not be discerned from a shopping list of the components. The same can be said of a troop of primates, in which a deep and complex social structure forms. "Popper has recently said, 'We live in a world of emergent novelty,' and this is very important in studying nature, especially in biology," observes Mayr. "New properties turn up in systems that could not have been predicted from the components, which means you have to study things hierarchically. Reductionism can be vacuous at best, and, in the face of emergence, misleading and futile." Strong words, both for the "arrogant physicists" and the narrowly focused geneticists.

One of the most characteristic features of evolutionary biology is in the type of questions it asks. Every issue in biology has two facets: a functional facet, in which one asks, "what?" and "how?" questions; and an evolutionary facet, in which one asks, "why?" questions. "You can ask, why are certain organisms similar to each other, while others are utterly different?" says Mayr. "You can ask, why are there two sexes in most species of organisms? Why is there such a diversity of plant and animal life? Why are the faunas of some areas rich in species while those of others poor? Asking 'why?' questions is the major task of evolutionary biologists."

The "why?" question has little or no part in the world of the physical scientist, and the taboo against it was impressed on the biologists. "It was regarded as an Aristotelian question and quite out of place," says Mayr. "But it is now legitimate, as well as necessary, to ask, why?"

Many physical scientists have inferred a tendency to vitalism in their biological colleagues' insistence that living nature is different from the inanimate world, and until a few decades ago this might have been correct in some cases. "Physical scientists must understand that biologists are not disclaiming physical phenomena," urges Mayr. "We are not setting up vitalism. We are not trying to produce a metaphysics. We simply claim that in complex, historically formed systems things occur that do not occur in inanimate systems. That is all that is being claimed."—ROGER LEWIN

Gene Family Controls a Snail's Egg Laying

The marine snail Aplysia *displays a stereotyped egg-laying behavior which appears to be under the control of a family of related genes*

The application of recombinant DNA technology to neuroscience is still in its infancy, but its promise of novel products is already being fulfilled. A recent example, tantalizing in its putative generality, comes from the combined efforts of the laboratories of Richard Axel, James Schwartz, and Eric Kandel, at Columbia University, New York. Early results* reveal insights into the organization and expression of a gene that is important in behavior of the marine snail *Aplysia*.

One of neuroscience's most fertile areas of research is in the discovery and characterization of behaviorally important peptides, of which the list stands currently at around 25. And one of the most intriguing features of neuroactive peptides is that, in a surprising number of instances, several peptides are coded for by the same gene, the resultant polyprotein being processed to release the individual peptides. A great deal remains to be discovered about the scope of action of neuropeptides, but it seems likely that under certain circumstances different combinations of such peptides might produce subtle variations on behavioral themes.

What the Columbia researchers have discovered is a family of genes in *Aplysia*, each member of which apparently has the potential to code for a small complement of neuropeptides. The genes are related in that each codes, among other things, for a peptide that initiates egg laying, or at least codes for something similar to the so-called egg-laying hormone (ELH). Axel, Schwartz, Kandel, and their colleagues think that different aspects of the snail's reproductive behavior might be elicited by the expression of different members of the gene family, depending on the nature of the peptides produced in association with ELH.

Aplysia is a simple organism, being blessed with only 20,000 central nerve cells which are arranged in four symmetrical pairs of ganglia—the cerebral, buccal, pleural, and pedal—and a single asymmetrical abdominal ganglion. With so limited a nervous system, it has been possible to relate the function of specific cells to certain behaviors. The extensive documentation of *Aplysia* behavior has been particularly useful in Kandel's earlier work. But perhaps the greatest advantage the snail has to offer is the large size of its nerve cells and the very large amount of genetic material each contains. For instance, one nerve cell may carry more than 1 microgram of DNA, which is up to 200,000 times more than that in other somatic cells.

In spite of *Aplysia*'s special endowments, conventional neurochemistry still has a problem in addressing some of the most important questions. Many of the interesting peptides are active at astonishingly small concentrations, and so it is difficult to learn very much about them when normal techniques are used. The tricks of recombinant DNA technology offered a way around this problem and so Axel, Schwartz, and Kandel began mulling over the idea of some kind of collaboration more than 2 years ago. When Richard Scheller arrived in Axel's laboratory from the California Institute of Technology late in 1980, he initiated a project that rapidly yielded results. The Columbia team was joined later by James Jackson and Linda Beth McAllister.

Egg-laying hormone was chosen as the target peptide in the project for a number of sound practical reasons. The peptide was known to be released from the bag cells, which are a pair of homogeneous clusters of neurons attached to the abdominal ganglion; access to source tissue would therefore be relatively easy. There is a rich background of information on the behavioral effects of ELH. The hormone is manufactured at relatively high concentration; therefore the chances were excellent that the messenger RNA could be isolated and the search for ELH genes could be undertaken.

Success was swift in coming. ELH messenger was used to fish out an ELH gene from fragmented DNA. And this gene was then used as a very specific

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probe with which to search for other ELH genes that might be present in the snail's genome. Very quickly the Columbia team was able to establish that there is a family of about five genes, but the exact number remains to be determined. This, clearly, was an intriguing discovery because the existence of multiple genes implied that the production of ELH was probably not as simple as might have been imagined.

Neuroscientists have in recent years become used to the idea that in certain cases several peptides might be encoded in a single gene which gives rise to a polyprotein. Perhaps the most prominent example of this is pro-opiomelanocortin, which is a portmanteau of at least three neuroactive peptides (ACTH, MSH, and β-endorphin). The polyprotein precursor is processed by cleavage at specific sites (a pair of basic amino acids, usually lysine and arginine) that flank the active peptide regions. Different processing in different parts of the pituitary, where pro-opiomelanocortin is synthesized, releases different combinations of neuroactive peptides.

A second example of a multipart precursor for neuropeptides, and related in small degree at least to pro-opiomelanocortin, is the precursor of enkephalin. Because pro-opiomelanocortin contains the sequence for one form of enkephalin (Met-enkephalin, as part of β-endorphin), it was expected to be the precursor of the small opiate. But it turns out that another gene is responsible for Met-enkephalin production, a gene that encodes six Met-enkephalins, together with one Leu-enkephalin, in one polyprotein. Once again, the individual peptides are snipped out of the precursor at lysine-arginine pairs flanking the required sequence. The deployment of multifunctional genes in the production of neuroactive peptides is therefore firmly established.

When Axel, Schwartz, and Kandel began work on ELH they knew from the results of Felix Strumwasser, of the California Institute of Technology, Earl Mayeri, of the University of California, San Francisco, and Steven Arch, of Reed College, Oregon, that other peptides were closely associated with the release of the hormone. There was, however, no information on how the synthesis of these various peptides might be related. Nor was there any reason to suspect that ELH would be produced as part of a polyprotein, still less a family of such proteins.

As the project progressed, however, it became apparent that a number of discrete ELH-coding messenger RNA's were produced in *Aplysia*, three in the bag cells and two in the atrial gland, a secretory organ which is part of the reproductive system. The genes are not expressed in any other tissue. "This made us wonder," says Scheller, "why should there be different messengers for ELH?" When Scheller and his colleagues translated the messenger RNA's in vitro the answer was clear: the different messengers yielded different polyproteins. "It all began to make sense," he says. "A family of genes gives rise to a family of polyproteins which are then processed to give different, though related, sets of peptides."

In order to determine what peptides might be associated with ELH, the Columbia group has begun to sequence one of their ELH clones. With a sequence of 600 to 700 nucleotides now complete, the polyprotein nature of the gene structure is clearly revealed. The code for the 36 amino acids of ELH are flanked by the codes for lysine-arginine pairs. Adjacent is the coding sequence for another peptide involved in egg-laying behavior, B peptide. And other putative peptide-coding regions, flanked by cleavage sites, are clearly discernible. As Axel observes, these preliminary results demonstrate the power of searching for new neuropeptides with recombinant DNA techniques rather than sifting through neuronal extracts.

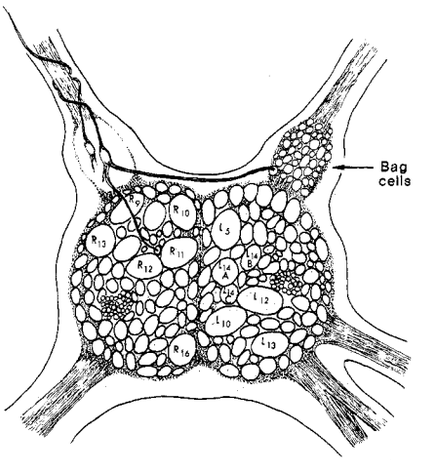
The constellation of active peptides in the ELH gene family is likely to be complex. A number of researchers have shown that when purified ELH, rather than a crude extract of bag cells, is used to elicit egg-laying behavior, only part of the normal activity occurs. Other peptides are required for the complete behavioral response, which includes cessation of walking and eating, a characteristic head waving, and finally the deposition of eggs.

In addition, there is a fascinating relation between egg-laying release hormone (ERH), which is produced by the atrial gland, and ELH, the hormone whose production it mediates. The first 19 amino acids of ERH correspond with those of ELH. Moreover, James Blankenship, of the University of Texas at Galveston, has shown that the last 12 amino acids of the release hormone are the same as two other atrial gland peptides, the A and B peptides, which are both involved in the control of ELH production. The relationships are intimate indeed.

Gene families of many kinds are of course widespread in most organisms, but what is important in the case of ELH is the existence of a family of multifunctional genes, the different components of which probably affect different aspects of a well-defined behavior. Expression of selected members of the gene family in different tissues, or in the same tissue at different points in development, might elicit distinct variants of the behavior. Such a system would be an important way of extending mechanisms of molecular control of behavior. "The concept of combinatorial sets of neuropeptides," conclude the Columbia researchers in their paper in *Cell*, "greatly expands the informational potential of a small set of genes." Specific probes for each ELH gene will have to be constructed in order to elucidate whatever differential expression might be operating.

If the five ELH genes of *Aplysia* do code for different sets of peptides that direct nuances of egg-laying behavior, then one can sketch possible evolutionary scenarios. For instance, a primordial coding sequence, an ancestral ELH gene perhaps, might have duplicated several times to give rise to a single transcription unit that specified a long polyprotein. Or, the ancestral gene might have specified a single multifunctional protein, in which case the evolution of cleavage signals and other control sequences would have allowed the production of individual peptides with interrelated functions. In any case, the origin of the capacity to release separate peptides is the crucial step in giving the organism an important degree of flexibility in molecular control of behavior.

Copying and translocation of the multifunctional gene to other parts of the genome would establish a gene family, the members of which could diverge in structure and, presumably, in function. The end result would be a gene family in which combinatorial control would be possible.—ROGER LEWIN



The source of egg-laying hormone

Bag cells, 800 homogeneous neurons, produce ELH with other neuropeptides.