Neuroendocrinology

Molecular approach appraised

from Stafford Lightman

THE mechanisms controlling neuroendocrine regulation can be modulated by a variety of hormones, neurotransmitters and neuromodulators both *in vivo* and *in vitro*. As these systems have been well characterized by physiologists, they clearly offer an extremely attractive model in which to study the control of gene expression. The benefits and problems of the application of molecular genetics to neuroendocrine models were brought up to date at a recent meeting*.

Characterization of the precursors of hormones continues to be an important step in understanding the regulation of hormone production and in the discovery of new candidate hormones. A good example is the recent description of the precursor to gonadotropin-releasing hormone (GnRH) with its carboxy-terminal sequence GAP, which itself inhibits prolactin and stimulates gonadotropin secretion (Nikolics *et al. Nature* **316,** 511; 1985). Further studies (P. Seeburg, Genentech) confirm that GAP coexists with GnRH in hypothalamic cells, but also show that it is present in portal blood and even in the ovary and testis where GnRH has previously been demonstrated.

Except in frog skin, where it is found in abundance (Richter, D. *et al. EMBO J.* **3,** 617; 1984), the precursor to the tripeptide (Gln–His–Pro) releasing hormone, TRH, has been particularly difficult to identify. The problem has now been circumvented with antibodies to a synthetic peptide (Cys–Lys–Arg–Gln–His–Pro–Gly–Lys–Arg–Cys) presumed to represent a portion of the TRH precursor (R. Goodman, New England Medical Center, Boston). The antibodies were used to screen a DNA library from rat hypothalami, which yielded a cDNA clone that encodes a protein containing five copies of the sequence Gln–His–Pro–Gly, flanked by pairs of basic amino acids. In support of the view that this protein is a TRH precursor, *in situ* hybridization with the cDNA was shown to detect neurones in those regions of the hypothalamus that are known to produce TRH. Thus, as with enkephalin, TRH has a precursor from which multiple copies of the biologically active peptide can be produced. Oddly, there is no homology between the frog-skin and rat hypothalamic precursors except for the TRH units themselves.

Defining the mechanisms of gene regulation is a major challenge to the molecular neuroendocrinologist. Quantitation of changes in pro-opiomelanocortin (POMC) mRNA in the anterior pituitary in response to corticotropin releasing factor and dexamethasone, or in the pars intermedia in response to bromocriptine, provide evidence of control at the level of gene transcription (J. Roberts, Columbia University, New York). The situation is more complex, however, in sex-hormone mediated changes in the arcuate nucleus of the hypothalamus. Treatment of a two-week ovariectomized adult rat with oestradiol results in a slow decline in the concentration of POMC mRNA in the arcuate nucleus and the expected corollary, a rapid decline in POMC transcription, which remains suppressed for days.

On the other hand, events controlling dopamine synthesis in the same system are not so clear. Oestradiol administration results within one hour in a fall of the transcription of the gene for tyrosine hydroxylase (TH) — the rate-limiting enzyme in catecholamine synthesis — with a gradual return to normal over 4 days, whereas the amount of TH mRNA begins to increase after 20 minutes and continues to rise for 4 days. This dissociation of the quantity of TH mRNA from the level of transcription of its gene implies that mRNA stabilization may be responsible for some of the quantitative changes in TH mRNA. Clearly, turnover of TH mRNA is the variable that we should like to measure, but the techniques are not available and, in particular, we have no idea how to study the rate of specific mRNA degradation.

Similar problems apply to the interpretation of *in situ* hybridization studies, in which the hybridization of intracellular mRNA with specific DNA probes is used to locate sites of gene expression in tissue sections. This technique cannot provide any measure of RNA turnover and suffers from a lack of sensitivity which still limits its application to quantifying intracellular mRNAs. Nevertheless, it is finding many applications. For example, POMC mRNA can be detected in the pars intermedia of the rat pituitary, but in the anterior pituitary it is only found after adrenalectomy (J. Coghlan, University of Melbourne). Atrial natriuretic peptide mRNA could not be detected in the hypothalamus but has been found in the cardiac atria; renin mRNA has been detected for the first time in the arcuate nucleus. Vasopressin and oxytocin cDNA probes have now been used to measure mRNA responses to different physiological conditions (J. McCabe, Rockefeller University). Rats whose drinking water has been substituted with two per cent saline have an increased proportion of hypothalamic magnocellular cells heavily labelled by vasopressin cDNA, and magnocellular cells from 15-day pregnant rats show an increase both in the number of heavily labelled cells and in the number of cells with any autoradiographic grains. Further experiments are needed to show whether this represents two different groups of responding cells within the magnocellular paraventricular nucleus.

Novel techniques to measure gene expression and post-translational processing are exciting considerable attention. One method of incorporating mRNA into cells is by the use of a vaccinia virus (VV) vector (G. Thomas, Oregon Health Sciences University). The infection cycle of VV, unlike that of other DNA viruses, occurs solely in the cytoplasm of the host cell. Insertion of a cDNA downstream from an early VV promoter leads to transcription of the cDNA in the host cell within minutes of infection and to efficient production of protein from the mRNA. The potential of the technique is illustrated by the infection of different cell lines with recombinant VV containing the cDNA for human proenkephalin. Infected BSC-40 cells contain 2 main peaks of enkephalin immunoreactivity of which only the larger precursor is secreted into the medium. Infected AtT-20 cells, however, contain five peaks of immunoreactivity, with met-enkephalin as the major secreted form. This is a good example of tissue-specific processing. The considerable versatility of the VV system will doubtless provide much further information on this and other processing systems. □

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