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Translational control of insulin biosynthesis

Evidence for regulation of elongation, initiation and signal-recognition-particle-mediated translational arrest by glucose

Michael WELSH,* Neal SCHERBERG,† Reid GILMORE‡ and Donald F. STEINER*

*Department of Biochemistry and Molecular Biology, and †the Thyroid Function Laboratory, University of Chicago, Chicago, IL 60637, U.S.A., and ‡Department of Biochemistry, University of Massachusetts Medical Center, Worcester, MA 01605, U.S.A.

The biosynthesis of insulin in the islets of Langerhans is strongly controlled at the translational level by glucose. We have used a variety of experimental approaches in efforts to dissect the mechanisms underlying the stimulatory effect of glucose. To assess its effects on rates of peptide-chain elongation, isolated rat islets were labelled with [^{3}H]leucine at different glucose concentrations in the presence or absence of low concentrations of cycloheximide. Under these conditions, at glucose concentrations up to 5.6 mM, endogenous insulin mRNA did not become rate-limiting for the synthesis of insulin, whereas stimulation of non-insulin protein synthesis was abolished by cycloheximide at all glucose concentrations, indicating either that insulin synthesis is selectively regulated at the level of elongation at glucose concentrations up to 5.6 mM, or that at these concentrations inactive insulin mRNA is transferred to an actively translating pool. Glucose-induced changes in the intracellular distribution of insulin mRNA in cultured islets were assessed by subcellular fractionation and blot-hybridization using insulin cDNA probes. At glucose concentrations above 3.3 mM, cytoplasmic insulin mRNA was increasingly transferred to fractions co-sedimenting with ribosomes, and relatively more of the ribosome-associated insulin mRNA became membrane-associated, consistent with effects of glucose above 3.3 mM on both the initiation of insulin mRNA and SRP (signal recognition particle)-mediated transfer of cytosolic nascent preproinsulin to the endoplasmic reticulum. When freshly isolated islets were homogenized and incubated with ^{125}I-Tyr-tRNA, run-off incorporation of ^{125}I into preproinsulin was increased by prior incubation of the islets at 16.7 mM-glucose. The addition of purified SRP receptor increased the run-off incorporation of [^{125}I]iodotyrosine into preproinsulin, especially when the islets had been preincubated at 16.7 mM-glucose. These findings taken together suggest that glucose (*a*) may stimulate elongation rates of nascent preproinsulin at concentrations up to 5.6 mM, (*b*) stimulates initiation of protein synthesis involving both insulin and non-insulin mRNA at concentrations above 3.3 mM, and (*c*) increases the transfer of initiated insulin mRNA molecules from the cytoplasm to microsomal membranes by an SRP-mediated mechanism that involves the modification of interactions between SRP and its receptor.

INTRODUCTION

The biosynthesis and secretion of insulin by pancreatic β-cells is regulated by many factors, including glucose, certain amino acids, peptide hormones and cyclic nucleotides (for reviews see Hedeskov, 1980; Permutt, 1981). Among these agents glucose appears to be a pre-eminent signal for regulation of both processes (Hedeskov, 1980; Howell & Taylor, 1966). Metabolism of glucose is believed to be essential for stimulation of insulin secretion and biosynthesis, as only metabolizable glucose analogues and other carbohydrate or amino acid substrates are effective (Ashcroft *et al.*, 1978). The action of glucose on insulin biosynthesis is very rapid, occurring within a few minutes, and does not require the synthesis of new mRNA (Ashcroft *et al.*, 1978; Permutt & Kipnis, 1972*a*). Although the enhancement of insulin synthesis is selective relative to total islet protein synthesis, little information is available on the molecular mechanism of this specificity. One earlier study suggested that glucose increased islet initiation rates (Permutt & Kipnis, 1972*b*), but conflicting results have been reported as to whether islet mRNA is shifted to a more rapidly sedimentable form in the presence of high glucose (Permutt & Kipnis, 1972*b*; Itoh & Okamoto, 1980).

Since insulin is a secretory product of the β-cell, it is possible that glucose may affect processes in the early stages of preproinsulin synthesis that lead to its synthesis and segregation as a secretory protein via membrane-bound ribosomes. Several factors essential for the formation of ribosome–membrane junctions have now been described, including the soluble signal-recognition particle (SRP; Walter & Blobel, 1981) and a membrane-associated protein of the rough endoplasmic reticulum (ER), called the docking protein (Meyer *et al.*, 1982) or SRP receptor (Gilmore *et al.*, 1982). SRP appears to bind to the nascent prepeptide as it emerges from the ribosome, and arrests further translation until the complex interacts with a receptor protein (the docking protein) on the rough ER (Walter & Blobel, 1981; Meyer *et al.*, 1982). The interaction between the SRP receptor and the elongation-arrested ribosome results in ribosome

Abbreviations used: SRP, signal-recognition particle; ER, endoplasmic reticulum; SSC, 0.15 M-NaCl/0.015 M-sodium citrate.

attachment to the membrane and dissociation of SRP from the ribosome, thereby allowing peptide-chain synthesis to continue to completion (Gilmore & Blobel, 1983). These early events, which are associated with the synthesis of most secreted proteins, including insulin (Eskeridge & Shields, 1983), and also some membrane-associated proteins (Anderson *et al.*, 1983) as well as lysosomal proteins (Erickson *et al.*, 1983), may provide control points for specific regulation of translation. Thus chemical modification(s) of either SRP or its receptor in the membrane could alter their ability to interact and thereby modulate the translation of insulin and other secreted proteins.

It was the aim of this study to characterize further the mode of translational control exerted by glucose on insulin biosynthesis. This was done by studying the rates of insulin biosynthesis in the presence of low doses of cycloheximide in isolated rat islets of Langerhans. We also determined the subcellular localization of insulin mRNA and SRP when islets had been incubated at a low or a high glucose concentration. Furthermore, islets were preincubated at low or high glucose and then homogenized and incubated with added ^{125}I-Tyr-tRNA, and the incorporation of radioactivity into preproinsulin was studied. Evidence is presented suggesting that glucose stimulates insulin biosynthesis at low glucose concentrations ($\leqslant$ 5.6 mM) either by increasing elongation rates or by mobilizing mRNA from a translationally inactive pool, whereas it augments the rates of initiation of insulin mRNA translation concomitantly with the release of SRP arrest of preproinsulin synthesis at higher concentrations.

EXPERIMENTAL

Materials

L-[4,5-^{3}H]Leucine (145 Ci/mmol), [α-^{32}P]dCTP (3000 Ci/mmol), ^{125}I, ACS and NCS were from Amersham, Arlington Heights, IL, U.S.A. A nick-translation system from BRL, Rockville, MD, U.S.A., was used. RNAsin was from Promega Biotech, Madison, WI, U.S.A. Glyoxal, cycloheximide, yeast tRNA, poly(A), polyvinylpyrrolidone, salmon sperm DNA and ribonuclease A were from Sigma Chemical Co., St. Louis, MO, U.S.A. Nitrocellulose membranes (pore size 0.44 μm) was purchased from Schleicher and Schuell, Keene, NH, U.S.A. Ficoll 400 was from Pharmacia, Uppsala, Sweden. Collagenase (CLS IV) was from Worthington, Freehold, NJ, U.S.A. Fetal-calf serum was from Biolabs, Northbrook, IL, U.S.A.

Preparation of islets

Islets were isolated on Ficoll gradients from collagenase-digested pancreata of male Sprague–Dawley rats weighing 150 g (Lernmark *et al.*, 1976), and in some instances were cultured overnight free-floating in RPMI-1640 supplemented with 10% fetal-calf serum (Andersson, 1978) before experimentation. These culture conditions have been shown to provide excellent maintenance of insulin-biosynthesis capability (Andersson, 1978).

Insulin biosynthesis

Groups of 20 freshly isolated islets were incubated at 37 °C for 60 min in 100 μl of Hanks' salt solution containing 10 μCi of L-[4,5-^{3}H]leucine with additions as stated in the Figure legends. The islets were then washed once with ice-cold buffer (containing 0.5 mg of bovine serum albumin/ml, and sonicated in 1 ml of water. Samples were precipitated by addition of 10% (w/v) trichloroacetic acid and counted for radioactivity by liquid scintillation after dispersion in NCS and ACS. Other samples were immunoprecipitated with anti-insulin antibody in excess [as determined by the completeness of precipitation of added ^{125}I-insulin and the linear precipitation of leucine-labelled (pro)insulin with increasing amounts of homogenate]. Normal guinea-pig serum served as a control. The immunoprecipitates were pelleted by adding formalin-fixed *Staphylococcus* A, washed and dissolved in NCS+ACS before liquid-scintillation counting.

Subcellular fractionation

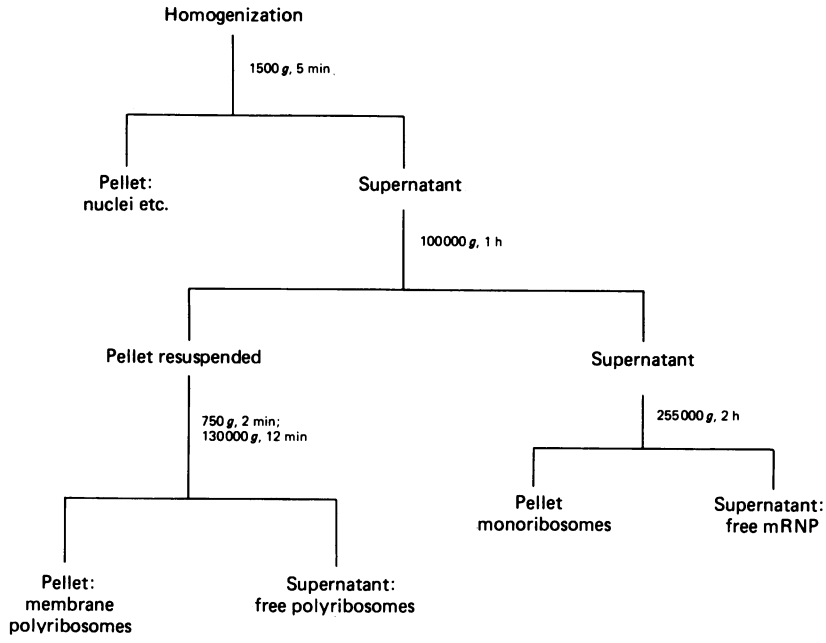
The procedure used for subcellular fractional was adopted from that of Bag & Sarkar (1975) and Ramsey & Steele (1976). After appropriate preincubation as described in Figure legends, 500–1000 islets were homogenized in 2 ml of the standard homogenization buffer (250 mM-sucrose, 250 mM-KCl, 10 mM-$MgCl_2$, 10 mM-Tris, pH 7.5, 1 mM-EDTA, 2 mM-dithiothreitol and 5–20 units of RNAsin/ml) with 12 strokes in a glass/Teflon-pestle Potter–Elvehjem homogenizer at 4 °C. Throughout the entire procedure, precautions were taken to avoid ribonuclease contamination, and the temperature was maintained at 4 °C. After an initial 5 min centrifugation at 1500 *g* to pellet nuclei, the supernatant was centrifuged for 1 h at 100000 *g* (Scheme 1). The pellet was resuspended with a Pasteur pipette and re-centrifuged for 2 min at 750 *g* and then for 12 min at 130000 *g*. This pellet (membrane-bound polyribosomes) contains insulin mRNA associated with membranes, whereas the supernatant contains free polyribosomes and some monoribosomes. The 100000 *g* supernatant was further centrifuged for 2 h at 255000 *g* to pellet most of the 80S monoribosomes. RNA was extracted from all fractions with phenol/chloroform/3-methylbutan-1-ol/2-mercaptoethanol (240:240:10:1, by vol.) equilibrated with 0.1 M-Tris/HCl, pH 7.5, and then ethanol-precipitated from the aqueous phase after addition of 30 μg yeast tRNA to ensure efficient precipitation of RNA.

Sucrose-gradient experiments

After homogenization of 500–1000 islets in 500 μl of homogenization buffer, the homogenate was placed on a linear 10–40% (w/v) sucrose gradient. The gradients were centrifuged at 30000 rev./min in an SW.60 rotor for 90 min at 20 °C and were stopped without braking. RNA was prepared from gradient fractions by phenol extraction, precipitated with ethanol and transferred to nitrocellulose sheets to quantify insulin mRNA by dot-blot analysis as described below. The linearity of the sucrose gradients was checked by refractometry.

Insulin mRNA and 7SL RNA quantification

Insulin mRNA and 7SL RNA were quantified by Northern-blot analysis (Thomas, 1980) or dot-blot analysis (White & Bancroft, 1982). The RNA precipitates were treated with 1 M-glyoxal and electrophoresed on 2%-agarose gels before transfer to nitrocellulose as described by Thomas (1980). Alternatively, glyoxylated samples were diluted with 4 parts of 20 × SSC and bound to nitrocellulose with a suction apparatus. The samples



Scheme 1. Protocol for the subcellular fractionation of islet homogenates

See the text for details. Abbreviation: RNP, ribonucleoprotein.

were prehybridized for 6–12 h (Thomas, 1980) at 42 °C and then hybridized at 42 °C with the appropriate nick-translated probe [rat insulin 1 cDNA insert separated from pRI-7 (Chan *et al.*, 1979) or the cDNA to the 7SL-RNA specific S fragment (Ullu *et al.*, 1982) cloned in pUC-8]. The hybridization solution (Thomas, 1980) also contained 200 μg of poly(A)/ml. After completion of hybridization, the filters were washed first in 2 × SSC/0.1% SDS (5 × 5 min) at room temperature and then in 0.1 × SSC/0.1% SDS (3 × 30 min) at 50 °C and then exposed at −70 °C to Kodak XAR-5 film with an intensifying screen. The appropriate labelled spots were cut out and counted for radioactivity by liquid scintillation. This assay was linear with added amounts of RNA in the range of interest, and there was no significant background hybridization of pBR-322 sequences with liver RNA or with 15 μg of tRNA. Recoveries were determined by removing samples for quantification of insulin mRNA or 7SL RNA immediately after homogenization.

Translation of islet extracts *in vitro*

Preincubated islets (500–1500) were homogenized in 150 μl of reaction buffer in a small glass–Teflon-pestle homogenizer at 4 °C (120 mM-potassium actate, 1.5 mM-magnesium acetate, 20 mM-Hepes, pH 7.5, 1 mM-dithiothreitol and 200 units of RNAsin/ml). After homogenization, 20% Triton X-100 was added to give a final 1% solution. Then 30 μl samples were transferred to tubes containing (final concns.) 3×10^5–6×10^5 c.p.m. of ^{125}I-Tyr-tRNA (Scherberg *et al.*, 1978), 0.1 mM-GTP, 0.8 mM-ATP, 10 mM-phosphocreatine and 30 μg of creatine kinase/ml. Incubations were carried out for various periods at 24 °C and were terminated by the addition of ribonuclease A (to 0.25 mg/ml; 15 min at 24 °C). After precipitation with 10% trichloroacetic acid and one wash with diethyl ether/ethanol (1:1, v/v), ^{125}I-Tyr incorporation into preproinsulin was detected after SDS/polyacrylamide-gel electrophoresis on gradient (20–25% acrylamide) slab gels (Laemmli, 1970) by radioautography and was quantified by densitometric scanning.

To demonstrate that the monoribosome fraction of islet homogenates contains SRP-blocked nascent preproinsulin, freshly isolated islets were homogenized in the subcellular fractionation buffer, and microsomes (microsomal fractions) were removed by centrifugation for 12 min at 130000 $g_{av.}$. The supernatant was centrifuged for 2 h at 255000 $g_{av.}$ to bring down the monoribosomes. The two pellets were each dissolved in 40 μl of the mixture for translation *in vitro* described above containing ^{125}I-Tyr-tRNA. These were then divided into two aliquots each, one serving as a control, and the other receiving SRP receptor (Gilmore *et al.*, 1982) (corresponding to about 5 μl of purified microsomes at 1 eq/μl) and were incubated for 10 min at 24 °C. Incorporation of ^{125}I was analysed by SDS/polyacrylamide-gel electrophoresis as described above.

Methodological considerations

To assess the validity of the subcellular-fractionation procedure, 200 islets were labelled overnight with [5,6-^{3}H]uridine (10 μCi/ml) in RPMI 1640, and the membrane-bound polyribosomal, free polyribosomal and monoribosomal fractions were separated as described above. The separated fractions were then each rehomogenized with 200 unlabelled islets and then again subjected to the same subcellular-fractionation technique. Of the trichloroacetic acid-precipitable [^{3}H]uridine initially found in the membrane polyribosomal fraction, 60% reappeared in this fraction, and the rest redistributed roughly equally among all the other fractions. For the monoribosomal fraction, 73% of the initial radioactivity reappeared in the monoribosome fraction, whereas the

initial radioactivity of the free polyribosomes was redistributed among the free polyribosomes, monoribosomes and the supernatant (14%, 35% and 29% respectively of total trichloroacetic acid-precipitable radioactivity). Thus, in presenting the data from subcellular-fractionation experiments, the results for the monoribosomal and free polyribosomal fractions were combined. The fractional distribution of 80 S rat liver monoribosomes suspended in homogenization buffer (as assessed by the A_{260}) after centrifugation at 100000 *g* was 36% in the pellet. When the supernatant was re-centrifuged at 255000 *g* for 2 h, 53% of the original monoribosomes were in the pellet.

Under the conditions used here for subcellular fractionations, 250 mM-K^+ was added to buffers to avoid non-specific binding of SRP and RNA to microsomes. A previous report (Walter & Blobel, 1983) indicated that significant amounts of SRP may be dissociated from microsomes when [K^+] is increased from 100 to 250 mM. Whether this fraction represents losses of specifically bound SRP is not clear. However, no differences in the intracellular distribution of 7SL RNA and insulin mRNA were observed when the above subcellular-separation procedure was performed in buffers containing 150 mM-K^+ instead of 250 mM.

RESULTS

Insulin biosynthesis

Fig. 1 shows the effects of increasing concentrations of glucose on islets (pro)insulin (*a*) and non-insulin protein (*b*) synthesis expressed as percentages of the rates of synthesis at 16.7 mM-glucose during a 1 h incubation period (○). This time was chosen to minimize interference caused by transcriptional effects of glucose. Even at a concentration as low as 3.3 mM, glucose exerted a significant degree of stimulation of both (pro)insulin and non-insulin islet-protein synthesis. These data are in agreement with previously published reports (reviewed by Permutt, 1981).

Previous studies have shown that low concentrations of cycloheximide selectively slow elongation, making it the rate-limiting step in protein synthesis (Lodish, 1971). Thus the rates of protein synthesis in the presence of low concentrations of cycloheximide are a function of the amounts of available mRNA rather than of the rates of initiation. Since the amount of insulin mRNA in rat islets is not affected by glucose in short incubations of 1 h or less (Itoh & Okamoto, 1980; M. Welsh, unpublished work), isolated islets incubated in the presence of cycloheximide should show the same rates of insulin biosynthesis at different glucose concentrations if glucose only regulates initiation of insulin-mRNA translation. Several concentrations of cycloheximide were tested for their effects on islet protein synthesis. The effect of 1.6 μg of cycloheximide/ml, ●, the highest concentration tested, on (pro)insulin and non-insulin protein synthesis in isolated rat islets is shown in Figs. 1(*a*) and 1(*b*) respectively. (Pro)insulin biosynthesis was stimulated by glucose in the concentration range 0–5.6 mM in the presence of cycloheximide, whereas non-insulin protein synthesis was not significantly affected by glucose. This finding suggests that glucose regulation of insulin synthesis between 0 and 5.6 mM mainly occurs via either stimulation of elongation or through recruitment of insulin mRNA from an untranslatable pool. It seems unlikely that the cycloheximide concentration selected for use in these studies was insufficient to make elongation rate-limiting for insulin biosynthesis, since the overall

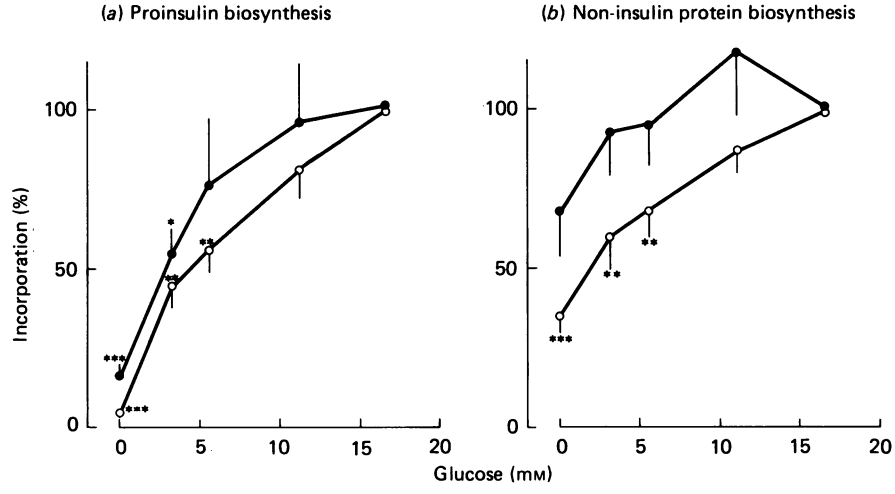


Fig. 1. Effects of glucose on (pro)insulin (*a*) and non-insulin protein (*b*) synthesis in the absence (○) or presence (●) of cycloheximide (1.6 μg/ml)

Freshly isolated islets (20) were incubated at the given glucose conentrations for 60 min at 37 °C in a Hanks buffer containing 10 μCi of L-[4,5-^{3}H]leucine/100 μl and 0.5 mg of bovine serum albumin/ml. Incorporation of [^{3}H]leucine into proinsulin was determined by immunoprecipitation as described in the Experimental section; non-insulin protein synthesis was estimated as tricholoroacetic acid-insoluble radioactivity after subtraction of the proinsulin radioactivity. All values (means ± S.E.M. for four to six experiments) are expressed as percentages of incorporation in the presence of 16.7 mM-glucose. [^{3}H]Leucine incorporation in the absence of cycloheximide was 2212 ± 200 d.p.m./h per islet for proinsulin and 10990 ± 2116 d.p.m./h per islet for total protein synthesis. In the presence of cycloheximide (1.6 μg/ml) these values were 295 ± 128 and 1459 ± 391 d.p.m./h per islet respectively. *, ** and *** denote $P < 0.05$, < 0.01 and < 0.001 respectively with a paired *t* test.

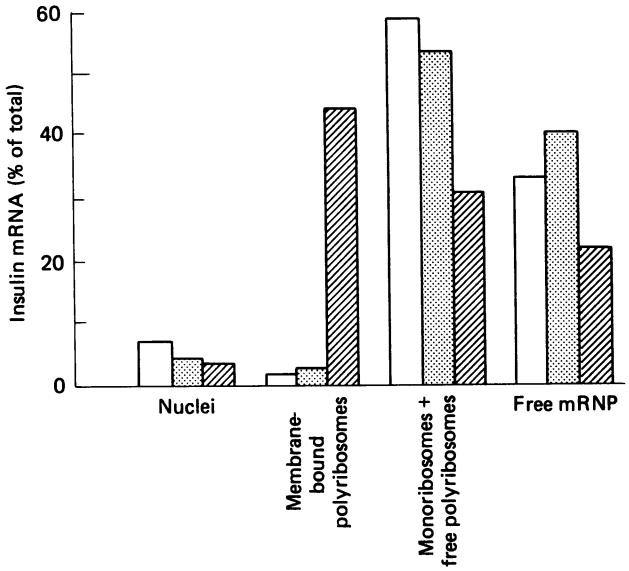


Fig. 2. Effect of glucose on the subcellular distribution of insulin mRNA

Freshly isolated islets were preincubated for 60 min at (□) 0, (▨) 3.3 mM- and (■) 16.7 mM-glucose, after which they were homogenized. Subcellular fractionation of the homogenate was carried out as in Scheme 1 and insulin mRNA was quantified after dot-blotting and hybridization to ^{32}P-labelled insulin cDNA as described in the Experimental section. The values in the Figure are the radioactivity (c.p.m.) in each fraction as a percentage of the total recovered for each homogenate. The recoveries were 53 (0 mM), 100 (3.3 mM) and 62% (16.7 mM) for each set of islets in this experiment. To assess the degree of degradation of insulin mRNA, the samples were occasionally subjected to Northern blot analysis. A band of about 600 nucleotides was seen without any high-M_r precursors. Although the degree of degradation of insulin mRNA varied between the different subcellular-fractionation experiments, the degree of degradation of free and ribosome-bound (both microsomal and free) insulin mRNA was always similar in each subcellular-fractionation experiment, thus not altering the relative proportionality of these fractions.

inhibition of both insulin and total islet protein synthesis was comparable (> 85%).

Effects of glucose on the subcellular distribution of insulin mRNA

Typically rat islets each contain about 0.02 ng of insulin mRNA, as quantified with rat insulin cDNA. Fig. 2 shows the effects of an incubation for 60 min with 0, 3.3 mM-and 16.7 mM-glucose on the distribution of insulin mRNA in the islets in a typical experiment. The nuclear fraction, which also probably contained nucleus-associated rough ER and some unbroken cells, contained only small amounts of insulin mRNA under all conditions. Glucose at 16.7 mM caused both a decrease in free cytoplasmic insulin mRNA, suggesting that it affects insulin-mRNA initiation, and a shift of ribosomal-bound insulin mRNA from the monoribosome and free-polyribosome fractions to the membrane-bound polyribosome fractions. The latter finding is consistent with the hypothesis that an SRP-related mechanism may control insulin-mRNA translation, i.e. that at low glucose SRP-induced arrest of insulin synthesis is more stable than at high glucose. No significant alteration in mRNA distribution was detected on addition of glucose at 3.3 mM. This finding is also consistent with the interpretation that glucose selectively stimulates elongation rates of preproinsulin in this glucose concentration range (0–5.6 mM).

Although changes described above in the distribution of insulin mRNA in response to glucose could be consistently obtained, the effect was variable in magnitude from experiment to experiment. To achieve greater reproducibility in the response, islets were first cultured for 24 h after isolation, a procedure known to stabilize and enhance their protein-synthetic capability (Andersson, 1978), and were then incubated for 90 min in 3.3 mM- or 16.7 mM-glucose before fractionation. The effects of high glucose concentration on intracellular distribution of insulin mRNA in these experiments are summarized in Table 1. As in freshly isolated islets (Fig. 2), glucose decreased the amount of free cytosolic insulin mRNA and increased the insulin mRNA found in the microsomal pellet. No consistent effects of preincubation with glucose

Table 1. Effects of glucose on islet insulin mRNA and 7SL RNA distribution

The subcellular distribution of insulin mRNA and 7SL RNA was determined by dot-blot hybridization after preincubation of islets at 3.3 mM- or 16.7 mM-glucose for 90 min at 37 °C, followed by homogenization and subcellular fractionation. The values given represent the percentages of total recovered RNA given in each fraction as means ± S.E.M. for the numbers of observations in parentheses: [a] denotes $P < 0.01$ with a regular t test and [b] denotes $P < 0.05$ with a paired t test when values are compared with those at 3.3 mM-glucose.

	Insulin mRNA (%)		7SL RNA (%)	
Glucose concn. ...	3.3 mM	16.7 mM	3.3 mM	16.7 mM
A. Nuclei	18.2 ± 4.1 (5)	26.9 ± 4.0 (7)	15.6 ± 3.9 (6)	13.0 ± 1.7 (5)
B. Membrane polyribosomes	14.7 ± 1.0 (5)	32.2 ± 3.3[a] (7)	10.8 ± 3.5 (6)	17.1 ± 3.9[b] (5)
C. Free polyribosomes and monoribosomes	42.0 ± 2.5 (4)	30.0 ± 5.8[b] (6)	48.0 ± 4.3 (6)	48.3 ± 3.2 (5)
D. Free RNA	22.9 ± 4.9 (4)	5.4 ± 1.1[a] (6)	22.3 ± 2.4 (5)	21.5 ± 2.8 (5)
E. B/C ratio	0.36 ± 0.03 (4)	1.73 ± 0.66[b] (6)	0.23 ± 0.08 (6)	0.38 ± 0.11[b] (5)
F. Recovery (%)	47 ± 8 (4)	65 ± 9 (6)	57 ± 14 (5)	82 ± 14 (5)

were found on the relative distribution of insulin mRNA sedimenting in the free-polyribosome and monoribosome fractions (results not shown). The cultured islets appeared to contain relatively less cytoplasmic insulin mRNA than did freshly isolated islets, possibly owing to the relatively high glucose concentration (11 mM) in the culture medium.

Effects of glucose on 7SL RNA distribution

The islet cells were found to contain 1.5–3 times as many copies of 7SL RNA as of insulin mRNA, as estimated by comparison of dot-blots or Northern analyses standardized with known amounts of 7SL RNA from an SRP preparation (Walter & Blobel, 1981). Assuming that SRP is the major reservoir of 7SL RNA in islet β-cells, it should be available in amounts roughly equal to or somewhat greater than the total rough-ER-associated mRNA. The subcellular distribution of 7SL RNA in the presence of 3.3 mM-glucose was similar to that of insulin mRNA (Table 1), although there was relatively less of it bound to the membrane fraction. Glucose induced the same changes in the distribution of 7SL RNA from free cytoplasmic ribosome-bound forms to membrane-bound forms as it did for insulin mRNA. However, the free cytoplasmic fraction was left unchanged by glucose, consistent with the possible involvement of SRP in the synthesis of other proteins and/or its rapid recycling once insulin-synthesizing polyribosomes have engaged the ER membrane (Gilmore & Blobel, 1983).

Sucrose-gradient analysis of islet homogenates

Fig. 3(*a*) shows the effect of incubation in high or low glucose on insulin-mRNA distribution during zonal sucrose-gradient centrifugation of islet homogenates. Slowly sedimenting insulin mRNA (fractions 1–4) was considerably more abundant when the islets had been incubated at 3.3 mM-glucose than at 16.7 mM. A peak appearing at fraction 6 corresponding to monoribosomes was relatively unchanged by preincubation with glucose, whereas the rapidly sedimenting microsomes at the bottom of the gradient (also containing nuclei) contained more insulin mRNA after preincubation in 16.7 mM-glucose. These data thus support those obtained in the subcellular-fractionation experiments.

To rule out the possibility that the increase in insulin mRNA binding to microsomes at the higher glucose concentration results from increased ribosome loading per molecule of insulin mRNA because of more rapid initiation rates, islets were preincubated in high glucose and then homogenized with or without 1% Triton X-100 to dissolve microsomal membranes. These homogenates were analysed on 10–40% sucrose gradients, and the sedimentation profile of insulin mRNA was determined (Fig. 3*b*). As in Fig. 3(*a*), some free insulin mRNA was found at the top of the gradient. A prominent monoribosome peak as well as a peak of insulin mRNA sedimenting at 120–160 S (presumably small polyribosomes) was seen for islets homogenized in either the absence or the presence of 1% Triton X-100. Since the

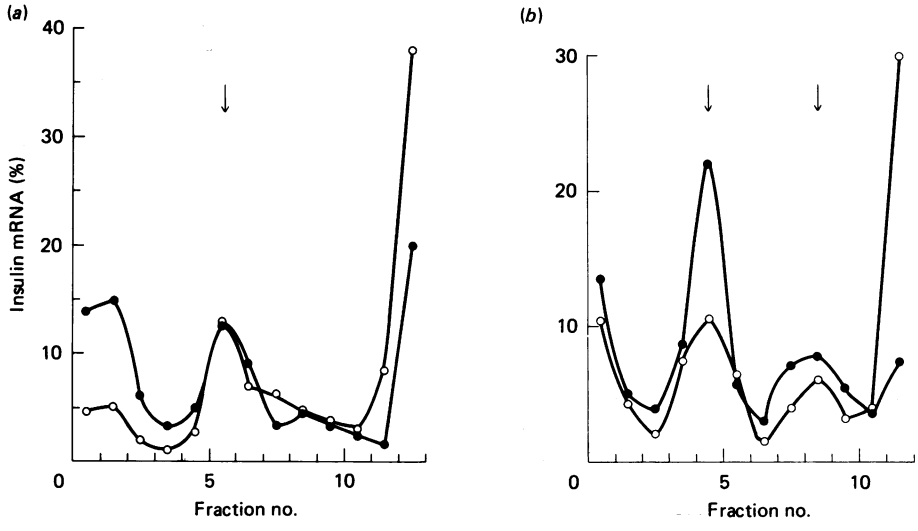


Fig. 3. Sucrose gradient analysis of insulin mRNA in islet homogenates

Freshly isolated islets were preincubated for 60 min at (●) 3.3 mM- or (○) 16.7 mM-glucose and then homogenized (*a*). The homogenate was placed on a 3 ml 10–40% sucrose gradient above a 0.4 ml 45% sucrose step, and centrifuged for 90 min at 30000 rev./min in a SW 60 rotor. Fractions (0.3 ml) were carefully collected, phenol-extracted, ethanol-precipitated, dot-blotted and hybridized to ^{32}P-labelled insulin cDNA. The percentage of total recovered insulin mRNA is shown for each fraction. The arrow indicates the position of 80 S monoribosomes. (*b*) Precultured islets were incubated for 60 min at 16.7 mM-glucose. Half of the islets were homogenized in 600 μl of the regular homogenization buffer (○), and the other half (●) were homogenized in the presence of 1% Triton X-100. The homogenates were centrifuged for a few seconds at 15600 *g* to remove nuclei, and the supernatants were then placed on 3.2 ml 10–40% sucrose gradients. After centrifugation as in (*a*), the fractions were collected with an ISCO gradient collector with upward flow. Percentages of recovered insulin mRNA in each fraction was determined as in (*a*). Means of two experiments are shown. The left arrow indicates the position of the monoribosome peak, and the right arrow denotes 150 S. Sedimentation is from left to right.

ratio of the peaks representing monoribosomes and small polyribosomes was essentially the same for both sets of islets, it can be concluded that rough-ER-bound insulin mRNA in glucose-stimulated islets is not present predominantly in the form of small polyribosomes.

Translation *in vitro* with islet homogenates

To obtain data bearing directly on the question of translational control of insulin synthesis, freshly isolated islets were preincubated at low or high glucose concentrations, disrupted and immediately incubated in a reaction mixture containing ^{125}I-Tyr-tRNA. Incorporation of ^{125}I into preproinsulin was dependent on the presence of phosphocreatine (results not shown), but was not inhibited (Fig. 4, lanes *c*, *d*, *g*, *h*) by concentrations of edeine (0.1 mM) sufficient to block synthesis in the reticulocyte-lysate or wheat-germ translation systems. Thus under these conditions preproinsulin synthesis was limited to run-off of pre-initiated polyribosomes. The incorporation of ^{125}I into preproinsulin was barely detectable at 2 min, and reached a maximum at 10 min of incubation (results not shown) regardless of the concentration of glucose to which the islets had been exposed during the preincubation. Preincubation of islets at a high glucose concentration for 90 min enhanced the incorporation of ^{125}I into preproinsulin (Fig. 4, lane *a* versus lane *e*). The magnitude of the enhancement of incorporation by glucose varied from 2- to 5-fold.

Although consistent with previous results showing increased incorporation of insulin mRNA into polyribosomes, the glucose stimulation of preproinsulin synthesis might alternatively reflect acceleration of association of the SRP initiation complex with SRP receptor. To test this possibility, islet homogenates were incubated as before but with the further addition of purified SRP receptor (Fig. 4). When 2 or 5 μl of a solution of SRP receptor was added during incubation of islet homogenates with ^{125}I-Tyr-tRNA, the incorporation of ^{125}I into preproinsulin was increased (Fig. 4). Addition of SRP receptor to reaction mixtures increased the incorporation of ^{125}I into preproinsulin by about 40% (as assessed by densitometer scanning) with islets preincubated at low glucose concentration, whereas islets preincubated in high glucose more than doubled their radioactivity incorporation on addition of SRP receptor. Addition of SRP to the islet homogenates did not affect incorporation into preproinsulin. Thus much of the labelling of preproinsulin occurring during these incubations may reflect that fraction of initiated insulin mRNA that has already interacted with SRP receptors. The lower degree of stimulation by addition of SRP receptor after preincubation of islets in low glucose, in contrast with our above observations that more insulin mRNA co-sedimented with SRP in cytosolic ribosome fractions under these conditions and was thus presumably in an SRP-arrested form, may indicate that SRP is chemically altered so as to be less able to interact with its receptor in β-cells exposed to low glucose.

The addition of purified SRP receptor to free polyribosome and monoribosome fractions prepared from freshly isolated islets enhanced the incorporation of ^{125}I-tyrosine into preproinsulin several-fold (Table 2). In the same experiment the incorporation of ^{125}I into preproinsulin in the microsome fraction was not quite doubled by addition of SRP receptor, although ^{125}I incorporation into preproinsulin was greater in this fraction than in the free polyribosome/monoribosome fraction. These data suggest that in normal (freshly isolated) islets the free monoribosomes contain relatively more SRP-blocked nascent preproinsulin than do the microsomes.

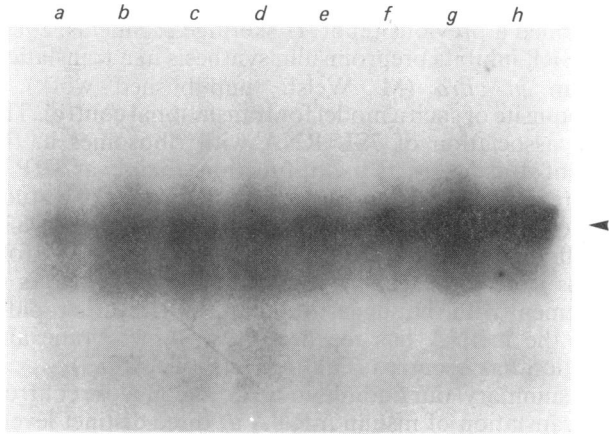


Fig. 4. Translation of islet homogenates *in vitro* with ^{125}I-Tyr-tRNA

Freshly isolated islets preincubated for 90 min at 3.3 mM-(lanes *a–d*) or 16.7 mM-glucose (lanes *e–h*) were homogenized and incubated for 10 min at 24 °C with ^{125}I-Tyr-tRNA as described in the Experimental section. Incubations shown in lanes *c*, *d* and *g*, *h* also contained purified SRP receptor corresponding to 5 eq of microsomes each (Gilmore *et al.*, 1982), whereas the incubations shown in lanes *b* and *f* contained SRP receptor corresponding to 2 eq of microsomes each. In lanes *d* and *h* 0.1 mM-edeine was also added to the incubation mixture. The samples were treated with ribonuclease, precipitated with trichloroacetic acid, washed with ether/ethanol and analysed by SDS/polyacrylamide-gel electrophoresis. The arrow indicates the position of preproinsulin as determined by immunoprecipitation and parallel electrophoresis of M_r markers. Occasionally some proinsulin-like material was seen migrating below the preproinsulin band. The trichloroacetic acid-precipitable radioactivities (c.p.m.) were 1795, 2301, 2086, 1626, 1869, 2378, 2093 and 2170 in lanes *a–h* respectively.

Table 2. Effects of SRP receptor on the incorporation of ^{125}I into preproinsulin in islet membrane-bound polyribosomes or free polyribosomes–monoribosomes

The two fractions were prepared and incubated with ^{125}I-Tyr-tRNA as described in the Experimental section. The products of the incubation were analysed on SDS/polyacrylamide-gel electrophoresis as described in the Experimental section, and the incorporation of ^{125}I into preproinsulin was assessed by densitometric scanning and is given in arbitrary units.

SRP receptor	Membrane-bound polyribosomes	Free polyribosomes–monoribosomes
–	1.26	0.04
+	2.15	0.26

DISCUSSION

Our results indicate that in isolated islets glucose influences insulin-mRNA translation through a combination of effects on both insulin-mRNA initiation and release of SRP-mediated arrest of preproinsulin translation and/or SRP-receptor interaction. Furthermore, it is possible that glucose below 5.6 mM specifically stimulated the elongation of nascent preproinsulin as assessed in experiments estimating rates of insulin synthesis after addition of low doses of cycloheximide. However, this experimental approach does not exclude the possibility that glucose also recruits insulin mRNA from some kind of inactive pool. However, the finding that glucose below 3.3 mM did not affect the subcellular distribution of insulin mRNA, despite marked changes in insulin-synthetic rates, argues in favour of effects on proinsulin elongation rates. It is of interest that these effects closely parallel glucose-induced changes in the islet energy state (Malaisse *et al.*, 1979; Hellman *et al.*, 1969). Thus, at these rather low concentrations glucose elicits a specific increase in islet ATP content that is not mimicked by the addition of many other non-stimulatory nutrients (Ashcroft *et al.*, 1973). An effect of ATP concentration might become manifest at several steps in peptide-bond formation. However, such effects would not be specific for insulin synthesis unless linked to translocation.

Glucose regulation of mRNA initiation

Effects of glucose on islet initiation rates regulate both insulin and total islet protein synthesis. This effect occurs between 3.3 mM- and 16.7 mM-glucose for insulin mRNA, as indicated by our observed changes in the distribution of insulin mRNA in subcellular fractions as well as by the effects of addition of cycloheximide on insulin and total protein synthesis in whole islets. It appears that alteration in initiation rates is a relatively non-specific response to glucose that accounts for a major portion of glucose effects on total islet protein synthesis. However, it may also have some degree of specificity of insulin mRNA in that initiation efficiency varies considerably between different mRNAs. Thus relatively minor changes in intracellular conditions (e.g. ion concentration etc.) might considerably alter the rates of initiation for a particular abundant cellular mRNA (Lodish, 1971; Lomedico & Saunders, 1977). It also has been shown that both free thiols (Jagus & Safer, 1981) and glucose phosphates (Jackson *et al.*, 1983) affect the reticulocyte translation machinery by affecting the phosphorylation of initiation factor eIF-2α. It is possible that similar mechanisms operate in islets, since both thiol (Anjaneyulu *et al.*, 1982) and glucose phosphate (Ashcroft *et al.*, 1972) concentrations are increased by glucose. However, our attempts to demonstrate phosphorylation of eIF-2α in isolated islets after partially purifying eIF-2 or eIF2–eIF2B complexes as described previously (see Towle *et al.*, 1984; Konieczny & Safer, 1983) have been unsuccessful thus far (M. Welsh, unpublished work).

SRP-mediated control of insulin translation

Glucose induced a shift of already-initiated (i.e. ribosome-bound) insulin mRNA from cytoplasmic to membrane-bound forms. Stimulation of elongation rates by glucose would rather decrease the ratio of membrane-bound/free polyribosomal forms of initiated insulin mRNA, and thus the observed increase in the membrane-associated insulin mRNA is distinct from stimulation of elongation. Moreover, it could be argued that larger polyribosomes may be associated more strongly with the ER membranes than are monoribosomes, and thus increased rates of initiation could explain the observed effect. However, we were able to show that the ER-associated polyribosomes were not larger than those free in the cytoplasm at high glucose concentrations, thus excluding enhanced initiation as the explanation for the increased association of insulin mRNA with rough ER. The incorporation of ^{125}I-tyrosine from its cognate tRNA into preproinsulin in islet homogenates was preferentially increased by adding purified SRP receptor after pre-incubation of islets at high rather than low glucose, despite the fact that less initiated insulin mRNA may be available in an SRP-blocked form under conditions of high glucose.

The above findings are in accord with a model for SRP-mediated control of translation in which it is proposed that the time from the induction of SRP arrest until its release by interaction with SRP receptor on the rough ER may be differentially regulated in islets. This mode of control is consistent with our observations that more SRP–7SL RNA was directed towards the microsomes along with insulin mRNA at high glucose concentrations and might be achieved via some form of chemical modification of SRP, or its receptor. We have confirmed a previous report (Eskeridge & Shields, 1983) that SRP inhibits preproinsulin synthesis in a translation system *in vitro* (M. Welsh, unpublished work), a prerequisite of such a model for translational control. The close association of 7SL RNA with ribosomes in the present study suggests that it functions mainly as SRP in pancreatic islets, in agreement with the results of a study on dog pancreas microsomes (Walter & Blobel, 1983). The finding that SRP RNA is present in roughly 2-fold excess relative to insulin mRNA in the islets also is in agreement with the suggestion that SRP recycles rapidly once the mRNA has reached the ER membrane and insertion has occurred (Gilmore & Blobel, 1983).

In summary, our findings indicate that glucose controls the translation of insulin mRNA at three distinct levels. Such a multifaceted translational control mechanism is appropriate, considering the vital importance of insulin for the organism and especially the need to be able to respond promptly to a glucose challenge with increased production of insulin. Operation of these three control mechanisms in an integrated fashion can give rise to a 20-fold increase in insulin biosynthesis in response to glucose. Moreover, if one of these mechanisms becomes defective through disease or mutation, the β-cell will retain considerable capacity to synthesize insulin and may thus be able to compensate under normal conditions. However, under conditions associated with increased demand for insulin, failure of one of these translational control mechanisms could contribute to the pathophysiology of diabetes.

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REFERENCES

Anderson, D. J., Mostov, K. E. & Blobel, G. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 7249–7253
Andersson, A. (1978) Diabetologia **14**, 397–404
Anjaneyulu, K., Anjaneyulu, R., Sener, A. & Malaisse, W. J. (1982) Biochimie **64**, 29–36
Ashcroft, S. J. H., Weerasinghe, L. C. C., Bassett, J. M. & Randle, P. J. (1972) Biochem. J. **126**, 525–532
Ashcroft, S. J. H., Weerasinghe, L. C. C., Bassett, J. M. & Randle, P. J. (1973) Biochem. J. **132**, 223–231
Ashcroft, S. J. H., Bunce, J., Lowry, M., Hansen, S. E. & Hedeskov, C. J. (1978) Biochem. J. **174**, 517–526
Bag, J. & Sarkar, S. (1975) Biochemistry **14**, 3800–3807
Chan, S. J., Noyes, B. E., Agarwal, K. L. & Steiner, D. F. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 5036–5040
Erickson, A. J., Walter, P. & Blobel, G. (1983) Biochem. Biophys. Res. Commun. **115**, 275–280
Eskeridge, E. M. & Shields, D. (1983) J. Biol. Chem. **258**, 11487–11491
Gilmore, R. & Blobel, G. (1983) Cell **35**, 677–685
Gilmore, R., Walter, P. & Blobel, G. (1982) J. Cell Biol. **95**, 470–477
Hedeskov, C. J. (1980) Physiol. Rev. **60**, 442–509
Hellman, B., Idahl, L.-Å. & Danielsson, Å. (1969) Diabetes **18**, 509–516
Howell, S. L. & Taylor, K. W. (1966) Biochim. Biophys. Acta **130**, 521–524
Itoh, N. & Okamoto, H. (1980) Nature (London) **283**, 100–102
Jackson, R. J., Herbert, P., Campbell, E. A. & Hunt, T. (1983) Eur. J. Biochem. **131**, 313–324
Jagus, R. & Safer, B. (1981) J. Biol. Chem. **256**, 1317–1323
Konieczny, A. & Safer, B. (1983) J. Biol. Chem. **258**, 3402–3408
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Lernmark, Å., Nathans, A. & Steiner, D. F. (1976) J. Cell Biol. **71**, 606–623
Lodish, H. F. (1971) J. Biol. Chem. **246**, 7131–7138
Lomedico, P. T. & Saunders, G. F. (1977) Science **198**, 620–622
Malaisse, W. J., Hutton, J. C., Kawazu, S., Herchuelz, A., Valverde, I. & Sener, A. (1979) Diabetologia **16**, 331–341
Meyer, D. I., Krause, E. & Dobberstein, B. (1982) Nature (London) **297**, 647–650
Permutt, M. A. (1981) in Islets of Langerhans (Cooperstein, S. & Watkings, D. T., eds.), pp. 75–95, Academic Press, New York
Permutt, M. A. & Kipnis, D. M. (1972*a*) J. Biol. Chem. **247**, 1194–1199
Permutt, M. A. & Kipnis, D. M. (1972*b*) Proc. Natl. Acad. Sci. U.S.A. **69**, 505–509
Ramsey, J. C. & Steele, W. J. (1976) Biochemistry **15**, 1704–1712
Scherberg, N., Seo, H. & Hynes, R. (1978) J. Biol. Chem. **253**, 1773–1779
Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 5201–5205
Towle, C. A., Mankin, H. J., Arruch, J. & Treadwell, B. V. (1984) Biochem. Biophys. Res. Commun. **121**, 134–140
Ullu, E., Murphy, S. & Melli, M. (1982) Cell **29**, 195–202
Walter, P. & Blobel, G. (1981) J. Cell Biol. **91**, 557–561
Walter, P. & Blobel, G. (1983) J. Cell Biol. **97**, 1693–1699
White, B. A. & Bancroft, F. C. (1982) J. Biol. Chem. **257**, 8569–8572

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