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Origins and Principles of Translational Control

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Proteins occupy a position high on the list of molecules important for life processes. They account for a large fraction of biological macromolecules—about 44% of the human body's dry weight, for example (Davidson et al. 1973); they catalyze most of the reactions on which life depends; and they serve numerous structural, transport, regulatory, and other roles in all organisms. Accordingly, a large proportion of the cell's resources is devoted to translation. The magnitude of this commitment can be appreciated in both genetic and biochemical terms.

Translation is a sophisticated process requiring extensive biological machinery. One way to estimate the minimal amount of genetic information needed to assemble the protein synthetic machinery would be to compile a "parts list" of essential proteins and RNAs and add up their sizes. However, this approach entails several questionable assumptions about the identity of the essential components and their minimal sizes. An alternative approach is to examine the genomes of simple living organisms. The smallest known cellular genome, that of the parasitic bacterium

Translational Control of Gene Expression

Mycoplasma genitalium, encodes 480 proteins, of which no fewer than 101 have been ascribed a function in protein synthesis (Fraser et al. 1995; Hutchison et al. 1999). Excluding genes that are less directly involved in translation per se (e.g., those for proteases and peptidases), *M. genitalium* has about 90 genes encoding proteins in the translation system. Additionally, 37 genes specify RNA molecules, chiefly ribosomal and transfer RNAs (rRNA and tRNAs), which fill critical translational roles. Thus, some 127 genes, a quarter of the *M. genitalium* complement, are involved in protein synthesis. Nearly all of these are held in common with *M. pneumoniae*, which has a somewhat larger genome (Himmelreich et al. 1996), and have been shown by transposon mutagenesis to be essential for growth under laboratory conditions (Hutchison et al. 1999). Discounting genes that are dispensable for mycoplasma growth in the laboratory, it can be calculated that the fraction of genes in a theoretical minimal genome that is devoted to translation may be as high as 35–45%.

Such a heavy genomic investment is not surprising in view of the high proportion of a cell's resources and energy budget that is allotted to translation. Protein synthesis consumes 5% of the human caloric intake but as much as 30–50% of the energy generated by rapidly growing *Escherichia coli* (Meisenberg and Simmons 1998). A portion of this is accounted for by the substantial input of energy required during translation itself (4 high-energy bonds per peptide bond, or ~25 kcal/mole, plus additional consumption for initiation and termination). Extensive resources are invested in the translation system—the ribosomes, tRNAs, and enzymes that constitute the physical plant for making proteins. A rapidly growing yeast cell, for example, contains nearly 200,000 ribosomes occupying some 30–40% of its cytoplasmic volume (Warner 1999). Growth alone demands that the yeast cell produce 2000 ribosomes per minute, an operation that absorbs ~60% of its transcriptional activity in manufacturing rRNA, as well as a large fraction of its translational capacity, since ribosomal protein messenger RNAs (mRNAs) account for almost one-third of the cell's mRNA population (for review, see Warner 1999). It would be surprising if a biological process of this importance were not closely monitored and regulated.

ORIGINS OF TRANSLATIONAL CONTROL

The central idea of translational control is that gene expression can be regulated by the efficiency of utilization of mRNA in specifying protein synthesis. This notion emerged only a few years after the articulation of the central dogma of molecular biology (Crick 1958) and very soon after

the formulation of the messenger hypothesis. In 1961, Jacob and Monod perceived that "the synthesis of individual proteins may be provoked or suppressed within a cell, under the influence of specific external agents, and . . . the relative rates at which different proteins are synthesized may be profoundly altered, depending on external conditions." They pointed out that such regulation "is absolutely essential to the survival of the cell," and went on to advance the concept of an unstable RNA intermediary between gene and protein as a key feature of their elegant model for transcriptional control (Jacob and Monod 1961). The idea that this mRNA could be subject to differential utilization depending on the circumstances was accorded scant attention in the bacterial culture of the time, but it was taken up enthusiastically by workers in other fields, to the extent that 10 years later, one writer could allude to the "now classical conclusion" that eggs contain translationally silent mRNA that is activated upon fertilization (Humphreys 1971).

The term Translational Control was certainly in use as early as 1968, by which date at least four clearly distinct exemplars had been recognized and were already coming under mechanistic scrutiny. The groundwork for these four paradigms—developing embryos, reticulocytes, virus- and phage-infected cells, and higher cells responding to stimuli ranging from heat to hormones and starvation to mitosis—had all been laid by the middle of the 1960s. They founded a thriving and expanding field of study that has advanced from its largely eukaryotic origins to embrace prokaryotes (although not yet the archaebacteria, as far as we are aware).

The Early History of Translation

The genesis of the translational control field took place at a time when translation itself was in its infancy; many (although not all) of the reactions had been observed, but most of the components were not yet characterized and mechanistic details were essentially unknown. To place the origins of translational control in context, we briefly outline the development of protein synthesis.

Biochemical investigations of the process began in the latter half of the 1950s, at the same time as the view of proteins as unique, nonrandom linear arrays of just 20 amino acid residues was solidifying. Enabled by the availability of radioactive amino acids as tracers, biochemistry ran ahead of genetics, as it continued to do in this field until the advent of cloning and the systematic exploitation of the yeast system, which began to make their mark in the 1980s. Siekevitz and Zamecnik produced a cell-free preparation from rat liver that incorporated amino acids into protein,

showing that energy was required in the form of ATP and GTP (Siekevitz 1951). The system was refined by stages and resolved into subfractions including a microsomal fraction that included ribosomes attached to fragments of intracellular membrane (for review, see Zamecnik 1960). It is salutary to recall that this was accomplished in advance of an understanding of the central role of RNA in the flow of genetic information to protein and in an era when theories of protein synthesis via enzyme assembly and peptide intermediates were entertained along with template theories (Campbell and Work 1953). Further biochemical work demonstrated that the ribonucleoprotein particles later called ribosomes comprise the site of protein synthesis, but it was not until the early 1960s that polysomes were observed and their function appreciated in light of the messenger hypothesis (Marks et al. 1962; Warner et al. 1963).

At much the same time, the role of aminoacyl-tRNA was being established. The existence of an intermediate, activated amino acid state was detected (Hultin and Beskow 1956) and characterized (Hoagland et al. 1959), then understood as the physical manifestation of the adapter RNA predicted on theoretical grounds (Crick 1958). Once its function had been realized, the name transfer RNA rapidly displaced the original descriptive term, "soluble" RNA. Later, chemical modification of the amino acid moiety of a charged tRNA confirmed that it is the RNA component which decodes the template (Chapeville et al. 1962). Thus, responsibility for the fidelity of information transfer from nucleic acid to protein rests in part on the aminoacyl-tRNA synthetases, which became the first macromolecular component of the protein synthetic apparatus to be purified (Berg and Ofengand 1958). These, together with the other enzymes, or protein "factors" as they became known, were steadily characterized and purified such that nearly all of the protein components have been known for more than 20 years. Yet, new ones continue to be reported (e.g., eIF5B; see Chapters 2 and 9), and even today there is no certainty that the full complement of protein factors involved in translation has been identified.

It was genetics rather than biochemistry that supplied the missing cornerstone of the protein synthetic system, mRNA. According to the messenger hypothesis, the ribosomes and other components of the protein synthesis machinery constitute a relatively stable decoding and synthetic apparatus that is programmed by an unstable template (Jacob and Monod 1961). This insight soon received confirmation in bacteria (Brenner et al. 1961; Gros et al. 1961) and in bacterial cell-free systems. The discovery that poly(U) can direct the synthesis of polyphenylalanine (Nirenberg and Matthaei 1961) was particularly fruitful, greatly speeding the elucidation of the genetic code by the mid 1960s. Because of the greater stability of most eukaryotic mRNAs, the applicability of the messenger hypothesis to

higher cells was less readily apparent. Nonetheless, the existence of a class of rapidly labeled RNA, heterogeneous in size and with distinct chromatographic properties, was recognized. Its essential features as informational intermediary were confirmed and it was universally accepted several years before the discovery (in the early 1970s) of 5′ caps and 3′ poly(A) tails, the modern hallmarks of most eukaryotic mRNAs. The mRNA concept immediately revolutionized thinking about gene expression in all cells.

To appreciate the pace at which protein synthesis advanced during the decade of the 1960s, it is instructive to compare the Cold Spring Harbor Symposium volume of 1962 (on Cellular Regulatory Mechanisms) with that of 1970, a much thicker book devoted to a narrower topic (the Mechanism of Protein Synthesis). By the end of the decade, much of the translational apparatus had been characterized (although much also remained to be done), many problems of regulation had been laid out, and translational control came to receive increasing attention.

General Features of Translational Control

In a multistep, multifactorial pathway like that of protein synthesis, regulation can be exerted at many levels. Examples of translational control are indeed found at different levels, but the overwhelming preponderance of known instances—including all of the earliest cases recognized—is at the level of initiation. This empirical observation conforms to the biological (and logical) principle that it is more efficient to govern a pathway at its outset than to interrupt it in midstream and have to deal with the resultant logjam of recyclable components and the accumulation of intermediates as by-products. Nevertheless, well-characterized cases do occur at later steps in the translational pathway, especially at the elongation level, where it seems that a translational block may be imposed as a safety measure to halt further peptide bond formation.

One of the chief virtues of translation as a site of regulation is that it offers the possibility of rapid response to external stimuli without invoking nuclear pathways for mRNA synthesis and transport. Predictably, the first cases to be recognized were those in which it was simplest to establish, if it was not self-evident, that transcription and other nuclear events were not responsible. By the same token, the relative scarcity of prokaryotic examples and their generally later recognition can be largely attributed to the lack of a nuclear barrier between the sites of mRNA synthesis and translation. The greater speed of macromolecular synthesis in bacteria and their lesser dependence on mRNA processing are other factors. These circumstances allow a coupling of transcription and translation that all but obviates the need for translational control. That it occurs at all in

prokaryotes is due to the exigencies of particular circumstances and to the potency of translational control mechanisms.

The earliest cases of translational control to be explored in depth, in fertilized invertebrate eggs and mammalian reticulocytes, were those in which the departure from the transcription-based regulatory model was the most obvious and extreme. Protein synthesis is abruptly turned on (in fertilized eggs) and off (in iron-starved reticulocytes) in the absence of ongoing transcription. A further distinction that made it easier to define and study these two particular cases is that the regulation is apparently indiscriminate in that it affects protein synthesis generically, rather than the synthesis of specific proteins. Not all translational controls are of this type, however. A distinction is often drawn between global and selective controls (sometimes referred to, rather misleadingly, as quantitative and qualitative controls). Global controls, such as those operating in eggs and reticulocytes, impact the entire complement of mRNAs within a cell, switching their translation on or off or modulating it by degrees in unison. This kind of regulation is usually implemented by substantial alteration in the activity of general components of the protein synthesis machinery that act in a nonspecific manner. Selective controls, on the other hand, affect a subset of the mRNAs within a cell, in the extreme case a single species only. This can be accomplished through mechanisms that target ligands to individual mRNAs or classes of mRNA, but it is achieved more commonly by exploiting the differential sensitivity of mRNAs to more subtle changes in the activity of general components of the translation system, e.g., eIF4E (Chapter 6) or eIF2 (Chapter 5). Although examples of all these exist and are discussed at length in this monograph, in the context of the historical origins of translational control, it should come as no surprise that the earliest examples were mainly of the global variety and that (with notable exceptions) definitive evidence in favor of selective translational control accumulated more slowly.

PARADIGMS OF TRANSLATIONAL CONTROL

In large part, the origins of translational control can be traced to the confluence of four early streams of investigation, which still continue to flow. Their early courses are described below, followed by an example involving elongation control.

Sea Urchin Eggs

The eggs of sea urchins and other invertebrates provide a striking example of regulated gene expression which, it was quickly realized, did not

harmonize with the emerging theme of transcriptional control. These cells are essentially quiescent until they are galvanized into action by fertilization. Egg ribosomes synthesize protein at a very low rate but are triggered to incorporate amino acids within a few minutes of fertilization (Hultin 1961). Although the rate of protein synthesis accelerates rapidly after fertilization, there is little or no concomitant RNA synthesis (Hultin 1961; Nemer 1962; Gross and Cousineau 1963). Translation in enucleated eggs can be activated parthenogenetically (Denny and Tyler 1964). Moreover, actinomycin D fails to block the first wave of increased translation, which lasts for several hours, and both cell division and many morphogenetic events proceed unimpeded by the transcriptional inhibition. A second wave of increased protein synthesis is prevented by actinomycin D, however, presumably because this wave does depend on new mRNA synthesis (Gross et al. 1964). Such observations are explained by the fact that the eggs contain preexisting mRNA in a form that is not translated until some stimulus dependent on fertilization is received. In principle, the limitation could be due to a deficiency in the translational machinery, but unequivocal evidence in this direction has been more difficult to obtain. For example, a comparison of polysome sizes and translation rates in eggs and embryos did not disclose any defect in the apparatus itself (Humphreys 1969). On the other hand, a good deal of evidence points to a defect in the availability of mRNA. Consistent with the conclusion that mRNA is largely sequestered in eggs, deproteinized egg RNA can be translated in a cell-free system (Maggio et al. 1964). The ribosomes from eggs—unlike those from embryos—display little intrinsic protein synthetic activity, although they are able to translate added poly(U) (Nemer 1962; Wilt and Hultin 1962), suggesting that they possess latent translational capacity. Egg mRNA exists in a masked form: Cytoplasmic messenger ribonucleoprotein (mRNP) particles have been observed (Spirin and Nemer 1965), and some studies even indicated that the template could be activated by trypsin treatment, presumably by removing masking proteins (Monroy et al. 1965). Since the assembly of masked mRNP complexes must take place during oogenesis, the sea urchin system exemplifies a reversible process of mRNA repression and activation. Developments in this arena are discussed in Chapters 7 and 27.

Reticulocytes

These immature red cells have endowed researchers with a unique and especially dynamic system for studying the mechanism and control of

translation. Because mammalian reticulocytes are enucleate, unlike those of most vertebrates, it was taken for granted that the regulation of protein production would be exercised at the translational level, an assumption that has been borne out in numerous studies. More than 90% of the protein made in the reticulocyte is hemoglobin, which consists of two α-globin and two β-globin chains together with four molecules of heme, an iron-containing porphyrin. In the intact rabbit reticulocyte, the synthesis of heme parallels that of globin (Kruh and Borsook 1956), and subsequent work showed that globin synthesis is controlled by the availability of heme or of ferrous ions (Bruns and London 1965). The phenomenon was made experimentally accessible by the development of the highly active unfractionated reticulocyte lysate translation system (Lamfrom and Knopf 1964), which became the forerunner of the widely used messenger-dependent system of Pelham and Jackson (1976). Regulation by heme is reversible in intact cells, and, to a limited extent, the repression of protein synthesis that ensues in the reticulocyte lysate soon after heme deprivation can also be rescued by restoring the heme level. When globin synthesis is inhibited in cells or extracts, the polysomes dissociate to monosomes (Hardesty et al. 1963; Waxman and Rabinovitz 1966), arguing that heme is involved in regulating translation initiation. Contrary to intuitive expectation, there is no necessary linkage between the role of heme as the prosthetic group of globin and its role as translational regulator. The effects of heme deprivation on protein synthesis in the reticulocyte or its lysate are mimicked by unrelated stimuli such as double-stranded RNA (dsRNA) and oxidized glutathione (Ehrenfeld and Hunt 1971; Kosower et al. 1971) and extend to all mRNAs in the reticulocyte lysate (Mathews et al. 1973). Such observations imply that a general mechanism of translational control is being invoked: In each of the conditions under which protein synthesis is down-regulated, inhibitors—now known to be the eIF2 kinases HRI and PKR—are activated (for reviews, see Chapters 13 and 14). By 1977, a unifying scheme could be advanced (Farrell et al. 1977), centering on the phosphorylation of the α-subunit of initiation factor eIF2 and the loading of the 40S ribosomal subunit with Met-tRNA$_i$. This mechanism has been found to have wide applicability in cells and tissues responding to a range of stimuli (see also Chapters 5 and 15).

Virus-infected Cells

During the 1960s, it came to be appreciated that cellular protein synthesis is suppressed during infection with many viruses (see Chapters 8, 31–35). This inhibition may begin before the onset of viral protein syn-

thesis and without any apparent interference with cellular mRNA production or stability. In poliovirus infection, an early example, the shutoff of host-cell translation can be complete within 2 hours after infection and is followed by a wave of viral protein synthesis (Summers et al. 1965). The first phase is accompanied by the reduction of polysomes to monosomes without any effect on the elongation or termination phases of protein synthesis (Penman and Summers 1965; Summers and Maizel 1967). In the second phase, virus-specific polysomes form (Penman et al. 1963), evidence that initiation has become selective for a class of mRNA—in this case viral, rather than cellular. Later studies showed that cellular mRNA remains intact in the infected cell (Leibowitz and Penman 1971) and is translatable in a cell-free system, although it is not translated in the infected cell. Furthermore, the inhibition extends to the mRNAs of several other viruses introduced together with poliovirus in a double infection (Ehrenfeld and Lund 1977), indicative of a general effect. Although circumstantial evidence aroused suspicions that viral dsRNA and PKR might be responsible for the phenomenon, later work incriminated a modification of the cap-binding complex, eIF4F. Cleavage of the eIF4G subunit of this complex prevents cap-dependent initiation on cellular mRNAs but does not interfere with initiation on the viral mRNA, which occurs by internal ribosome entry (see Chapters 4, 6, 8, and 31).

Bacteriophage f2 provided the first evidence for translational control in a prokaryotic system, as well as the first clear case of mechanisms specific for the synthesis of individual protein species. The phage RNA genome encodes four polypeptides, the maturation protein, coat protein, lysis protein, and replicase, that are initiated individually but produced at dissimilar rates. Several regulatory interactions among them are now known. One was revealed by the observation that a nonsense mutation early in the cistron coding for the viral coat protein down-regulates replicase synthesis (Lodish and Zinder 1966). Apparently, passage of ribosomes through a critical region of the coat protein cistron is required to melt RNA structure and allow replicase translation. In contrast, a second nonsense mutation leads to overproduction of the replicase, suggesting that the coat protein acts as a repressor of replicase translation. This inference has been amply confirmed, and the binding of the coat protein to the hairpin structure containing the replicase AUG has become one of the best-characterized cases of RNA–protein interaction (Witherell et al. 1991). Subsequent studies have disclosed translational control mechanisms in the DNA phages as well as in bacterial genes themselves (see Chapter 8), but it was eukaryotic systems that made most of the early running.

Physiological Stimuli

The cells and tissues of higher organisms have been reported to regulate the expression of individual genes or of whole classes of genes at the translational level in response to a wide variety of stimuli or conditions. These include cell state changes, such as mitosis (Steward et al. 1968; Hodge et al. 1969; Fan and Penman 1970) and differentiation (Heywood 1970); stress resulting from heat shock (McCormick and Penman 1969), treatment with noxious substances or the incorporation of amino acid analogs (Thomas and Mathews 1984); and normal cellular responses to ions (Drysdale and Munro 1965) and hormones (Eboué-Bonis et al. 1963; Garren et al. 1964; Martin and Young 1965; Tomkins et al. 1965). Not in every case was the evidence for regulation at the translational level complete, and in a few instances, the trail has gone cold or been erased upon more detailed examination, but the accumulated volume of information added conviction to the view that translational control is both widespread and important. One of the chief stumbling blocks in this arena lay in determining that the level at which control was exerted was indeed translational. This can be a difficult task in nucleated cells, let alone in a tissue or whole animal (or plant), and it was addressed in various ways. A popular approach exploited selective inhibitors of transcription or translation, such as actinomycin D and cycloheximide, but the results were liable to be complicated (if not confounded) by side effects of the drugs or their indirect sequelae in complex systems. Another argument that could be made for an effect at the translational level, although not without some reservations, came from its rapidity (see below). Timing alone cannot provide a definite assignment, however, and the most convincing proofs often came from subsequent investigations of the underlying biochemical processes—for example, by demonstrating changes in polysome profiles or initiation factor phosphorylation states as discussed below and in Chapters 6, 13–17, 20, and 23. The ultimate goal is to achieve an understanding of the regulatory mechanisms set in train by the stimuli applied, and within this wide array of phenomena lie many of the challenges for the future.

Secretory Proteins

No overview of the principal themes of translational control would be complete if it dwelt exclusively on the initiation phase. One of the best-studied examples of regulation during the elongation phase is found in the synthesis of proteins that are destined for secretion. These are made on polysomes that are attached to the endoplasmic reticulum, isolated from

cellular homogenates in the form of microsomes. In the early 1970s, it began to seem likely that ribosomes become associated with cell membranes only after protein synthesis has been initiated (Lisowska-Bernstein et al. 1970; Rosbash 1972). Contemporaneously, the existence of what came to be called a signal peptide was reported on an immunoglobulin light chain (Milstein et al. 1972) and other secreted proteins (Devillers-Thiery et al. 1975). These findings lent substance to the signal hypothesis (Blobel and Sabatini 1971), which suggested that an amino-terminal sequence might ensure secretion, and prompted the development of cell-free systems that enabled the biochemical dissection of the secretory pathway (Blobel and Dobberstein 1975). One of the surprising discoveries to emerge was the involvement in secretion of a ribonucleoprotein particle, the signal recognition particle (SRP), which interacts with the signal peptide, the ribosome, and the endoplasmic reticulum. Remarkably, binding of the SRP to a nascent signal peptide protruding from the ribosome causes translational arrest in the absence of cell membranes (Walter and Blobel 1981). This elongation block is relieved when the ribosome docks with the endoplasmic reticulum, allowing the protein chain to be completed and simultaneously translocated across the lipid bilayer. It has been speculated that this mechanism serves to ensure cotranslational protein export and to prevent the accumulation of secretory proteins in an improper subcellular compartment (the cytosol). Interestingly, a similar rationale has been offered to account for control at the elongation level during heat shock (for review, see Chapter 17). In this situation, it has been proposed that a translational arrest is imposed to prevent the synthesis of proteins that might be folded abnormally. Thus, elongation blocks might be used under exceptional circumstances to preserve cellular integrity when it is threatened by the production of protein at the wrong time or in the wrong place, or perhaps in the event of a sudden shortage of energy or an essential metabolite.

WHAT LIMITS PROTEIN SYNTHESIS IN PRINCIPLE?

Given that translational controls are so widespread in eukaryotic cells, it is appropriate to examine the fundamental principles on which these controls are based. Translational control is defined as a change in the rate (efficiency) of translation of one or more mRNAs, i.e., the number of completed protein products changes per mRNA per unit time. It is generally believed that during protein synthesis, the number of protein chains initiated is about the same as the number of proteins completed; in other words, few nascent polypeptides abort and fall off the ribosome (Tsung et

al. 1989). Therefore, under steady-state conditions, the number of initiation events per unit time approximates the number of protein products produced during the same time interval. It follows logically that the rate of protein synthesis is determined by the number of initiation events, i.e., the rate of initiation. What determines the number of initiation events per unit time? Four major parameters may influence or define the rate of protein synthesis. Each is considered briefly below.

The Activity of the Protein Synthesis Machinery

Numerous examples exist of cells that possess ribosomes and mRNAs in excess of those actively engaged in protein synthesis. This may occur if a single translational component (e.g., a soluble factor) is limiting in amount or if one or more components have reduced specific activities. Such regulation frequently involves the phosphorylation status of translational components, as detailed in numerous chapters in this monograph. Regulation of the overall activity of the translational apparatus is expected to affect the translation of essentially all mRNAs. As argued earlier by Lodish (1976), down-regulation of the initiation steps that occur prior to the binding of mRNAs is expected to lead to greater inhibition of those mRNAs whose initiation rate constants are relatively low ("weak" mRNAs), as compared to "strong" mRNAs. Reciprocally, activation of such steps may stimulate more greatly the translation of weak mRNAs. Alteration of the activities of components that interact with mRNAs and affect their binding to ribosomes also would be expected to generate differential effects on the translation of the mRNA population (Godefroy-Colburn and Thach 1981). The mechanisms affecting mRNA binding and differences in the translational efficiency of specific mRNAs are reviewed in Chapter 6.

The Rate of Elongation

The initiation rate on an mRNA can be inhibited if a ribosome, having already initiated, vacates the initiation region too slowly. A ribosome bound at the AUG initiation codon occupies about 12–15 nucleotides (4–5 codons) downstream from the AUG and about 20 nucleotides upstream. Another ribosome can occupy the initiation site only after the first ribosome has moved about 7 codons down the mRNA. When the time needed to vacate the initiation region approaches or exceeds the time required for initiation, the elongation rate becomes limiting. In general, it is believed that the elongation rate is about the same for all mRNAs (3–8

amino acids per second per ribosome in eukaryotes, faster in prokaryotes), because measurements of a few specific examples gave similar results in this range (Lodish and Jacobsen 1972; Palmiter 1974). Nevertheless, the rate of elongation is not uniform throughout the coding region of an mRNA, as pausing may occur at specific locations, possibly due to the occurrence of rare codons or RNA secondary structure (Wolin and Walter 1988). If ribosome pausing occurs such that it impedes initiation, mRNA efficiency is decreased. The question of which translation phase is rate-limiting, initiation or elongation/termination, is addressed in greater detail below.

The Amount or Efficiency of mRNAs

The level of mRNA in the cytoplasm is determined by the rate of transcription, the proportion of primary transcripts that are processed and transported into the cytoplasm, and the degradation rate of cytoplasmic mRNAs. In actively translating mammalian cells, mRNAs often are found entirely in polysomes, as shown for actin (Endo and Nadal-Ginard 1987); thus, the rates of synthesis of such specific proteins are mRNA-limited. However, total mRNA in the cytoplasm frequently appears to be present in excess, with about 30% of the mRNA in cultured cells present as free mRNP particles (Geoghegan et al. 1979; Kinniburgh et al. 1979; Ouellette et al. 1982). Therefore, the level of mRNA appears not to limit the overall number of translational initiation events in these cells. In cells exhibiting low translational activity, many mRNAs are repressed and apparently unavailable to the translational apparatus (masked), as seen most dramatically in oocytes and unfertilized eggs as described above, but also in somatic cells in culture (Lee and Engelhardt 1979). Such repression sometimes appears to be all or none, as some mRNAs are distributed bimodally in polysome profiles; a fraction of the specific mRNA is completely repressed (nontranslating mRNP particles), whereas a portion is actively translated as large polysomes (Yenofsky et al. 1982; Agrawal and Bowman 1987). In instances of specific regulation of protein synthesis, mRNA repression and availability to the translational apparatus likely have a dominant role, for example, in the translation of ferritin mRNA (see Chapter 21) and ribosomal protein mRNAs (see Chapter 22). Furthermore, individual activated mRNAs differ greatly in their efficiencies of translation as deduced from polysome sizes, thereby contributing to regulation of gene expression. These innate efficiencies are determined in large part by the primary and higher-order structures of the mRNAs (for reviews, see Chapters 2, 4, and 8).

The Abundance of Ribosomes

The cellular levels of ribosomes may be rate-limiting under some circumstances. Cells active in protein synthesis, for example, liver cells from fed rats, engage 90–95% of their ribosomes in protein synthesis (Henshaw et al. 1971), suggesting that still higher rates of protein synthesis might have been possible were there a greater number of ribosomes. On the other hand, in translationally repressed cells, such as liver cells from fasted rats (Henshaw et al. 1971) or in quiescent cells in culture (Duncan and McConkey 1982; Meyuhas et al. 1987), fewer than half of the ribosomes may be actively translating mRNAs. The level of ribosomes surely is not limiting in these cells, since a rapid increase in the rate of protein synthesis can be induced within 20 minutes, before the assembly of more ribosomes is possible (Duncan and McConkey 1982). Translation also may be limited by the levels (as opposed to specific activities) of other components of the translational apparatus, e.g., eIF2 and eIF4F, the latter likely through its eIF4E subunit (see Chapter 6). When amino acids become limiting, global protein synthesis is rapidly repressed by inhibiting the activity of initiation factors (Clemens et al. 1987; Chapter 16).

WHICH PHASE OF PROTEIN SYNTHESIS IS RATE-LIMITING AND REGULATED?

The analysis above identifies three ways in which the rate of protein synthesis may be limited and thus regulated over a relatively short time period (on the order of minutes): the rate of initiation, the rate of elongation/termination, and the repression/activation of mRNAs/mRNPs. How is the rate of protein synthesis measured and how is the rate-limiting step identified? The overall rate of protein synthesis can be measured by assaying the time course of incorporation into protein of radioactively labeled amino acids added to the culture medium. The method is complicated only by the uncertainty of the specific radioactivities of the precursors within cells, as intracellular de novo synthesis of amino acids and degradation of proteins may influence these values. A second method measures the absolute number of active ribosomes and the elongation rate, from which the number of amino acids incorporated per unit time can be calculated. The elongation rate is obtained by dividing the number of amino acids in the average protein by the ribosome transit time (Fan and Penman 1970), the time it takes to translate an average-sized mRNA. The fraction of total ribosomes that is active is assessed by high-salt sucrose gradient centrifugation of cell lysates to generate a polysome pro-

file: Active ribosomes in polysomes are separated from nonactive, free ribosomal subunits. The second method, although more laborious than the measurement of labeled amino acid incorporation, is not complicated by uncertainties of amino-acid-specific radioactivities. Both methods serve to analyze global rates of protein synthesis. The relative synthesis rates of specific proteins can be measured by radioactively labeling proteins, followed by immunoprecipitation or fractionation of proteins by high-resolution two-dimensional gel electrophoresis.

Which phase of protein synthesis is rate-limiting, initiation or elongation/termination? Although most mRNAs are thought to be limited by their initiation rate, others are limited by the rate of elongation/termination. Therefore, the question is best addressed to specific mRNAs rather than to the whole population. Insight into which phase is rate-limiting is gained by an examination of polysome profiles, where the specific mRNA is located in the sucrose gradient fractions by hybridization techniques. The rate of initiation, i.e., the number of initiation events per minute, can be calculated from the number of ribosomes translating an mRNA (polysome size) and the ribosome transit time (the time required for the ribosome to traverse the mRNA). As elegantly determined for ovalbumin mRNA in chick oviducts (Palmiter 1975), ovalbumin polysomes average 12 ribosomes and the ribosome transit time is 1.3 minutes, giving a rate of initiation of 9.2 events per minute (or one initiation every 6.5 seconds). Since the elongating ribosome requires only about 2 seconds to vacate the initiation site, it is clear that the initiation rate is slower than potentially possible and thus is rate-limiting. Parenthetically, if the number of mRNA molecules in the polysomes is known, an absolute rate of specific protein synthesis can be calculated.

A second way to determine whether initiation or elongation/termination is rate-limiting for an mRNA is to treat cells with low concentrations of an elongation (e.g., cycloheximide and sparsomycin) or initiation (e.g., pactamycin) inhibitor. If translation of the specific mRNA is limited by the elongation rate, its synthesis will be sensitive to the inhibitors of elongation. Conversely, if initiation is rate-limiting, such mRNAs will be insensitive to elongation inhibitors but sensitive to initiation inhibitors. For example, when mRNAs encoding α- and β-globin (Lodish and Jacobsen 1972) or reovirus proteins (Walden et al. 1981) were analyzed, initiation was the sensitive step. Because the majority of mRNAs in cells are resistant to low concentrations of cycloheximide, it is thought that the translation of most mRNAs is limited at the initiation phase.

Further evidence that the rate of initiation limits the translation of most mRNAs is obtained by examining polysome sizes from sucrose gra-

dients. On the average, ribosomes in polysomes occur once every 80–100 nucleotides. For example, the average polysome size for globins is about 5 ribosomes per mRNA, or 1 ribosome per 90 nucleotides. When protein synthesis is inhibited by cycloheximide such that elongation becomes rate-limiting, polysomes increase in size (to more than 12 ribosomes per globin mRNA, for example). Therefore, polysome densities of one ribosome per 30–40 nucleotides are possible. This approaches the limit for close packing, since a ribosome occupies about 30 nucleotides of mRNA. That average polysome densities are much less is due to the relatively low rate of initiation.

Changes in the size (number of ribosomes per mRNA) or amounts (amplitude) of polysomes may be diagnostic of the phase of global protein synthesis that is being modulated. If the size of polysomes decreases, either initiation is inhibited or elongation/termination is stimulated, or a combination of both occurs. Conversely, an increase in polysome size can be caused by an increased rate of initiation and/or a decreased rate of elongation/termination. To interpret polysome profiles unambiguously, it is advisable to measure the elongation rate by determining the ribosome transit time and average length of mRNAs being translated. In cases where the overall rate of protein synthesis is repressed and polysomes are smaller, initiation has clearly been inhibited. Regulation of a specific mRNA is readily evaluated by these methods, since the average size of its polysomes is readily determined by hybridization techniques with cloned probes. Repression or activation of protein synthesis need not always affect polysome size. Instead, the number of translating mRNAs may be affected by masking mRNAs or mobilizing them into polysomes. In this case, there is a change in the *amount* (i.e., amplitude) of polysomes, but the average *size* of the polysomes may remain the same.

Are there cases where the elongation rate is regulated? Examination of a number of specific mRNAs shows that rather modest changes in the rate of elongation are found following treatment of cells with hormones and other agents (Chapter 24). A dramatic example is the fivefold stimulation of the rate of elongation of tyrosine aminotransferase seen when rat hepatoma cells are treated with dibutyryl-cAMP (Roper and Wicks 1978). Similarly, the elongation rate on vitellogenin mRNA drops about fourfold when cockerel liver explants are treated with 17β-estradiol (Gehrke and Ilan 1987). Even small changes in the elongation rate will affect the efficiencies of those mRNAs that are elongation-limited; whether or not moderate inhibition of elongation affects initiation-limited mRNA expression depends on the degree that initiation is limiting.

TARGETS AND MECHANISMS OF TRANSLATIONAL CONTROL

Having defined the rate-limiting steps in the protein synthesis pathway, we now turn to the means by which its regulation is accomplished in the cell. Translational control is realized through multiple mechanisms that target structural features of the mRNA and *trans*-acting components; the latter may be either protein or (less commonly) RNA in nature. The survey that follows takes stock of the principal targets of translational control and the mechanisms which they coordinate, giving reference to chapters in this monograph where these topics are considered in greater detail.

mRNA

The intrinsic translational efficiency of an mRNA is dependent on several *cis*-acting elements, which also have critical roles in the regulation of mRNA utilization, as discussed in many chapters of this work. It is convenient to divide the *cis*-acting elements into two categories: those that act alone or with general translation factors; and those whose actions are mediated by specific *trans*-acting factors.

In prokaryotes, the first category is of overriding importance. Translational efficiency is heavily influenced by mRNA primary structure, especially the Shine-Dalgarno sequence, as well as by the degree of secondary structure that can be modulated by various mechanisms (Chapters 2, 4, and 8). In eukaryotes, *cis*-acting elements distributed along the length of the mRNA modulate translational efficiency. Primary structure, notably the 5′ cap, the sequence flanking the initiator AUG (its "context"), and the presence of upstream AUG triplets all determine translational efficiency (Chapters 2 and 4). Secondary structure, particularly in the 5′ -untranslated region (5′UTR), can also have a determinative role. Upstream open reading frames (uORFs) participate in translational control in yeast and higher eukaryotes. Regulation of the translation of uORF-containing mRNAs is dependent on many factors, including the amino acid sequence encoded by the uORF, the length of intercistronic regions, and the sequence context of the termination codon of the uORFs (Chapters 5 and 18).

Within the coding sequence of some mRNAs are elements that signal ribosome frameshifting, hopping, termination codon readthrough, and the incorporation of selenocysteine (for review, see Chapters 11, 25, and 26). Some of these processes are known to be regulated. For example, ribosomal frameshifting is regulated in both eukaryotes (in antizyme) and prokaryotes (in RF2 and tryptophanase).

cis-Acting elements belonging to the second category also occur throughout the mRNA. The iron-responsive element (IRE) is a sequence- and structure-specific negative regulatory element, found in the 5′UTR of ferritin mRNA (and subsequently in other mRNAs), that modulates its translation in accordance with the level of cellular iron. This regulation is mediated by a *trans*-acting iron repressor protein (IRP) that binds to the IRE and inhibits translation (Chapter 21). It is reasonable to expect that other such *negative* mRNA-specific *trans*-acting regulators of translation are awaiting discovery. *Positive* mRNA-specific regulators of translation have been described in bacteriophages. For example, the Com protein of bacteriophage Mu activates translation of *mom* mRNA by binding near its initiation site and altering its secondary structure (Chapter 8). Although no factor with similar activity has yet been reported in eukaryotes, several proteins interact with the internal ribosome entry site (IRES) of picornavirus RNAs and stimulate their translation (Chapters 4 and 31).

The past decade has seen the surprising discovery that the 3′UTR is a rich repository of *cis*-acting elements that determine mRNA stability and localization in the cytoplasm and also serve to regulate translation initiation. These controls are most likely mediated by *trans*-acting factors (Chapters 7, 27, and 29). Most such examples of translational control occur during early development, but some cases have been described in somatic cells. An unusual case is seen in the developmentally regulated *Caenorhabditis elegans* gene *lin-14*. Translation of this mRNA is inhibited by a short (22-nucleotide) RNA transcribed from the *lin-4* gene, which can base-pair with sequences in the 3′UTR of the *lin-4* mRNA. At the 3′ end of eukaryotic mRNAs, the poly(A) tail also has an important role as an enhancer of translation. Intriguingly, the poly(A) tail acts in synergy with the mRNA 5′ cap structure, and the translational activity of the poly(A) tail may be mediated by the poly(A)-binding protein (Chapter 10).

mRNA stability is an important determinant of cytoplasmic mRNA levels and therefore of protein synthesis. In many instances, translation has a direct role in determining mRNA stability, as mRNA degradation may be coupled to translation (Chapter 29). Most but not all of the *cis*-acting elements that trigger mRNA degradation are localized to the 3′UTR; the poly(A) tail influences the degradation of mRNAs via the poly(A)-binding protein, and short-lived mRNAs possess sequence-specific elements that mediate mRNA degradation. A separate pathway exists to degrade mRNAs that contain premature termination codons (nonsense-mediated decay) (Chapters 29 and 30). This pathway has most probably evolved to prevent the synthesis of truncated proteins that might function in a dominant-negative manner. It is puzzling that this degrada-

tive pathway seems to operate in the cytoplasm in yeast, whereas it is nuclear in mammals. The nuclear mode of nonsense-mediated decay poses intriguing questions concerning the mechanism whereby nonsense codons are recognized in the nucleus, and the possible coupling between translation and nuclear-cytoplasmic mRNA transport.

Initiation Factors

The effects of the various *cis*-acting elements in the mRNA 5′UTR are modulated through the activity of initiation factors and other *trans*-acting factors. Phosphorylation of initiation factors provides the chief means to control the rate of mRNA binding. Several factors that promote mRNA binding to ribosomes (eIF4E, eIF4G, eIF4B, and eIF3 in mammalian cells; also eIF4A in plants) are phosphorylated, and the phosphorylation status of these proteins correlates positively with both translational and growth rates of the cell (Chapter 6). The phosphorylation state of these initiation factors is modulated in a wide variety of circumstances and affects translation during the cell cycle, during infection with viruses, after heat shock, or in response to growth factors and hormones (Chapters 6, 8, 17, and 20). Although there is some biochemical evidence that the phosphorylation of eIF4E potentiates its cap-binding activity, for eIF4B and eIF4G, the consequences of phosphorylation are not yet established (Chapter 6).

Phosphorylation of eIF2 also has a central role in regulating translation by affecting the binding of Met-tRNA$_i$. In contrast to the eIF4 group, phosphorylation of eIF2 inactivates its ability to recycle, as the exchange of GDP for GTP on the factor is blocked, leading to inhibition of translation (Chapter 5). Phosphorylation of eIF2, like that of the eIF4 proteins, has a role in differentiation (Chapter 7) and occurs under conditions of stress, including heat shock (Chapter 17), viral infection (Chapters 8, 32–35), and serum deprivation (Chapters 16 and 17). Extensive analyses of the mechanisms of eIF2 phosphorylation led to the identification and characterization of four mammalian protein kinases, PKR, HRI, PERK, and GCN2 (Chapters 5, 13–15), the first having a key role in the antiviral host defense mechanism that is mediated by interferons (Chapter 8). GCN2 in yeast regulates translation reinitiation on the 5′UTR of GCN4 mRNA and mediates the response to amino acid deprivation (Chapter 5). It would be of interest to know whether GCN2 plays a similar role in vertebrates. Thus, phosphorylation of eIF2 controls the rate of reinitiation of translation on mRNAs that contain uORFs. Phosphorylation also controls the activity of eIF2B, the eIF2 guanine nucleotide exchange factor (Chapters 5 and 16).

Apart from phosphorylation, translation initiation factor activity can be modulated in principle by other reversible or irreversible modifications. One important example that occurs as a result of infection with certain picornaviruses is the cleavage of eIF4G. This cleavage is responsible in part for the shutoff of host-protein synthesis after viral infection (Chapters 8 and 31) and a different cleavage pattern occurs in cells undergoing apoptosis (Bushell et al. 2000).

An important recent development is the discovery that initiation factor activity can be modulated by proteins that interact with initiation factors. For example, polypeptides (4E-BPs) that bind eIF4E and inhibit cap-dependent translation initiation have been identified; their activity is modulated by phosphorylation under the control of growth factors and hormones (Chapter 6). Also, proteins exhibiting homology with eIF4G (p97/DAP5/NAT1 and Paip1) have been described (Chapter 6). These proteins modulate translation most likely via their interaction with eIF4G-binding proteins. Similarly, eIF2 activity may be modulated by an accessory protein, p67, which binds to eIF2 and prevents its phosphorylation by eIF2 kinases (Chapter 5).

Elongation Factors

Elongation rates are also modulated by phosphorylation, particularly through the activity of the translation elongation factor eEF2. This factor undergoes phosphorylation in response to growth-promoting stimuli, calcium ion fluxes, and other agents, to affect translation (Chapter 24). eEF2 and the other elongation factors are also altered posttranslationally by other modifications. For example, eEF2 is a substrate for ADP-ribosylation by diphtheria toxin on the unique diphthamide residue (derived from histidine). There is evidence that diphthamide has a role in polypeptide chain elongation (Chapter 3). Both bacterial EF1A and eukaryotic eEF1A also contain modifications, but their functions are not yet clear.

Ribosomes

Phosphorylation of ribosomal proteins may also affect translational initiation. Of these, ribosomal protein S6 provides the best-studied example: Its phosphorylation promotes the initiation of translation on mRNAs encoding ribosomal proteins and elongation factors. Recent studies have revealed that the mechanism underlying this selectivity involves an oligopyrimidine tract in the 5′UTR of the target mRNAs and have shed

light on the signal transduction pathways that link growth-promoting stimuli to S6 phosphorylation (Chapters 22 and 23).

WHY CONTROL TRANSLATION?

Thus far, we have considered the basis and principles of translational control. As mentioned above, there is a clear-cut rationale for regulating a biochemical pathway at its first step; this principle holds true, by and large, for protein synthesis, in that regulation is most often exercised at the initiation phase. From a broader perspective, however, matters become less clear-cut. Viewing gene expression in totality, translation occupies a position somewhere in the middle of a complex pathway that begins with transcription, continues with RNA processing and transport, and ends with protein translocation, modification, folding, assembly, and degradation. Each of these steps is known to be regulated in one or another biological system. Yet, two of the steps in this grand scheme, transcription and translation, are especially critical for the cell. Both are biosynthetic steps in which the cell makes large investments of energy. Consequently, both are steps at which the cell's expenditure of resources is checked. Indeed, transcription is subject to a multitude of controls. So, why control translation, too? And where and when is this option exercised?

To these frequently asked questions there is no single answer. Rather, there are several compelling reasons for cells to deploy translational control in their arsenal of regulatory mechanisms. Some of the advantages offered by translational control are considered briefly below. Evidently, the benefits more than compensate for the energetic and other penalties paid for the privilege of exerting regulation over a downstream reaction in a long pathway.

Directness and Rapidity

Immediacy is the most conspicuous advantage of translational control over transcriptional and other nuclear control mechanisms. Whereas transcriptional control affects the first step in the flow of genetic information, translational control affects the last step. When control is applied at a step prior to translation, the cell has to confront subsequent biochemical reactions (splicing, nuclear transport, etc.) that might be rate-limiting and inevitably entail a delay in implementing changes in protein synthesis. No such time lag applies in the case of translational control.

Reversibility

Most translational controls are effected by reversible modifications of translation factors, chiefly through phosphorylation. The readily reversible nature of translational control mechanisms is economical in energetic terms, a feature that is of particular biological significance in energy-deprived cells.

Fine Control

There are numerous examples of genes that are under both transcriptional and translational control (e.g., TNF-α, C/EBPB, VEGF, ornithine decarboxylase). In most instances, but not all, the changes in transcription rates are considerably greater in magnitude than the changes in translation rates. Thus, regulation of gene expression at the translational level provides a means for fine control.

Regulation of Large Genes

Some genes are extremely long (e.g., dystrophin, >2000 kb), and their transcription is estimated to take an extended period of time (>24 hours for dystrophin). It is reasonable to assume that if their expression needs to be regulated in a relatively short period, it is likely to be accomplished at the level of translation.

Systems That Lack Transcriptional Control

In some systems (e.g., reticulocytes, oocytes, and RNA viruses), there is little or no opportunity for transcriptional control, and gene expression is modulated mostly at the translational level. The widespread use of translational controls to regulate gene expression during development suggests that this mode of control preceded transcriptional control in evolution. Such a hypothesis is consistent with the notion of the existence of an RNA world prior to the emergence of DNA. Is it therefore possible that translational control was more prevalent early in evolution and that we are now witnessing only the relics of such control mechanisms?

Spatial Control

Regulation of the site of protein synthesis within the cell can generate concentration gradients of proteins. Such gradients are known to affect

the translational efficiency of other specific mRNAs that determine patterning in early development (Chapter 7). Similar mechanisms are likely to explain synaptic plasticity (see section below).

Flexibility

Because of the wide variety of mechanisms for translational control, it can be focused by specific effector mechanisms on a single or a few gene(s) or cistrons, such as the coat protein and replicase of RNA phages, antizyme, and ferritin (see Chapters 8, 21, and 25); alternatively, by influencing general factors, it can encompass whole classes of mRNAs, as in heat shock and virus-induced host-cell shutoff (see Chapters 8, 17, and 22). Such flexibility affords the cell a powerful and adaptable means to regulate gene expression.

FUTURE TRENDS

Applied Genomic Approaches to Translational Control Studies

In the past two years, the development of cDNA microarray technology has provided a powerful means to explore the control of gene expression at a genome-wide level (Iyer et al. 1999). This technology has been applied primarily to studies of global expression profiles at the transcriptional level, but has recently been adapted for studies at the translational level (Johannes et al. 1999; Zong et al. 1999). The basis of this modification is the fact that the number of ribosomes associated with an mRNA reflects, under most circumstances, the rate of translation initiation, the rate-limiting step in translation as described above. Thus, mRNAs associated with a small number of ribosomes (light polysomes) are translated inefficiently, whereas those associated with a large number of ribosomes (heavy polysomes) are translated efficiently (see Chapter 19). The cDNA microarray technology has already been used, albeit on a small scale, to identify mRNAs that are translationally regulated in response to mitogens (Zong et al. 1999). Another study identified mRNAs, which are likely to translate via an IRES-dependent mechanism, because they could be translated in poliovirus-infected cells (Johannes et al. 1999). It is certain that this approach will be extended to identify translationally controlled mRNAs during development, differentiation, proliferation, and through the cell cycle, with the prospect of exciting findings. The results will be of importance in the understanding of translational control in diseases such as cancer and virus infection where there are clear indications that normal translation patterns are disrupted (see Chapters 8 and 20).

mRNA 5′–3′ Interactions

In the past four years, it has become abundantly clear that the 5′ end of the eukaryotic mRNA physically interacts with the 3′end. This interaction, which was first discovered in yeast (Tarun and Sachs 1996), is phylogenetically conserved and is mediated primarily by the interaction of eIF4G with the poly(A)-binding protein. The interaction of the mRNA ends brings about circularization of the mRNA, a phenomenon observed occasionally as polysome circles or spirals by electron microscopy by several investigators during the past four decades (see, e.g., Christensen et al. 1987). mRNA circularization could explain the synergistic activation of translation by the mRNA 5′ and 3′ ends (Gallie 1991). Although the mechanism of translational activation is not clear, it may involve the direct shunting of ribosomes (following termination) from the 3′ to the 5′ end of the mRNA. What is tantalizing about the circularization model is that it holds much promise to explain how sequences in the mRNA 3′UTR affect translation initiation from the 5′UTR. Such examples of translational control abound in development and in response to extracellular stimuli (see Chapters 7 and 27). One attractive hypothesis is that proteins, which interact with the 3′UTR positively or negatively, affect mRNA circularization. A likely mechanism is that 3′UTR-binding proteins interact with PABP or eIF4G or their partners to modulate their binding affinity. The circularization model could well explain the difference between initiation and re-initiation (recycling) with respect to their dependence on the cap structure (see Chapter 2). Future work is bound to shed light on these mechanisms.

Synaptic Plasticity

Synaptic plasticity is the mechanism that leads to changes in neurons in response to experience. Local translation of specific proteins in individual synapses plays a key role in effecting synaptic plasticity. This translation occurs on mRNAs that are localized at synapses, and on mRNAs that are transported after learning to synapses. Regulation also occurs at the level of the localization of mRNAs to synapses. For example, the mRNA for Arc, which is inducible, is specifically localized to previously activated synapses (Steward et al. 1998)

Translation inhibitors block long-term facilitation (L-LTP) in the snail, *Aplysia*, by specific blockade of synaptic translation. Translation at synapses in *Aplysia* is increased after treatment with serotonin, a response that is partially blocked by rapamycin. This suggests a role for the

FRAP/TOR rapamycin-sensitive pathway in synaptic plasticity (Casadio et al. 1999). Indeed, local application of rapamycin to synapses blocks the retention of long-term facilitation in this system.

The mechanism by which translation is activated in synapses is not understood, but some clues have recently been obtained. For example, polyadenylation of α-CaMKII, which is localized to synapses and is important in synaptic plasticity, is increased in dark-reared rats that are exposed to light. This is accompanied by enhanced translation (Chapter 27). CaMKII synthesis is also increased at synapses after NMDA stimulation. Interestingly, this is coupled to a decrease in general translation mediated by calcium-dependent phosphorylation of eEF2 (Sheetz et al. 2000). It is possible that this decrease is required to facilitate the enhancement of the translation of specific mRNAs.

mRNPs and mRNA Localization

Proteins that interact with mRNA and mediate its genesis, transport, activity, and destruction continue to be characterized in profusion. Elucidation of their interactions with one another and with mRNA, and the determination of their precise functions in the cell, remain formidable challenges, as discussed in many chapters of this monograph. The central roles played by such RNA–protein interactions are illustrated by recent work on mRNA localization in developing embryos and neural tissue. For example, the *cis*-acting elements known as "zip codes," which specify the sorting of some mRNAs within the cytoplasm, are recognized by a variety of *trans*-acting proteins such as ZBP and Vg1 (Ross et al. 1997; Deshler et al. 1998; Havin et al. 1998); similarly, Staufen recognizes structured RNA elements in the 3′UTR of mRNA species and targets them to specific locations (St Johnston et al. 1991; Broadus et al. 1998; Kiebler et al. 1999). How the resulting mRNP complexes are transported through the cytoplasm is not well understood. Evidence suggests that the mRNPs are assembled into large granules or "locusomes," which may contain ribosomes and other components of the translation apparatus, and implicates cytoplasmic structures such as microfilaments, microtubules, and the endoplasmic reticulum as migration pathways (for review, see Bassell et al. 1999; Kiebler and DesGroseillers 2000). Presumably, additional factors mark the final destinations of the mRNAs and govern their translational activity once they have been delivered there. The full elucidation of these mechanisms presents an exciting prospect as well as a technical challenge.

CONCLUDING REMARKS

The recognition of translational control formally requires the measurement of two parameters—the rate of protein synthesis and the concentration of the corresponding mRNA—so its rigorous demonstration can be demanding. Nevertheless, appreciation of the range of biological processes that entail translational control is expanding rapidly. At the same time, our understanding of the underlying protein synthetic apparatus is well advanced and provides a solid platform to address the mechanisms exploited by cells to control gene expression at this level. Goals for the future lie in many directions: to identify and characterize the *cis*- and *trans*-acting elements that mediate translational control, to visualize the interactions at the atomic level, and to integrate this information within the framework of the physiology and evolution of intact cells and organisms. The next few years will undoubtedly see progress toward all of these goals, as well as insights and unlooked-for discoveries that will open further vistas in this dynamic field.

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