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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 genetic, biochemical, and cell biological terms.

Translation is a sophisticated process requiring extensive biological machinery. One way to gauge the amount of genetic information needed to assemble the protein synthetic machinery is to compile a "parts list" of essential proteins and RNAs. Analyses of the genomes of several microorganisms have converged on similar estimates (Hutchison et al.

1999; Tamas et al. 2002; Kobayashi et al. 2003; Waters et al. 2003). These organisms get by with about 130 genes for components of the translation machinery, including about 90 protein-coding genes (specifying 50–60 ribosomal proteins, about 20 aminoacyl-tRNA synthetases, and 10–15 translation factors) and about 40 genes for ribosomal and transfer RNAs (rRNA and tRNAs). A somewhat larger number of genes are involved in eukaryotes, which have more ribosomal proteins and initiation factors, for example. Discounting genes that are dispensable for growth in the laboratory, it can be calculated that approximately 40% of the genes in a theoretical minimal cellular genome are devoted to the translation apparatus.

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This heavy genomic commitment is matched by the high proportion of a cell's energy budget and components that are devoted 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 ~28 kcal/mole, plus additional consumption for initiation and termination). Extensive resources are invested in the ribosomes, tRNAs, and enzymes required for making proteins. A rapidly growing yeast cell, for example, contains nearly 200,000 ribosomes occupying as much as 30–40% of its cytoplasmic volume (Warner 1999). Growth alone demands that the yeast cell produce 2000 ribosomes/min, an operation which absorbs about 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 (Warner 1999).

It would be surprising if a process of such importance were not closely monitored and regulated. In this chapter, we review the origins, mechanisms, and targets of translational control, a topic that impinges on biological fields as varied as medicine, agriculture, and biotechnology.

ORIGINS OF TRANSLATIONAL CONTROL

The central idea of translational control is that gene expression is 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 at the time, but it was taken up enthusiastically by workers in related 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).

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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 bacteria (although not yet the archaea, as far as we are aware).

Early History of Translation

The genesis of the translational control field took place at a time when studies of the translation system itself were in their 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 1950s, at the same time as the concept of proteins as unique, nonrandom linear arrays of just 20 amino acid residues was solidifying (Sanger and Tuppy published the first protein sequence, that of the insulin B chain, in 1951; Sanger and Tuppy 1951). Radioactive isotopes had begun to revolutionize many areas of biomedical science in the late 1940s, and labeled amino acids came into use as tracers around 1950. Initially, the radiolabeled amino acids had to be synthesized from simple labeled compounds such as formaldehyde or cyanide by the researchers themselves as a first step in their experiments (see, e.g., Borsook et al. 1950; Levine and Tarver 1950), but they became commercially available in the latter part of the decade. Enabled by this profound technical advance, 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 (1951) 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. The system was refined by stages and resolved into subfractions including a microsomal fraction that contained ribosomes attached to fragments of intracellular membrane (for review, see Zamecnik 1960). Elegant pulse-chase experiments demonstrated that the ribosomes are the site of protein synthesis, not an easy task in bacterial cells where protein synthesis was found to be very rapid: The assembly of a protein chain on a ribosome was estimated to take only about 5 seconds (McQuillen et al. 1959). 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, well before the first RNA sequence was completed (Holley et al. 1965), 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). However, 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). Technical advances in electron microscopy and high-speed centrifugation made indispensable contributions during this phase of the field's development.

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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. 1958), then understood as the physical manifestation of the adaptor RNA predicted on theoretical grounds (Crick 1958). Once its function had been realized, the name transfer RNA rapidly displaced the original term, "soluble" RNA (sRNA). Later, chemical modification of the amino acid moiety of a charged tRNA confirmed that it is the RNA component that 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, the activities of some factors remain obscure (e.g., EFP and its homolog eIF5A; Kang and Hershey 1994; Aoki et al. 1997) while others are still emerging (e.g., eIF2A; Komar et al. 2005; Ventoso et al. 2006). 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 eukaryotic mRNAs (apart from those histone mRNAs that lack poly(A) and some viral mRNAs that lack one or even both of these modifications). The mRNA concept immediately revolutionized thinking about gene expression in all cells.

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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 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, processing, and transport. Predictably, the first cases to be recognized were mostly in eukaryotes and were those in which it was either self-evident or relatively simple to establish 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 bacteria is due to the exigencies of particular circumstances and to the potency of translational control mechanisms.

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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 which made it easier to define and study these two particular cases is that their 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, as evidenced early on during studies of phage-infected bacteria. 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, affect 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 mRNAs, or by exploiting the differential sensitivity of mRNAs to more subtle changes in the activity of general

components of the translation system, e.g., eIF4E (Chapters 14, 15, 16, and 20) or eIF2 (Chapters 9, 13, 16, and 20). Although examples of all these exist and are discussed at length in this volume, 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.

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Early Paradigms of Translational Control

In large part, the origins of translational control can be traced to studies of four early examples. These are described below, followed by an example involving elongation control.

Sea Urchin Eggs

The eggs of sea urchins and other invertebrates synthesize protein at a very low rate but are triggered to incorporate amino acids within a few minutes of fertilization with little or no concomitant RNA synthesis (Hultin 1961; Nemer 1962; Gross et al. 1964). The first wave of increased translation, which lasts for several hours, is not blocked by actinomycin D (Gross et al. 1964) because the eggs contain preexisting mRNA in a masked form that is not translated until a stimulus dependent on fertilization is received. In principle, the limitation could be due to a deficiency in the translational machinery, but there is little evidence to support this possibility (Humphreys 1969). On the other hand, egg ribosomes are able to translate added poly(U) even though they display little intrinsic protein synthetic activity (Nemer 1962; Wilt and Hultin 1962). The deproteinized egg RNA can be translated in a cell-free system (Maggio et al. 1964; Monroy et al. 1965), and cytoplasmic messenger ribonucleoprotein (mRNP) particles have been observed (Spirin and Nemer 1965). Because the assembly of masked mRNP complexes must take place during oogenesis, the sea urchin system exemplifies a reversible process of mRNA repression and activation. Recent developments in this active area of research are discussed in Chapter 19.

Mammalian Reticulocytes

Because they were enucleate, it was taken for granted that protein synthesis in these immature red cells—mainly hemoglobin—would be regulated at the translational level. In the intact rabbit reticulocyte, the synthesis of heme parallels that of globin (Kruh and Borsook 1956), and

globin synthesis is controlled by the availability of heme or of ferrous ions (Bruns and London 1965). Regulation by heme occurs in the highly active unfractionated reticulocyte lysate translation system (Lamfrom and Knopf 1964), the forerunner of the widely used messenger-dependent system (Pelham and Jackson 1976) and of commercially available coupled transcription–translation systems. When globin synthesis is inhibited in cells or extracts, the polysomes dissociate to monosomes (Hardesty et al. 1963; Waxman and Rabinowitz 1966), arguing that regulation affects translation initiation. The effects of heme deprivation are mediated by the protein kinase HRI (heme-regulated inhibitor) and are mimicked by unrelated stimuli such as double-stranded RNA (dsRNA) and oxidized glutathione (Ehrenfeld and Hunt 1971; Kosower et al. 1971). They extend to all mRNAs in the reticulocyte lysate (Mathews et al. 1973), implying that a general mechanism of translational control is being invoked. This mechanism centers on the phosphorylation of the α-subunit of initiation factor eIF2, which results in reduced levels of ternary complex (eIF2:GTP:Met-tRNA$_i$) and impaired loading of the 40S ribosomal subunit with Met-tRNA$_i$ (Farrell et al. 1977). Considerable attention has been given to the family of eIF2 kinases, which confer sensitivity to a wide range of stimuli. HRI, PKR, GCN2, and PERK are activated by heme deprivation, structured RNA, uncharged tRNA, and endoplasmic reticulum stress, respectively, inter alia, whereas PKZ, recently found in fish, is potentially regulated by Z-DNA (for review, see Chapter 12).

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Virus-infected Cells

Translation of cellular mRNAs is suppressed during infection with many viruses (Chapter 20). This inhibition may begin before the onset of viral protein synthesis and without any apparent interference with cellular mRNA production or stability. In polioviral infection, for 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). In the first phase, polysomes break down without any effect on translation elongation or termination (Penman and Summers 1965; Summers and Maizel 1967). In the second phase, virus-specific polysomes form (Penman et al. 1963). Cellular mRNA remains intact and translatable in a cell-free system (Leibowitz and Penman 1971), evidence that initiation has become selective for viral mRNA. Translational inhibition extends to mRNAs produced by several other viruses introduced together with poliovirus in a double infection (Ehrenfeld and Lund 1977), indicative of a general effect that later work ascribed to modification of the cap-bind-

ing 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 (Chapter 20).

Bacteriophage f2 provided the first evidence for prokaryotic translational control, 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 phage 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 long-range RNA structure and allow replicase translation. In contrast, a second nonsense mutation leads to overproduction of the replicase because the coat protein acts as a repressor of replicase translation. The binding of phage coat protein to the hairpin structure containing the replicase AUG is now one of the best-characterized RNA–protein interactions (Witherell et al. 1991). Subsequent studies have disclosed translational control mechanisms in the DNA phages as well as in bacterial genes themselves (Chapter 28), but it was eukaryotic systems that made most of the early running.

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Physiological Stimuli

The cells and tissues of higher organisms 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. Early examples 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). These reports strengthened the view that translational control is widespread and important even though in some cases the trail has gone cold or been erased upon further investigation. Proving that control is being exerted at the translational level can be a challenging task in nucleated cells, let alone in a tissue or whole organism, and this constituted one of the chief stumbling blocks. Although several methods are available

that can give rigorous evidence (described below), simpler approaches can be misleading. One popular approach took advantage of selective inhibitors of transcription or translation, such as actinomycin D and cycloheximide, but the results were liable to be complicated (if not confounded) by the drugs' side effects or 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 definite evidence, however, and the most convincing proofs often came from investigations of the underlying biochemical processes for example, by demonstrating changes in polysome profiles or initiation factor phosphorylation states as discussed later in this chapter and in a number of chapters in this volume (see, e.g., Chapters 13, 14, 17, and 20). The 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.

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Secretory Pathway

One of the best-studied examples of regulation during the elongation phase is found in the synthesis of proteins that are destined for secretion or for a life within a cellular membrane (for review, see Chapter 21). Most such proteins are made on polysomes that are attached to the endoplasmic reticulum (ER), 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), and the existence of what came to be called a signal peptide was reported on secreted proteins (Milstein et al. 1972; Devilliers-Thiery et al. 1975). These findings lent substance to the signal hypothesis which proposed that an amino-terminal sequence might ensure secretion (Blobel and Sabatini 1971). The development of cell-free systems enabled the biochemical dissection of the secretory pathway (Blobel and Dobberstein 1975) and led to the discovery of the signal recognition particle (SRP). This RNP particle interacts with the signal peptide, the ribosome, and the ER. 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). The elongation block is relieved when the ribosome docks with its receptor on the ER, allowing the protein chain to be completed and simultaneously translocated