generation to generation. Thus there is no reason why the simplest solution to a problem should be the one actually used by the nervous system. As long as both the end result and all the intervening stages work, elegance of design counts for little. The result, in the case of the crayfish escape response, is a confusing mixture of conflicting excitatory and inhibitory pathways. In fact, a common feature of all the examples we have described is that their organization could not have been predicted. It may not be possible to deduce the design of neural circuits by considering the patterns of inputs and outputs, as has been attempted (2, 38). Such a process of deduction is essentially logical, whereas the evolution of neural circuits is not. Therefore, the chance of arriving at the correct answer is not high. Of course, the theoretical approach to understanding nervous systems is essential, but the manner in which it can be applied is limited (39). An evolutionary perspective helps us understand why.

3) We have referred only in passing to developmental constraints, but the interposition of developmental processes between the genome and the neural circuits suggests that these effects will be significant. In general, since developmental processes usually affect more than one cell, altering such a process during evolution will tend to have multiple effects, not all of which may be desirable. For example, connections between homologous neurons in different ganglia may be determined by the same sequence of events. Hence, altering them independently may present problems. This could explain why the strength of LG to FF connections in the crayfish shows a smooth transition instead of a stepwise change half-way down the abdomen.

The H cell in grasshoppers shows a similarly graded, though much more marked, change in cellular properties over several ganglia (10). Thus, selection of an adaptive feature may have side effects that may or may not be adaptive, but, as long as the overall effect is beneficial, they will still be selected for. Also, when synapses are weak or ineffective, the rearrangement necessary to eradicate them might cause more problems than it would solve, thus favoring their persistence. This is not always the case, as the degeneration of visual systems in cave-dwelling animals indicates. However, this change occurred on a much larger scale, and probably greater benefits were derived from a more parsimonious design.

Although we have emphasized the difficulties in understanding neural circuits, certain potentially useful conclusions can be drawn.

(i) Organizational principles may have an evolutionary (therefore developmental, historical, or architectural) basis as well as a functional one. (ii) Comparative studies may help us separate the differences between features of neural circuits that are functionally significant from those that are not. Analogous systems are those that have converged functionally from disparate evolutionary pasts as a result of the selection of features designed to perform the same function. Therefore, the common features of analogous systems should be functionally related (40). Conversely, homologous systems share a common evolutionary past. Therefore, if they differ,

comparison can help us understand the relatively limited divergent evolution that they have undergone. (iii) Although weak or ineffective connections between neurons may be functionally useless, they may serve as the substrate for further evolution or may become strengthened during learning.

Finally, we can ask whether our discussion is applicable to vertebrate nervous systems, since almost all our examples are drawn from arthropods. The development of the nervous systems of higher animals probably differs from that of the invertebrates in that experience plays a greater role. For example, in mammals the connections of visual interneurons are modified according to the animal's early visual experience (41). Such modification might provide a means of eradicating functionally useless connections. However, as Gould has pointed out (42), the human brain, because of its complexity, is perhaps the most striking example of the effects of nonadaptive processes in evolution. Our brains, which evolved under the selective pressures experienced by our prehistoric ancestors, have enabled us to conceive of our own deaths and compose music, functions that can scarcely have been selected for.

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9. Homologous structures are those that have evolved from a common precursor, and thus they develop from genomic sequences that were identical in a common ancestor. Serially repeated structures of the same organism are recognized as being serial homologs, deriving not from the same precursor but from equivalent precursors. It is difficult to demonstrate conclusively that particular neural elements are homologous. Compelling evidence is a common developmental lineage [for example (10)]; see also M. J. Katz, R. J. Lasek, J. Silver, *Proc. Natl. Acad. Sci. U.S.A.* 80, 5936 (1983)]. In the absence of such information, homology can be inferred only on the basis of shared characteristics; a greater number of shared characteristics allows a conclusion for homology to be made with greater confidence. Analogous structures also share characteristics, but since they are the result of convergent evolution, these similarities will be mainly adaptive and less numerous or diverse than those of homologous structures (11). The putative homologs mentioned in this article are sufficiently similar in morphology and physiology that there is little doubt about the homology.
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Research Articles

The Complete Primary Structure of Protein Kinase C—the Major Phorbol Ester Receptor

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Protein kinase C, the major phorbol ester receptor, was purified from bovine brain and through the use of oligonucleotide probes based on partial amino acid sequence, complementary DNA clones were derived from bovine brain complementary DNA libraries. Thus, the complete amino acid sequence of bovine protein kinase C was determined, revealing a domain structure. At the amino terminal is a cysteine-rich domain with an internal duplication; a putative calcium-binding domain follows, and there is at the carboxyl terminal a domain that shows substantial homology, but not identity, to sequences of other protein kinase.

ANALYSIS OF GROWTH FACTORS AND THEIR ACTION HAS provided important insights into the mechanisms used to subvert the control of normal cell proliferation. Thus there is evidence that certain genes capable of transforming cells encode growth factors (1, 2) or abnormal growth factor receptors (3–5); the expression of these genes allows cells to divide in a constitutive manner. In elucidating the responses of cells to growth factors, it has become evident that postreceptor events may also be in some way involved in cellular transformation. Therefore, a detailed molecular description of the intracellular pathways responsible for cell division induced by growth factors is necessary if we are to understand the normal mechanisms involved in growth factor action and in so doing to identify critical links open to subversion.

The phosphorylation of proteins plays a key role in regulating cellular functions (6–8). The kinases and phosphatases responsible for governing such phosphorylations are themselves targets for the action of growth factors, hormones, and other extrinsic agents

participating in the control of cellular events (6–8). One of the major signal transduction pathways defined recently involves the enzyme protein kinase C (9–11), a multifunctional kinase that appears to play a central regulatory role akin to that of cyclic nucleotide-dependent and calcium-calmodulin-dependent enzymes.

Protein kinase C is a serine- and threonine-specific protein kinase that is dependent upon calcium and phospholipid for activity (12). However, at physiological calcium concentrations diacylglycerol is required for activity (13). Thus diacylglycerol has been defined as a second messenger responsible for the activation of protein kinase C in vivo (9–11). Agonist-induced generation of diacylglycerol has been widely described and forms part of a bifurcating signal pathway (14). It is thought that agonist-induced receptor-mediated activation of phospholipase C acts to generate two important second messengers from inositol phospholipids; the first, inositol 1,4,5-triphosphate, appears to be responsible for the release of calcium from intracellular stores (15) and the second, diacylglycerol, leads to protein kinase C activation (13). There is as yet only circumstantial evidence for the functioning of such a pathway in vivo (16).

From studies on protein kinase C in vitro, it has become apparent that those phorbol esters capable of tumor promotion can mimic the effect of diacylglycerol in enzyme activation (17). More recently, other structurally related and unrelated tumor promoters have also been shown to activate protein kinase C in vitro (18–21). The implication is that these tumor promoters elicit responses through protein kinase C and that activation of this enzyme is at least in part responsible for the activity of these hyperplasiogenic tumor-promot-

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