Appendix A14.20

Regulation of Eukaryotic Gene Expression

Gene expression leading from DNA to the final, functional protein is a complex, multistep process, with each step subject to regulatory control. Theoretically, the steady-state level of a particular mRNA can be controlled at any step in the sequence of: i) transcription (initiation and elongation), ii) RNA processing (addition of the 5'cap , Poly(A) tail, and splicing), iii) mRNA transport from the nucleus to the cytoplasm, iv) translation, v) regulation of cytoplasmic RNA stability, accumulation and degradation, vi) protein processing and localization (see Atwater et al., 1990, and Karin, 1992 for excellent reviews).

Although eukaryotic gene expression is controlled at several different levels, these metabolic events can be broadly classified into transcriptional and post-transcriptional regulation. The first of these is the rate at which new mRNA transcripts are initiated by RNA polymerase; regulation of this event controls both when and where a gene is transcribed. The elucidation of transcriptional control mechanisms has resulted in the identification of both small cis-acting DNA elements that control cell-specific transcription as well as trans-acting proteins (transcription proteins). The most common way to regulate gene expression in response to signals is to modulate the activity of sequence-specific transcription factors (Angel et al., 1987; Malviya et al., 1990; Ofir et al., 1990 Atwater et al., 1990; Bohmann, 1990; Hunter & Karin, 1992; Jackson, 1992; Karin, 1992).

Cells respond to both intra- and extracellular cues by turning certain genes on or off, and by modulating the extent of transcription of active genes. In higher eukaryotes, transcriptional changes in the context of a developmental program can have profound long term physiological and pathophysiological consequences. Although the mechanisms and biochemical pathways by which neurons integrate environmental, physiological and pharmacologic cues to bring about appropriate transcriptional changes are still largely unknown, it is clear that the frequency of initiation of mRNA synthesis depends ultimately on factors that interact with specific elements in gene promoters.

Transcriptional selectivity of eukaryotic genes is mediated by complex control regions composed of different combinations of promoter and enhancer elements arrayed in tandem that appear to allow multiple distinct regulatory factors to function coordinately to potentiate RNA synthesis (Ofir et al., 1990; Atwater et al., 1990; Bohmann, 1990; Hunter & Karin, 1992; Jackson, 1992; Karin, 1992).

In the CNS, the role of receptor-mediated generation of intracellular second messengers and events coupling of cell activation has been explored in detail using recombinant DNA techniques to localize signal-responsive elements within regulatory DNA sequences that control gene transcription; this in turn, has allowed the identification and the cloning of a number of transcription factors directly modulated by signaling pathways. When cells are stimulated by neurotransmitters or depolarizing stimuli, signal transduction mechanisms converge on their cytoplasmic and nuclear substrates, and generate and amplify an appropriate cellular event or response. In the nucleus, the second messenger activities converge on stimulated expression of a small group of genes called primary response genes (PRG), the most prominent and best-studied of which are c-fos and c-jun. These primary response genes are characterized by rapid induction of their RNAs and protein. Their induction is rapid and transient and is not dependent on de novo protein synthesis, indicating that the transcriptional machinery necessary for rapid PRG induction is poised, awaiting stimulation through the transduction of extracellular stimuli. The mRNAs transcribed from these genes often have a very short half-life (in the case of c-fos, approximately 10-15 min); this tightly controlled expression of PRGs suggests a regulatory role for their protein products in cellular response to external stimuli. Fos contains a heptad repeat of five leucine residues which allows it to form stable heterodimer complexes with a variety of other transcriptional factors which share this motif, most notably the related protein Jun. These complexes are thought to influence transcription by binding to a regulatory DNA site termed AP-1(activating protein-1).

Within the framework of the current overview, the key feature of c-fos and other PRGs is that they can be viewed as "master switches" to turn on a "second wave" of specific neuronal genes of functional importance, resulting in long-term and enduring changes in the CNS. Such long-term control could result from a modification in receptors, G proteins, effectors, proteins involved in neurotransmitter release, and enzymes involved in neurotransmitter biosynthesis, thereby forming the biochemical basis for long-term synaptic adaptations.

In the mammalian CNS, downstream or posttranscriptional regulation also plays a general role in modulating the expression level of a particular molecule. In general, gene products which must be rapidly produced function within a relatively narrow time-frame, and thereafter be rapidly cleared, tend to be regulated posttranscriptionally. Such regulation may be as direct or rapid as phosphorylation or dephosphorylation of pre-existing protein, or more complex and involving the stabilizing or destabilizing of the pool of coding, cytosolic mRNAs. The type of control employed reflects the functional and physiological significance of the molecule. It is clear that regulated neuronal transcription requires the coordinated expression of multiple and specific trans-acting factors, enzymes, and ribonucleotide-protein complexes, and most of the primary transcript sequences are spliced before the mature mRNA is transported to the cytosol. In addition, repression of gene expression requires the continued production of various "silencer factors", contributing to the requirement of temporal precision and tissue and cellular specificity for the process of regulated gene expression.

Regulation of MRNA Stability by RNA Binding Proteins

Increasing recent evidence suggests that far from being "passive way-stations" of encoded information and simple intermediates in the pathway from gene to protein, mRNA molecules may exhibit markedly distinct properties based on structural features embedded in discrete regions of the molecule. The regulation of mRNA stability has now emerged as a critical control step in determining cellular mRNA levels, with individual mRNAs displaying a wide range of stability that has been linked to discrete sequence elements and specific RNA-protein interactions. Evidence is accumulating that strongly implicates the 3' UTR of mRNA in the regulation of transcript stability, and thus steady-state mRNA levels. A common cis element found in the 3' UTR of rapidly decaying mRNA is an AU-rich element (ARE), containing various numbers of AUUUA pentamers, sometimes associated with a general AU-richness with a surplus of uridylic residues. In hybrid constructs, AREs are able to confer rapid degradability to otherwise stable reporter transcripts.

Most relevant for the present discussion, it is noteworthy that various hormones and signaling pathways, including glucocorticoids and MAP kinases, are now known to control the levels of certain mRNAs in part by regulating transcript stability. Furthermore, the expression of a number of proteins that are important for CNS function is known to be markedly regulated by alterations in transcript stability. These proteins include the GLUTI glucose transporter, nerve growth factor (NGF), GM-CSF tumor necrosis factor (TNF), interferons (INF), interleukins (IL1, IL3, IL6), tyrosine hydroxylase, growth cone associated protein (GAP-43), the period (per) protein (a circadian rhythm regulator), c-fos, c-myc, and even a major target for lithium and valproate—the neuroprotective protein bcl-2.

Several classes of RNA-binding proteins have been implicated in regulating mRNA stability and turnover. More recently, a subset of smaller (ranging from Mr 30,000 to 40,000) mRNA-binding proteins have been identified that display recognition of AU-rich domains in the 3'-UTR . A human A+U-binding protein (AUH) has recently been cloned, and a growing body of data suggests that the regulation of mRNA stability by AUH plays an important role in a variety of physiological and pathophysiological processes. This has generated considerable excitement about the possibility that certain disorders of neuronal plasticity may also arise from pathological processes mediating the proper expression and targeting of genes by 3'UTR mediated processes.

In sum, cooperation among various levels of regulatory control points described above further increases the possibility for fine tuning the cellular response to physiologic, pathologic and pharmacologic perturbations. The relative contributions of individual steps of gene expression are likely to differ depending on the nature, duration and intensity of the perturbation. Although this level of complexity may seem cumbersome and inefficient, it allows for highly precise regulatory control, and endows neurons with specialized properties that permit the reception, transmission, and long-term storage of information and experiences. Thus, the induction of new programs of gene expression plays an important role in development and in the adaptive plasticity of the CNS, exerting control over processes such as neuronal sprouting, and learning-dependent gating between various synaptic pathways and neuronal circuits.

References

Angel, P., M. Imagawa, R. Chiu, B. Stein, R. J. Imbra, H. J. Rahmsdorf, C. Jonat, P. Herrlich, and M. Karin. 1987. "Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor." Cell 49:729-39.

Atwater, J. A., R. Wisdom, and I. M. Verma. 1990. "Regulated mRNA stability." Annu Rev Genet 24:519-41.

Bohmann, D. 1990. "Transcription factor phosphorylation: a link between signal transduction and the regulation of gene expression." Cancer Cells 2:337-44.

Hunter, T., and M. Karin. 1992. "The regulation of transcription by phosphorylation." Cell 70:375-87.

Jackson, S. M., C. A. Keech, D. J. Williamson, and A. Gutierrez-Hartmann. 1992. "Interaction of basal positive and negative transcription elements controls repression of the proximal rat prolactin promoter in nonpituitary cells." Mol Cell Biol 12:2708-19.

Karin, M. 1992. "Signal transduction from cell surface to nucleus in development and disease." Faseb J 6:2581-90.

Malviya AN, Rogue P, Masmoudi A, Labourdette G, Vincendon G. Gene transcription: a role for nuclear protein kinase C? Int J Cancer. 1990 Mar 15;45(3):580-2. No abstract available.

Ofir, R., V. J. Dwarki, D. Rashid, and I. M. Verma. 1990. "Phosphorylation of the C terminus of Fos protein is required for transcriptional transrepression of the c-fos promoter." Nature 348:80-2.