GENE EXPRESSION & CELL-CELL INTERACTIONS IN THE DEVELOPING NERVOUS SYSTEM

Edited by Jean M. Lauder
and Phillip G. Nelson

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PREFACE

The dramatic advances in molecular genetics are becoming incorporated into neurobiologic studies at an ever increasing rate. In developmental neurobiology, the importance of cell-cell interactions for neurogenesis and gene expression is beginning to be understood in terms of the molecular bases for these interactions. This book seeks to emphasize the importance of molecular technology in the study of neurogenetic mechanisms and to explore the possible relationships between specific cell-cell interactions and regulated gene expression in the developing nervous system.

This volume consists of nineteen chapters which address questions of gene expression and the importance of cell-cell interactions as key factors in the developing nervous system. Rather than viewing these two processes as separate mechanisms, as the organization of these chapters might suggest, we would like to emphasize the interplay of these genetic and epigenetic influences in all phases of neural ontogeny, a concept which is made clear by the subject matter of the contributions themselves. The authors of these chapters were participants in selected symposia from the Fourth Congress of the International Society of Developmental Neuroscience held in Salt Lake City, Utah, July 3-7, 1983.

The first two papers, under the heading "Perspectives", were written by the two plenary speakers fo this meeting who were asked to provide a theoretical overview of their work, based on the content of their original lectures. The chapter by Karl Pfenninger presents a model of the molecular events involved in growth cone motility and chemotaxis, based on his recent work with isolated growth cone fractions. In the chapter by Marshall Nirenberg, the growth cone is followed to the synapse where the importance of cAMP as a regulator of synaptogenesis is discussed.

In the section on "Molecular Genetics and Gene Expression" we have collected papers which range from the use of recombinant DNA technology to probe the molecular basis for development of cellular diversity in the nervous system to the use of mutants to study

mechanisms of circuitry construction in the developing brain.

In the chapter by Devillers-Thiery and co-workers, the molecular cloning of the acetylcholine receptor is described, including their hypothesis of the transmembrane organization of this molecule. This chapter illustrates the power of biotechnology for the elucidation of molecular mechanisms of receptor development and provides a context for the following chapters which make use of this technology for studies of genetic mechanisms at play in neurogenesis.

In the following contribution by Hahn and Chaudhari, the macromolecular complexity of both the developing and adult brain is discussed, including the importance of both genetic and epigenetic mechanisms in creating this diversity. These studies employ the strategy of making recombinant DNA (cDNA) libraries from adult brain and screening them by hybridization against brain messenger RNA (mRNA) from various stages of development to detect developmentally regulated genes and gene products.

Joh, on the other hand, discusses the molecular biology of a specific class of brain-specific molecules, those enzymes involved in the synthesis of the catecholamine neurotransmitters. In this contribution, a case is made for homology between the genes coding for these enzymes and for their derivation from a common ancestral gene. He also postulates that these enzymes share common protein domains with nerve ending proteins with which they may be intimately associated either in the cell membrane or in the membranes of synaptic vesicles. He concludes that the coordinate expression of the genes coding for these enzymes may be important in determining the catecholaminergic phenotype.

In the chapter by Pintar, another class of neurotransmitters is discussed, the pro-opiomelanocortin (POMC) peptides. This chapter describes studies designed to ascertain mechanisms of differentiation of the pituitary using both classical biochemical approaches as well as recombinant DNA technology to determine the timing and regulation of POMC gene expression. The appearance of monoamine oxidase is also studied as another transmitter-related marker of pituitary differentiation.

The interplay of genetic and epigenetic influences in brain development is made especially clear in the chapter by Villa-Komaroff and colleagues who discuss the molecular biology of the insulin-like growth factors which appear to be localized in the developing brain and may play important roles in the regulation of its development. This chapter will be especially useful to those neurobiologists new to recombinant DNA technology, since the authors give a detailed and well organized description of the methodology they have used, including the rationale behind the various techniques chosen.

The chapter by Dreyer and Roman concludes the portion of this section devoted to the use of biotechnology by discussing an hypothesis for gene expression during embryogenesis based on the molecular biology of the immune system, in which the editing of chromosomes by the splicing of genes plays a key role in the regulated expression of selected gene sequences leading to the formation of specific cell lineages.

With the chapter by Crepel and colleagues, we return to the organismal level for a discussion of how mutations of the nervous system can be used to study mechanisms of synaptogenesis, using the cerebellum as an example. The model system is the multiple innervation of the Purkinje cells by climbing fibers which regresses to innervation by single fibers during postnatal development. In three different mutants (weaver, reeler, and staggerer) in which other key competitive elements are absent, this synaptic rearrangement does not occur. Thus the case is made for the importance of synaptic competition in the correct wiring of this brain circuitry.

The use of mutants to study developmental mechanisms in the nervous system is further explored in the chapter by Wolf and Billings-Gagliardi who describe their studies of three mutants which exhibit disorders of myelination. The locus of the mutation in all three cases appears to lie in the oligodendrocyte rather than in the developing axon, as determined from tissue culture studies where mutant and normal neurons and glia are mixed and the consequences for myelin formation analyzed.

The importance of ploidy for development of normal neuronal connections is discussed by Tompkins and colleagues who have studied the effects of polyploidy in amphibians on the development of the retino-tectal system. They postulate that alterations in cell size and number as a result of gene duplication can lead to a complex miswiring of the nervous system due to changes in cell-cell interactions. This chapter provides another example of the interplay of genetic and epigenetic mechanisms in neurogenesis, as discussed in detail in the next group of chapters.

The second section of this book contains a series of chapters which discuss various types of "Cell-Cell Interactions and Epigenetic Influences" which appear to play roles in shaping the developing nervous system, including possible molecular mechanisms involved in these processes.

This section begins with two chapters on cell-adhesion and recognition molecules which appear to be important in mediating these cell-cell interactions.

In the chapter by Hoffman and Edelman, a mechanism of cell adhesion is discussed based on the binding of cell adhesion mole-

cules (CAMs) on one cell to those on another adjacent cell. It is suggested that a small number of these cell adhesion molecules could mediate pattern formation in the developing nervous system by undergoing chemical modification or changes in the spatio-temporal patterns of their expression as a result of modulation through such cell-cell interactions or other epigenetic influences.

Marchase discusses another cell surface molecule, ligatin, which appears to be important in mediating the adhesion of retinal cells to each other and to tectal cells via glycoproteins attached to the ligatin molecule which is embedded in the cell membrane. Such a mechanism may also be involved in cell-cell interactions in other parts of the developing nervous system. The trafficking of the ligatin molecule and its associated glycoproteins from their sites of synthesis within the cell to their final destinations at the cell surface is discussed in detail based on recent experimental findings.

New instrumentation to facilitate the microchemical analysis of cell surface molecules is described in the chapter by Dreyer and colleagues who have developed an ultrasensitive protein sequenator and a miniaturized mass spectrometer which make possible the structural analysis of picomole quantities of proteins and peptides. These peptide sequences are then used to synthesize oligonucleotides which are used to probe cDNA or genomic libraries for the genes related to these cell surface molecules. This instrumentation, together with advanced computer technology, is being used to analyze molecules important in cell-cell interactions in various parts of the developing nervous system, including the cerebellum and the neural retina.

The functional consequences of specific cell-cell interactions in the deveoping nervous system are discussed in the chapters by Linser and Moscona and Fisher who correlate specific neuronal-glial interactions with the development of glial marker enzymes in the neural retina and cerebellum, respectively. In the neural retina, the glial enzymes glutamine synthetase and carbonic anhydrase are regulated differently, such that neuronal-glial interactions are required for normal expression of glutamine synthetase, but not for carbonic anhydrase. In cerebellar mutants, changes in the expression of the glial marker, glycerol-3-phosphate dehydrogenase (GPDH) are attributed to alterations in cell-cell interactions between Bergmann glia and Purkinje cell dendrites.

The possible role of neurons and their neurotransmitters in the development of their target cells is discussed in the chapter by Wolff and co-workers with respect to the GABAergic system of the developing visual cortex, based on the presence of the cellular machinery for synthesis, uptake and release of GABA prior to the formation of GABAergic synapses with other cortical cells.

In the last two chapters on synaptogenesis, Fishman and

Fishman and Nelson discuss the different phases which developing synapses pass through during synaptogenesis: targeting, stabilization and rearrangment. This sequence is similar to that described by Devillers-Thiery and colleagues in their discussion of the formation of the cholinergic synapse in the first chapter of this volume. The progression of developing synapses through these phases appears to be dependent on competition, as also suggested by Crepel in his chapter on cerebellar synaptogenesis in mutant mice, and is related to neuronal activity, as determined in cell culture experiments. The molecular basis for this activity-dependence may involve transported proteins or other, smaller molecules, such as peptides or ions.

The editors wish to thank all of the authors for their contributions to this volume, the International Society for Developmental Neuroscience for the hosting of these Symposia and Plenary lectures, and especially Mr. Philip Alvarez of Plenum Publishing Corporation for his own initiation of this book and his efficient handling of its production.

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CONTENTS

MOLECULAR BIOLOGY OF THE NERVE GROWTH CONE: A PERSPECTIVE

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"I had the good fortune to behold for the first time that fantastic ending of the growing axon. In my sections of the three-days chick embryo, this ending appeared as a concentration of protoplasm of conical form, endowed with amoeboid movements. It could be compared to a living battering-ram, soft and flexible, which advances, pushing aside mechanically the obstacles which it finds in its way, until it reaches the area of its peripheral distribution. This curious terminal club, I christened the growth cone" (Ramón y Cajal, 1937).

INTRODUCTION

As so vividly described by Cajal, the nerve growth cone is the amoeboid, leading edge of the growing neurite. It occurs only during a narrow segment of the neuron's life, during the time between terminal mitosis and synaptogenesis and, possibly in somewhat different form, during regeneration. As the growing, advancing tip, the nerve growth cone plays a crucial role in nervous system development. It is critical for the establishment of the neuron's unusual geometry and high degree of polarity because it appears to be the main site of insertion of new plasma-

lemmal components for surface expansion (Pfenninger and Maylié-Pfenninger, 1981b; Pfenninger and Johnson, 1983). Furthermore, the growth cone is the structure responsible for vectorial growth into the appropriate target area because it is capable of locomotion, pathfinding and chemotaxis (see below). Last but not least, it is the structure which recognizes the target cell and triggers synaptogenesis. Nerve growth cones, devoid of ribosomes and Golgi apparatus, are apparently not capable of performing many of the synthetic functions carried out in the perikaryon (e.g., protein synthesis), but they seem to be competent to execute a variety of complex short-term functions, such as chemotaxis, without the direct involvement of the perikaryon. Indeed, the time frame of the growth cone's motile behavior (minutes) is so short that constant signaling between growth cone and perikaryon for the control of chemotaxis appears highly unlikely. Thus, the nerve growth cone is endowed with a certain degree of autonomy, especially with regard to the control of vectorial growth: One may actually envision the nerve growth cone as a "leukocyte on a leash", spinning out the neurite behind it (Figure 1). This analogy may be useful for the formulation of hypotheses on growth cone function and for the design of appropriate experimentation to test them.

Progress in research on nervous system development will depend to a large extent on the identification of growth-cone-specific components expected to be involved in functions unique to the structure and on the elucidation of mechanisms controlling vectorial growth and triggering synaptogenesis. Any research in these directions necessitates the use of methods of modern cell and molecular biology and, thus, the availability of substantial amounts of growth cone material in relatively pure form. For this reason, my laboratory has invested several years of intense effort

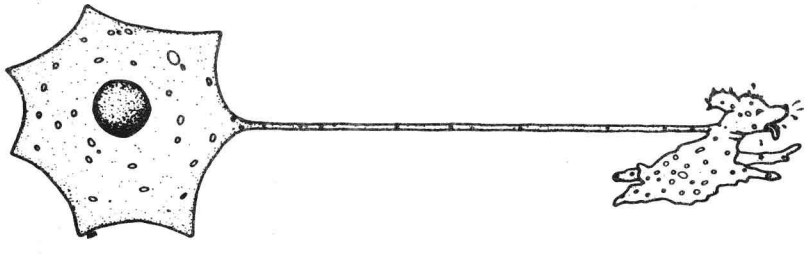

Fig. 1. The nerve growth cone, a "leukocyte on a leash".

in developing methods for the isolation of nerve growth cones from fetal mammalian brain. We have recently succeeded with this endeavor (Pfenninger et al., 1983) and many of the observations and conclusions summarized here are based on new results obtained from this growth cone fraction.

RESULTS AND DISCUSSION

Because the subcellular fraction enriched in fragments of nerve growth cones, so-called "growth cone particles", plays a critical role in the studies discussed below, it is briefly described here: The homogenate of fetal rat brains (17 days gestation) is first spun to produce a low-speed supernatant. This supernatant is layered on a discontinuous sucrose density gradient and spun to equilibrium (Pfenninger et al., 1983). The fraction at the interface between the load (0.32 M sucrose) and 0.75 M sucrose is highly homogeneous and contains membrane-bound elements that bear the morphological characteristics of axonal nerve growth cones (Figure 2). These particles also co-purify with identified nerve growth cones microdissected from

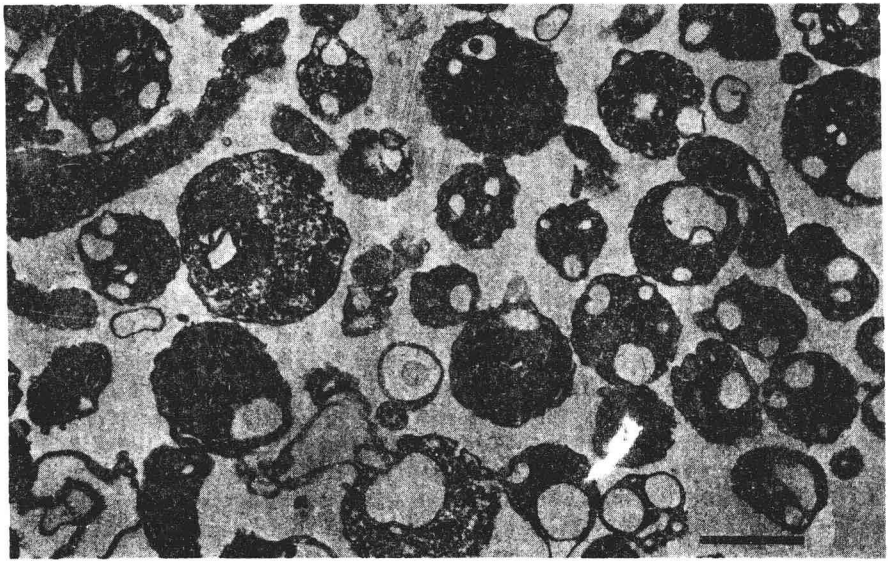

Fig. 2. Growth cone particles isolated by subcellular fractionation from fetal rat brain. The fraction is highly homogenous and consists of membrane-bound particles filled with organelles characteristic of nerve growth cones. For further description, see text. Calibration, 1μm.

primary cultures. A number of biochemical markers have recently been identified, and they support the cytological findings as follows: (i) One of the major membrane proteins of growth cone particles seems to have the same electrophoretic properties as GAP 43, a "growth-associated protein" which is neuron-specific and expressed during growth and regeneration, but not in mature axons (e.g., Skene and Willard, 1981; Ellis and Pfenninger, 1984; Meiri, Katz, Ellis, Willard, Pfenninger, in progress). (ii) Growth cone membranes are highly enriched in a large glycoprotein (250 to 300 kd) which is present during axonal growth, but neither in the dividing neuroblasts nor in the mature neuron (Wallis et al., 1983). (iii) The synaptic terminal's characteristic pattern of protein kinases and substrates is present in growth cone particles and highly enriched in this fraction relative to fetal brain homogenate (Ellis et al., 1983; Ellis et al., 1984; Katz et al., 1984). The presence of these neuronal markers, together with the ultrastructural features of growth cone particles, is strong evidence for their origin from nerve growth cones. Therefore, biochemical analyses of growth cone particles can be used to investigate nerve growth cone properties and functions.

Growth-Specific Gene Products of the Neuron

The fact that the nerve growth cone is a unique structure reserved for vectorial growth of the neurite has long suggested that the neuron should be synthesizing specific components during sprouting. This view is further supported by the fact that the growth cone membrane has unusual properties, both from the standpoint of lectin labeling (Pfenninger and Maylié-Pfenninger, 1981a) and of intramembranous structure revealed by freeze-fracture (Pfenninger and Bunge, 1974; Small and Pfenninger, 1984). However, biochemical identification of the first growth-associated components was accomplished only recently by Hall et al. (1978), Theiler and McClure (1978), Bisby (1980), Benowitz et al. (1981), Heacock and Agranoff (1982), and by Skene and Willard (e.g., 1981), who named them "GAPs". GAPs are either components of membranes or, possibly, contained in the lumen of vesicular structures shuttled by rapid transport to the tip of the growing axon. As already mentioned, a collaborative effort between the Willard and Pfenninger laboratories has indeed suggested that GAP 43 co-migrates in two-dimensional gel electrophoresis with one of the major bona-fide membrane proteins of isolated growth cones (Meiri, Katz, Ellis, Willard, Pfenninger, in progress). It appears that, in our gel system, this protein forms a doublet with an apparent molecular weight of 38,000. A second major membrane protein of the growth