12. In animals injected with [^{125}I]T_3, radioactivity from trapped plasma did not account for more than 3 percent of ^{125}I found in the pituitary. In T_4-injected animals, after washing with cold saline as described, plasma accounted for 20 to 30 percent of the radioactivity found in the extranuclear fraction and 0 to 18 percent of the nuclear radioactivity. The plasma contribution, P, was calculated as (^{131}I in pituitary fraction/TCA-precipitated ^{131}I per microliter of plasma) × (TCA-precipitated ^{125}I per microliter of serum). The amount of T_3 generated from T_4 was determined as follows. First, a correction factor for [^{125}I]T_3 in nucleus or plasma derived from [^{125}I]T_3 contaminating the [^{125}I]T_4 was subtracted. This was computed as $P \times T$, where P is the percentage of [^{125}I]T_3 in the [^{125}I]T_4 injected, and T is the fraction of injected [^{125}I]T_3 found in the nucleus (or plasma) at a particular time after [^{125}I]T_3 injection. In experiments C and D (Table 1), the correction factor was 5 to 12 percent of the nuclear [^{125}I]T_3 and 90 to 100 percent of the plasma [^{125}I]T_3. Second, the corrected [^{125}I]T_3 (derived from [^{125}I]T_4 conversion) as a percentage of the dose of [^{125}I]T_4 was then used to calculate the mass of T_3 produced (nanograms) as follows: (corrected [^{125}I]T_3/total dose of [^{125}I]T_4) × 2 × total dose of T_4 (nanograms) × (651/777). The factor 2 derives from the fact that [^{125}I]T_4 (Cambridge Nuclear) was prepared by iodinating 3,5-diiodothyronine with carrier-free ^{125}I. All T_4 molecules are therefore labeled at the 3′ and 5′ positions. Thus, each molecule of T_3 formed from T_4 has one-half the radioactivity of the original.
13. E. Silva and P. R. Larsen, in *Program of the 59th Annual Meeting of the Endocrine Society* (Endocrine Society, Bethesda, Md., 1977), abstr. 462. The half-time of disappearance of TSH is 30 minutes in thyroidectomized animals, whether measured by immunoprecipitation of injected [^{125}I]TSH or by the immunoassay of TSH after thyrotropin-releasing hormone stimulation or T_3 administration [with appropriate corrections for nonsuppressible TSH; see M. I. Surks and J. H. Oppenheimer, *Endocrinology* **99**, 1432 (1976)].
14. V. A. Galton, in *Thyroid Research*, J. Robbins and L. E. Braverman, Eds. (Excerpta Medica, Amsterdam, 1976), p. 251.
15. J. L. Bakke and N. Lawrence, *Eur. J. Pharmacol.* **2**, 308 (1968).
16. S. Reichlin, E. M. Volpert, S. C. Werner, *Endocrinology* **78**, 302 (1966).
17. C. R. DeFesi and M. I. Surks, *Clin. Res.* **25**, 462 (1977).
18. G. M. Abrams and P. R. Larsen, *J. Clin. Invest.* **52**, 2522 (1973).
19. P. R. Larsen, *Metabolism* **21**, 1073 (1972).
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Cell-Free Modulation of Proinsulin Synthesis

Abstract. *In vivo, glucose preferentially stimulates proinsulin biosynthesis; at least part of this process is independent of new RNA synthesis and is accompanied by increases in the overall rate of polypeptide chain initiation. The cell-free translation of proinsulin messenger RNA is very sensitive to changes in the protein-synthesizing system. Proinsulin synthesis is preferentially inhibited by the addition of increasing quantities of polyadenylate-containing RNA from the fetal bovine pancreas or by the addition of the drug, aurintricarboxylic acid, which blocks polypeptide chain initiation. These results suggest that proinsulin messenger RNA competes less efficiently for rate controlling initiation factors. We propose that glucose stimulates proinsulin biosynthesis by allowing the less competitive proinsulin messenger RNA to be translated more efficiently.*

In slices of pancreas, insulinomas, or isolated pancreatic islets, it appears that glucose (and certain other metabolites) will stimulate the synthesis of proinsulin, relative to other proteins synthesized within the beta cell (*1*-*5*). For example, in studying [^{3}H]leucine incorporation into rat islet proteins, Permutt and Kipnis (*2*) demonstrate that proinsulin comprises 6.1 and 21.8 percent of the radioactive protein synthesized during incubation with low (2.8 m*M*) and high (15.3 m*M*) concentrations of glucose, respectively. This stimulation is accompanied by a twofold increase in total protein synthesis and is independent of new RNA synthesis (*2*, *3*). Furthermore, Permutt (*4*) has determined that the glucose stimulation brings about a twofold increase in the overall rate of polypeptide chain initiation, but does not alter the overall rate of chain elongation or the total amount of messenger RNA (mRNA) available for translation. There are some data compatible with glucose regulation at the transcriptional level, but clearly translational modulation is present. The mechanisms responsible for the translational control of proinsulin synthesis remain undefined.

We have studied the translation of the mRNA for bovine proinsulin in a cell-free protein-synthesizing system derived from wheat germ (*6*) and have found that it is possible to modulate proinsulin synthesis by changing the conditions of translation. These results suggest that proinsulin mRNA competes less efficiently for rate controlling initiation factors. This finding suggests that, in vivo, increases in the overall rate of polypeptide chain initiation modulate proinsulin biosynthesis in accordance with Lodish's kinetic model for translational regulation (*7*).

For the experiments described here, RNA containing a polyadenylate [poly(A)] sequence at its 3′ end was isolated from the fetal bovine pancreas (*6*) and translated in the wheat germ system as described by Roberts and Paterson (*8*). A sensitive double antibody immunoprecipitation technique (*6*) was used to quantitate the radioactive amino acids incorporated into preproinsulin (*6*, *9*). Figure 1A shows that the optimum Mg^{2+} concentration for proinsulin synthesis in vitro was 1 m*M* lower than the optimum for total protein synthesis. Under conditions in which small variations in the Mg^{2+} concentration had no effect on total protein synthesis, proinsulin synthesis was severely depressed. This phenomenon was observed consistently at all mRNA concentrations and with different combinations of wheat germ lysates and mRNA samples. Increases in the K^+ concentration also appeared to inhibit selectively the synthesis of proinsulin (Fig. 1B). Figure 1C shows that at concentrations of mRNA below 1 μg per 50-μl assay, total protein and proinsulin were synthesized proportionally. However, above 1 μg/50 μl (Fig. 1, C and D) the synthesis of proinsulin was depressed.

Other investigators have been able to modulate different proteins synthesized in cell-free systems by varying mRNA competition (*10*–*12*), polyamines (*13*), and ionic conditions (*14*). Many other investigators use low background cell-free systems with the implicit assumption that the resultant translation products reflect the true complexity and abundancy of the input mRNA population. This concept has not been directly proved. Since it is so easy to change the percentage of the translation products that react with proinsulin antiserums, it is difficult to determine the proportion of the mRNA population coding for proinsulin. These results argue against the indiscriminate extrapolation of cell-free translation data to estimate mRNA concentration.

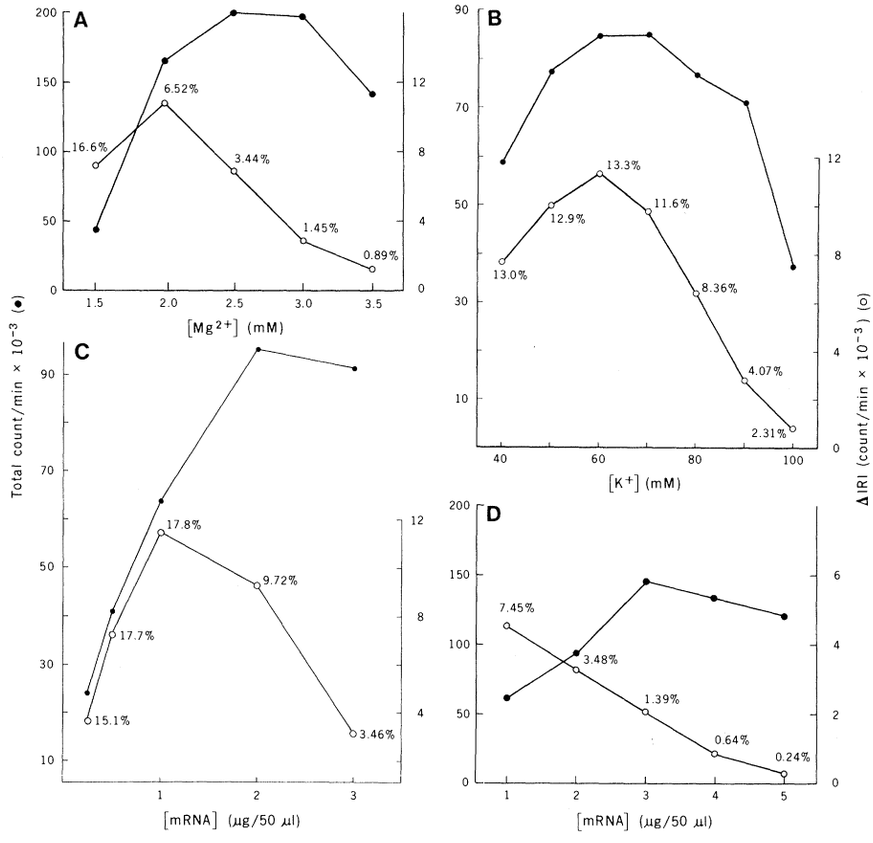
Our results are similar to those obtained by McKeehan (*11*) in a study of globin chain synthesis in a reconstituted translation system. He found that the concentrations of mRNA, ribosomal subunits, different initiation factors, Mg^{2+}, and K^+ all affect the ratio of α- to β-globin synthesized. Lodish (*15*) and Lodish and Jacobson (*16*) had previously shown that β-globin mRNA initiates polypeptide chain synthesis more efficiently than α-globin mRNA. McKeehan (*11*) and Lodish (*7*, *15*) conclude that α-globin mRNA has a lower affinity for active 40*S* ribosomal subunits.

Our results can be interpreted in a similar fashion. At low concentrations of mRNA, the competition for rate controlling elements is less severe and the proinsulin mRNA is efficiently initiated. With increasing quantities of mRNA the translational apparatus becomes saturated and less proinsulin is synthesized. Apparently, those mRNA's that are preferentially translated possess higher affinities for the relevant rate controlling ele-

ments. Proinsulin mRNA appears to have a relatively lower binding affinity and thus competes less efficiently compared to other mRNA's in the same population. Increasing quantities of Mg^{2+} or K^+ also inhibit proinsulin mRNA translation without greatly affecting total protein synthesis. This may result from a combination of factors: (i) an alteration in the secondary structure of proinsulin mRNA; (ii) an alteration in the structure of some critical component of the translation system; and (iii) a decrease in the overall rate of polypeptide chain initiation. Factors (i) and (ii) may affect the proinsulin and mRNA binding affinity for the rate controlling element, while factor (iii) will influence proinsulin chain initiation as predicted in Lodish's kinetic treatment (*7*).

It is possible to amplify cell-free mRNA competition by partially blocking polypeptide chain initiation with aurintricarboxylic acid (ATA). Figure 2 shows that ATA inhibition of protein synthesis directed by fetal bovine pancreatic mRNA affects proinsulin synthesis to a greater extent than it does total protein synthesis. This result supports the conclusion that proinsulin mRNA is initiated less efficiently when mRNA is present in saturating concentrations.

It is possible to correlate the evidence of mRNA efficiency in vitro with observations in vivo. Sonenshein and Brawerman (*12*) have shown that the mRNA's coding for the immunoglobulin light and heavy chains appear to compete more efficiently when poly(A)-containing RNA from a mouse myeloma is translated in the wheat germ system. With intact myeloma cells, treatments (that is, starvation, exposure to actinomycin D, and hypertonicity) that reduce the rate of polypeptide chain initiation result in the preferential synthesis of immunoglobulin chains (*17*). Data from experiments in vivo supporting our results in vitro were obtained by Pipeleers *et al.* (*5*). They found that within the rat β cell the rate of proinsulin synthesis varies as a simple function of the overall rate of protein synthesis. Treatments that increase the rate of protein synthesis, that is, increasing concentrations of various carbohydrates, preferentially stimulate the rate of proinsulin synthesis. Treatments that reduce the rate of protein synthesis, that is, changing the *p*H of the culture medium or replacement of Na^+ with other monovalent cations in the presence of a high glucose concentration, result in a preferential decrease in proinsulin synthesis. A similar mathematical expression describes both the stimulation and inhibition of proinsulin synthesis during



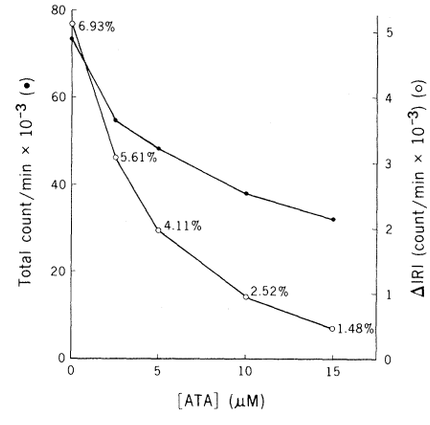


Fig. 1 (top). Modulation of immunoreactive proinsulin and total protein synthesis in the wheat germ system. (A) The concentration of Mg^{2+}. Poly(A)-containing RNA from fetal bovine pancreas was translated in the wheat germ system. Each 200-μl reaction contained 80 m*M* KCl, 2 μg of mRNA per 50 μl, [^{3}H]leucine (3.7 μ*M*; 54 c/mmole) and varying amounts of $MgCl_2$. Portions (5 μl) were precipitated with trichloroacetic acid to measure the total protein synthesized. Assays (at 2 m*M* $MgCl_2$) without added RNA generated approximately 10,000 count/min per 50-μl assay; this background synthesis was not subtracted in any of the immunoassay calculations. The reaction mixture was treated with ribonuclease, diluted with immunoassay buffer, and analyzed for the synthesis of immunoreactive proinsulin by means of a double antibody immunoprecipitation (*6*). Four precipitates (two of which contained 10 μg of proinsulin as competitor) were used for each point; the average difference in radioactivity represents the specific immunoreactive proinsulin (ΔIRI). Only 85 μl (equivalent to 42.5 μl of the original reaction) of the translation products were added to each precipitate, so the total protein synthesized (•) and the ΔIRI synthesis (○) is based on 42.5 μl. The percentage of the translation products that are proinsulin immunoreactive is shown above each point. (B) The concentration of K^+. The translation, at these different concentrations of KCl, and the immunoassay reactions were performed as described in (A). Reaction parameters were 2 m*M* $MgCl_2$ and 1 μg of mRNA per 50 μl. Incorporation is based on a 37.5-μl portion of each 200-μl reaction mixture. (C) The concentration of mRNA. The translation, at these different concentrations of input mRNA, and the immunoassay reactions were performed as described in (A), with 2 m*M* $MgCl_2$ and 60 m*M* KCl. Incorporation is based on a 37.5-μl portion of each 200-μl reaction mixture. (D) The concentration of mRNA. The translation, at these different concentrations of input mRNA, and the immunoassay reactions were performed as described in (A), with 2 m*M* $MgCl_2$ and 80 m*M* KCl. Incorporation is based on a 42.5-μl portion of each 200-μl reaction mixture. The mRNA used for this experiment was different from that used in (A), (B), and (C). Fig. 2 (bottom right). Inhibition of immunoreactive proinsulin and total protein synthesis by aurintricarboxylic acid (*ATA*). Poly(A)-containing RNA from fetal bovine pancreas was translated in the wheat germ system in the presence of varying amounts of aurintricarboxylic acid and 2 m*M* $MgCl_2$ and 1 μg of mRNA per 50 μl. The immunoassay reactions were performed as described in Fig. 1. Incorporation is based upon a 42.5-μl portion of each 200-μl reaction mixture. Both the mRNA and the wheat germ lysate used for this experiment were different from those used in Fig. 1.

these changes in the overall rate of protein synthesis. This implies that a common mechanism is involved. Moreover, Pipeleers *et al.* (*5*) found two treatments (cycloheximide and 2,4-dinitrophenol) which reduce the rate of protein synthesis without affecting the preferential synthesis of proinsulin. These two treatments primarily influence the rate of elongation, and thus support the hypothesis that the rate of polypeptide chain initiation determines how efficiently the proinsulin mRNA is translated.

Lodish's kinetic model for translational regulation (*7*) predicts that any increase in the overall rate of polypeptide chain initiation will allow mRNA's with lower rate constants (that is, affinities) to be translated more efficiently. Hence, under low glucose conditions, proinsulin mRNA may compete (relative to the other mRNA's in the β cell) less effectively as a result of its lower intrinsic binding affinity for rate controlling translation elements. This competition may be so severe that, at any one time, only a fraction of the proinsulin mRNA will be found engaged on an active ribosome. The high concentration of glucose increases the overall rate of polypeptide chain initiation (for example, by increasing the concentrations of guanosine triphosphate) (*18*). This permits the less competitive proinsulin mRNA to be initiated more rapidly, and mobilization of the proinsulin mRNA pool. This combination will account for the relative increase in proinsulin synthesis. Hence, if the rate of glucose oxidation within the β cell regulates the overall rate of polypeptide chain initiation, which in turn regulates the rate of proinsulin synthesis, then the β cell possesses a simple mechanism to replenish insulin levels following glucose-stimulated insulin secretion.

Our results are clearly compatible with Lodish's kinetic model for translational regulation. However, they in no way prove the hypothesis, nor do they exclude the possibility that glucose modulates either the concentrations of specific initiation factors for proinsulin mRNA or the amount of proinsulin mRNA available for translation. Experiments to test these possibilities await production of a labeled complementary DNA probe to measure the amount of proinsulin mRNA.

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References and Notes

1. K. W. Taylor and D. G. Parry, *Nature (London)* **203**, 1144 (1964); D. G. Parry and K. W. Taylor, *Biochem. J.* **100**, 2c (1966); S. L. Howell and K. W. Taylor, *Biochim. Biophys. Acta* **130**, 519 (1966); J. M. Martin and J. J. Gagliardino, *Nature (London)* **213**, 630 (1967); J. L. Clark and D. F. Steiner, *Proc. Natl. Acad. Sci. U.S.A.* **62**, 278 (1969); B. J. Lin and R. E. Haist, *Can. J. Physiol. Pharmacol.* **47**, 791 (1969); G. E. Morris and A. Korner, *Biochim. Biophys. Acta* **208**, 404 (1970); T. Tanese, N. R. Lazarus, S. Devrim, L. Recant, *J. Clin. Invest.* **49**, 1394 (1970); H. Schatz, J. Otto, M. Hinz, V. Maier, C. Nierle, E. F. Pfeiffer, *Endocrinology* **94**, 248 (1974); P. Zucker and J. Logothetopoulos, *Diabetes* **24**, 194 (1975); K. Jain, J. Logothetopoulos, P. Zucker, *Biochim. Biophys. Acta* **399**, 384 (1975); T. O. Tjioe and P. R. Bouman, *Horm. Metab. Res.* **8**, 261 (1976); A. Andersson, *Biochim. Biophys. Acta* **437**, 345 (1976); N. M. Baird and G. E. Bauer, *Proc. Soc. Exp. Biol. Med.* **151**, 16 (1976); A. Andersson, *Acta Endocrinol.* **82**, 318 (1976).
2. M. A. Permutt and D. M. Kipnis, *J. Biol. Chem.* **247**, 1194 (1972).
3. G. E. Morris and A. Korner, *FEBS Lett.* **10**, 165 (1970); D. F. Steiner, W. Kemmler, J. L. Clark, P. E. Oyer, A. H. Rubenstein, *Handbook of Physiology,* section 7, *Endocrinology,* vol. 1, *Endocrine Pancreas* (American Physiological Society, Washington, D.C., 1972), pp. 175–198.
4. M. A. Permutt, *J. Biol. Chem.* **248**, 2738 (1974).
5. D. G. Pipeleers, M. Marichal, W. J. Malaisse, *Endocrinology* **93**, 1001 (1973); *ibid.*, p. 1012.
6. P. T. Lomedico and G. F. Saunders, *Nucleic Acids Res.* **3**, 381 (1976); P. T. Lomedico, S. J. Chan, D. F. Steiner, G. F. Saunders, *J. Biol. Chem.*, in press.
7. H. F. Lodish, *Nature (London)* **251**, 385 (1974).
8. B. E. Roberts and B. M. Paterson, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2330 (1973).
9. S. J. Chan, P. Keim, D. F. Steiner, *ibid.* **73**, 1964 (1976).
10. A. G. Stewart, E. S. Gander, C. Morel, B. Luppis, K. Scherrer, *Eur. J. Biochem.* **34**, 205 (1973); N. D. Hall and H. R. V. Arnstein, *FEBS Lett.* **35**, 45 (1973); Y. Beuzard and I. M. London, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2863 (1974); G. Temple and H. F. Lodish, *Biochem. Biophys. Res. Commun.* **63**, 971 (1975).
11. W. L. McKeehan, *J. Biol. Chem.* **249**, 6517 (1974).
12. G. E. Sonenshein and G. Brawerman, *Biochemistry* **15**, 5501 (1976).
13. J. F. Atkins, J. B. Lewis, C. W. Anderson, R. F. Gesteland, *J. Biol. Chem.* **250**, 5688 (1975).
14. A. J. M. Vermorken, J. M. H. C. Hilderink, W. J. M. Van De Ven, H. Bloemendal, *Biochim. Biophys. Acta* **414**, 167 (1975).
15. H. F. Lodish, *J. Biol. Chem.* **246**, 7131 (1971).
16. ——— and M. Jacobson, *ibid.* **247**, 3622 (1972).
17. D. L. Nuss and G. Koch, *J. Mol. Biol.* **102**, 601 (1976); G. E. Sonenshein and G. Brawerman, *Biochemistry* **15**, 5497 (1976).
18. M. A. Permutt and D. M. Kipnis, *Mount Sinai J. Med.* **40**, 323 (1973).
19. This study was supported by grants from the Kroc Foundation, Kelsey and Leary Foundation (No. 973), the Robert A. Welch Foundation (G-267), and National Institutes of Health (AM20383). P.T.L. was the recipient of a Rosalie B. Hite Predoctoral Fellowship from the University of Texas System Cancer Center, Houston. We thank Harvey Lodish for suggesting the experiment shown in Fig. 2.

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Deuterolysis of Amino Acid Precursors: Evidence for Hydrogen Cyanide Polymers as Protein Ancestors

Abstract. *Deuterolysis experiments suggest that hydrogen cyanide polymers rather than aminoacetonitriles are major precursors of α-amino acids obtained from spark reactions and other studies on chemical evolution. These results are consistent with the hypothesis that the original heteropolypeptides on the earth were synthesized spontaneously from hydrogen cyanide and water without the intervening formation of α-amino acids.*

After the pioneering demonstration by Miller and Urey (*1*) that α-amino acids are readily obtained from methane, ammonia, and water subjected to electric discharges, it has become widely accepted that the prebiotic formation of primitive proteins occurred in two stages: α-amino acid synthesis initiated by the action of natural high-energy sources on the components of a reducing atmosphere, followed somehow by polycondensation of the accumulated monomers in the oceans or on land (*1, 2*). Limited progress in research on possible condensation reactions (*2*), however, suggests that the inherent thermodynamic barrier to spontaneous polymerization of α-amino acids is not easily overcome. An alternative model for the origin of proteins (*3–5*) bypasses this problem by

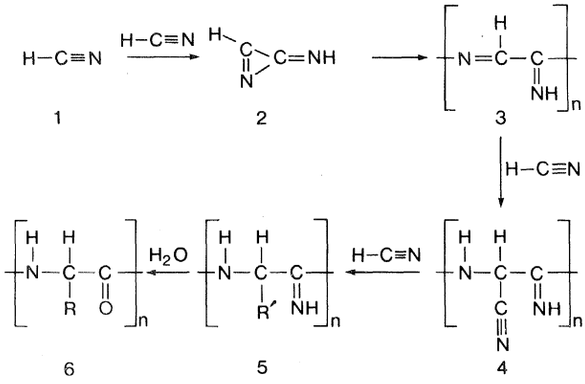

Fig. 1. Proposed route for heteropolypeptide synthesis from hydrogen cyanide and water (**1** → **6**). R′ represents precursors of protein side chains R.