Perspective

Molecular Neurobiology—A Conference Sponsored by the NIMH

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OVER THE PAST DECADE, THE NATIONAL INSTITUTE OF Mental Health (NIMH) has gone through a transformation that has included a renewed commitment to contemporary biological science and its applications to the problems of mental illness. At the beginning of May a conference on molecular neurosciences was sponsored by the Neurosciences Research Branch of NIMH. The topics discussed at the meeting stressed basic research areas that are likely to have implications for the study of mental illness and also highlighted how far the concerns of NIMH have moved. Our discussion will focus on three themes: neuronal plasticity, the molecular biology and structure of ion channels and receptors, and developmental neurobiology. A more complete discussion will be presented in the forthcoming symposium volume.

Several presentations including the keynote talk of the meeting centered on molecular processes of "memory" in various model systems ranging from bacteria to the mammalian hippocampus. Eric Kandel of Columbia University dealt with molecular and behavioral changes associated with short- and long-term memory in the marine snail *Aplysia*. In this system short-term sensitization results from modulation of synaptic strength by transmitters such as serotonin, which act through second messenger systems and protein kinases to increase the duration of the action potential. Protein phosphorylation is thought to decrease the number of potassium S channels, resulting in a greater calcium influx and more transmitter release.

In addition to the modulation of ion channels, other synaptic phosphoproteins play important roles mediating transmitter release and metabolism. Paul Greengard of Rockefeller University discussed mechanisms of synaptic vesicle mobilization mediated by synapsin I. Phosphorylation of the collagenase-sensitive "tail" region by the calcium-calmodulin–dependent protein kinase II results in dissociation of synapsin I from both actin and synaptic vesicles. These presentations, along with the studies of phosphorylation of membrane receptors important in modulation of bacterial chemotaxis presented by Daniel Koshland of the University of California, Berkeley, underscore the important role of covalent modification of preexisting molecules in mediating cellular responses to environmental stimuli. However, in spite of this impressive work, over 70 phosphoproteins, many of which are neuron-specific, have been identified that do not have precisely defined roles in the brain.

Eric Kandel also discussed long-term memory, which may involve some of the same loci that mediate the short-term modulation of synaptic strength. Major differences in these processes are that the long-term changes in *Aplysia* and other organisms are blocked by inhibitors of RNA or protein synthesis and involve the growth of new synaptic connections. Investigations are now centered around identification of the genes and protein products that may mediate the long-term changes.

Studies of the modulation of neuropeptide gene expression by neuronal activity and second messengers may provide insight into the genetic mechanisms of long-term memory. Ira Black, from Cornell University Medical School, presented studies that demonstrate that levels of messenger RNAs encoding the tachykinins are modulated by denervation and electrical activity. Jack Dixon from Purdue University went on to show that the somatostatin promoter region contains an eight-nucleotide sequence that confers adenosine 3′,5′-monophosphate (cAMP) regulation of transcription. The Dixon group has used DNA affinity chromatography to purify a 43-kD trans-acting factor that binds the octanucleotide site. It will be interesting to determine if this factor is a phosphoprotein and if transcription is directly influenced by the phosphorlyation state of the molecule. Perhaps the greatest challenge will be to unravel the molecular mechanisms whereby phosphorylation alters the activities of such a great diversity of molecules.

Another type of neuronal plasticity, discussed by Roger Nicoll from the University of California, San Francisco, and Chuck Stevens from Yale, involves modulation of excitatory synapses in the mammalian brain. Two types of glutamate receptors—the Q/K (quisqualate/kainate) type, which produces a brief (5 ms) increase in conductance to Na^+ and K^+ ions, and the NMDA (*N*-methyl-D-aspartate) type, which causes a prolonged (400 ms) increase in conductance to Na^+, K^+, and Ca^{2+}—are the key players in this process. The NMDA receptor is only activated by glutamate when depolarization of the postsynaptic membrane relieves a voltage-dependent block by Mg^{2+}, allowing Ca^{2+} influx. Thus, the pairing of glutamate release and depolarization provide the basis for an associative synaptic plasticity referred to as long-term potentiation (LTP) in hippocampal neurons. The Nicoll group demonstrated that a rise in the intracellular concentration of postsynaptic Ca^{2+} can mimic the short-term potentiation caused by NMDA. However, the longer lasting events of LTP require stimulation of presynaptic afferents by an as yet unknown mechanism. The biochemistry of this modulation must now be investigated and integrated with the physiology. In addition to increasing our understanding of plasticity, studies of LTP may also eventually lead to an understanding of the effects of some hallucinogenic drugs such as phencyclidine, which block the opening of NMDA-gated channels.

A second theme focused on the three important families of proteins involved in membrane excitibility of neurons. These are the voltage-gated ion channels, the ligand-gated ion channels, and the guanyl nucleotide binding (G) protein–coupled receptors. Members of these families show sequence as well as overall structural similarities, and various reports at the meeting revealed the fact that the diversity within the families is tremendous. The brain $GABA_A$ receptor discussed by Eric Barnard from the MRC Centre at Cambridge was shown by biochemical studies to consist of α and β subunits whereas the nicotinic acetylcholine receptor discussed by Steve Heinemann from the Salk Institute is comprised of the four different subunits α, β, γ, and δ. It is now clear that both of these ion channel–receptor systems are comprised of a variety of subtypes encoded by independent genes. For instance, there are at least four genes encoding α-like subunits of the acetylcholine receptor. The genes are differentially expressed not only in muscle and nerve but in

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different brain regions as well.

In the case of the voltage-sensitive potassium channel presented by Lily Jan of the University of California, San Francisco, alternate RNA splicing generates different voltage-sensitive ion channels from a single gene. In an exciting development, this group used the gene originally isolated based on the *Shaker* mutation in *Drosophila* to clone a homologous cDNA from rat that probably encodes a mammalian potassium channel. Thus the powerful tool of *Drosophila* genetics has made it possible to clone a mammalian channel for which no enriched tissue source or high affinity ligand is available.

Functional assays with the *Xenopous* oocyte system can also be used to isolate clones encoding novel G protein–coupled receptors. Oocytes injected with RNAs that have been synthesized in vitro are tested for sensitivity to various transmitter or modulatory substances. This technique was first used by Shigetada Nakanishi and his collegues at Kyoto University to isolate the receptor for the neuropeptide substance K and more recently by the labs of Richard Axel and Thomas Jessell at Columbia to isolate a cDNA encoding a functional serotonin receptor. Both of these receptors belong to the seven transmembrane domain, G protein–coupled receptor family. The serotonin receptor clones have been useful in clarifying regions of the central nervous system that express this serotonin receptor because in situ hybridization is usually more specific than ligand binding in differentiating among receptor subclasses.

Because there is such a diversity of channels and receptors, it is important to understand the functional significance of the individual proteins. Are the amino acid sequence differences among members of a receptor gene family crucial in specifying unique interactions with other molecules, including protein kinases? Furthermore, how is the expression of these genes regulated to generate the intricate distribution of receptors and channels in the brain? Certainly cloning the genes is a first step, yet the answers to at least some of these questions are still likely to be in the distant future.

Talks too numerous to mention in this short report focused on issues in developmental neurobiology. What guides axonal growth, how neuronal cell fate is determined, how glial cell development is controlled, and how gene expression in developing neurons is regulated were but a few of the topics covered.

Naturally a great deal of interest was generated by the studies using the two classic genetic systems in biological research, *Caenorhabditis elegans* and *Drosophila*, where mutations affecting neuronal development can be investigated at the molecular level. H. Robert Horvitz from Massachusetts Institute of Technology stressed the power of the *C. elegans* system for studies of cell lineage. The precise lineage of every cell including the 302 neurons and 56 glial cells is defined in this species making it possible to obtain mutants that alter normal development and behavior. Horvitz described genetic studies of egg-laying that have defined 34 genes that perturb the function of the serotonergic motorneurons that innervate the vulval muscles. It is hoped that further genetic studies and molecular cloning of these genes will shed light on the development and function of these cells. A complete physical map of the *C. elegans* genome is about 50% completed, so many genes can now be more easily isolated.

Many of the genes that control early development and segmentation in *Drosophila* have been cloned and characterized. The proteins encoded by many of these genes contain homeodomains, are localized to the nucleus, and are believed to interact directly with DNA to modulate gene expression. Corey Goodman of the University of California, Berkeley, is studying the role of these genes in neuronal development in *Drosophila*. Many of these genes are expressed at times other than early development, including neurogenesis. Using either promoter constructs or temperature-sensitive mutations Goodman's lab can express the segmentation genes early in development but then disrupt their subsequent reexpression during crucial periods of neuronal development. These studies demonstrate a crucial role for *fushi tarazu* and *even-skipped* genes in determining neuronal cell fate in the developing *Drosophila* embryo.

Yuh Nung Jan of the University of California, San Francisco, and Gerald Rubin from the University of California, Berkeley, described their exciting studies on the cloning of new genes that control neuronal fate, the *cut* and *rough* genes, respectively. Both of these genes contain homeodomains and are localized to the nucleus, supporting a common theme for the control of gene expression and cell fate in the nervous system. As is the case with the *Drosophila* potassium channel, it is hoped that studies of simple systems will bring forth fundamental principles and also aid in the isolation and characterization molecules important in mammalian development.

The presentations of this meeting indicate that behavioral neurochemistry as a field is now firmly established at the cellular and molecular levels. Tremendous progress has been made since the Cold Spring Harbor Symposium on Molecular Neurobiology in 1983, and the discipline has a broader range of application. Now there is a need to facilitate through NIMH many of the approaches described at this meeting. How do these research areas contribute to the mental illness research supported by NIMH? Eric Kandel challenged the audience as to whether new improved therapies will come from the knowledge that is being accumulated. Many areas discussed fit in directly with the goals of NIMH, which include elucidation of severe disorders, such as schizophrenia and manic depression in which neuroregulators, receptors, ion channels, and the fundamentals of neural connectivity will be key factors in understanding the molecular bases of these illnesses. Through the types of studies described at this meeting it should also be possible to develop novel therapeutic agents with increased specificity and to decrease the side effects of existing drugs. In addition, there may be multiple forms of what are now considered unitary disorders. Only when the mechanisms of the diseases are understood will it be possible accurately to classify them.

The meeting had another underlying theme: the future of its sponsor, NIMH and its Neurosciences Research Branch. Will NIMH become an institute capable of broadly supporting the type of research it is encouraging by such a meeting? The Neurosciences Research Branch is now headed by a neuroscientist with both basic and clinical credentials, Steve Koslow; Steve Zalcman, a clinically trained investigator with a background in basic science, is head of its Neurobiology Program. The NIMH has strong ties to academic and scientific centers and, with the recent appointment of Lewis Judd as director of NIMH, this is expected to continue.

The total budget of the Neurosciences Research Branch is $33 million. As a report of the Institute of Medicine of the National Academy of Sciences observed several years ago, that amount is inadequate to support critical areas of neuroscience that are key to the goals of NIMH. At the request of the Congress, the NIMH has prepared a report dealing with opportunities for advances in the clinical and basic neurosciences of mental illness. That report, *The Decade of the Brain: Opportunities for NIMH Neuroscience Research*, provides a description of basic research that is imperative for ongoing and future clinical research.

In an era of constraints, the goals of the *Decade of the Brain* may be difficult to bring to fruition. Budget increases at NIMH over the past several decades have not kept up with inflation. Nevertheless, mental illness has an enormous societal cost, creates a devastating social impact, and disproportionately strikes the young. These forces, combined with the facts that basic neuroscience has recently made vigorous advances and that basic researchers and families of patients are lobbying on behalf of NIMH and its research budget, may bode well for the success of the institute.