Journal of Cellular Biochemistry 20:29–39 (1982)
Evolution of Hormone-Receptor Systems 93–103

Insulin Binding Sites on the Nuclear Envelope: Potential Relationship to mRNA Metabolism

I.D. Goldfine, F. Purrello, G.A. Clawson, and R. Vigneri

Cell Biology Research Laboratory, Harold Brunn Institute, Mount Zion Hospital and Medical Center, San Francisco, California 94120 (I.D.G., F.P., R.V.), and Departments of Medicine (I.D.G.), Physiology (I.D.G., F.P., R.V.) and Pathology (G.A.C.), University of California, San Francisco, California 94143

Insulin regulates the growth and metabolism of most tissues. The hormonal potency of insulin results, to a large extent, from its ability to regulate target cells at a variety of subcellular sites. For many years, the effects of insulin on membrane transport, enzyme activity, and protein synthesis have been studied extensively. Less attention, however, was given to how insulin regulates nuclear functions. Recently the presence of specific binding sites for insulin on nuclei and nuclear envelopes have been documented and characterized. These binding sites have biochemical characteristics that are different from insulin binding sites on the plasma membrane. Moreover, direct in vitro effects of insulin on messenger RNA (mRNA) metabolism have recently been reported. These effects include the stimulation of mRNA efflux from intact nuclei, and stimulation of nucleoside triphosphatase activity (NTPase), the enzyme that regulates mRNA efflux. Thus, significant insight is now being gained concerning the action of insulin on the cell nucleus.

ACTIONS OF INSULIN ON DNA AND RNA SYNTHESIS

Insulin in vitro is known to stimulate the growth of cells in tissue culture [1]. In most studies, however, higher than physiological concentrations of insulin are necessary for this effect [2,3] and it is likely that in many instances insulin is interacting with receptors for the varius insulin-like growth factors, such as somatomedin, MSA, or nonsuppressible insulin-like activity (NSILA-s) [2–4]. In a few instances, however, physiological concentrations of insulin increase cell division. It has been shown that insulin is necessary for the regeneration of liver that follows partial hepatectomy in rats and other animals [5,6]. Also, in cultured cells from regenerating rat liver, there is evidence indicating that insulin stimulates DNA synthesis [7]. Finally, physiological concentrations of insulin stimulte DNA synthesis in H35 hepatoma cells [8].

Received June 29, 1982; revised and accepted August 17, 1982.

0730-2312/82/2001-0029$03.50

Insulin regulates RNA levels in many tissues. It is possible that insulin has multiple effects on RNA metabolism, including the regulation of transcriptional and posttranscriptional events. Effects of insulin on transcription have been reported in liver, pancreas, adipose tissue, and mammary gland. Insulin in vivo increases both RNA polymerase activity and template activity of liver [9,10] (Table I). Inhibition of RNA synthesis by actinomycin has been reported to block the insulin-stimulated synthesis of several enzymes in diabetic rats, including fatty acid synthetase, glycogen synthetase, hexokinase, phosphofructokinase, and pyruvate kinase [9,11–13]. In liver of diabetic rats, insulin administration inhibits the activities of glucose-6-phosphatase, fructose-1, 6-diphosphatase, pyruvate carboxylase, and phosphoenolpyruvate carboxykinase; actinomycin D pretreatment of animals also blocks this inhibition [9,11–13].

Insulin also influences mRNA levels both in vivo and in vitro. Recent studies have indicated that the production of mRNA for tyrosine aminotransferase [14] is decreased in the liver of the adrenalectomized rat, and albumin [15], fatty acid synthetase [16], and α_{2u} globulin [17] are decreased in the liver of diabetic rats. These diminished levels of mRNA can be restored by insulin administration in vivo. In the adrenalectomized rat, insulin causes a generalized increase in mRNA content [14].

Administration of insulin to diabetic rats both restores diminished pancreatic acinar cell amylase levels [18,19] and reduces increased trypsinogen levels; actinomycin treatment blocks this effect [19]. Further, amylase mRNA levels fall dramatically in the pancreas of diabetic rats and this fall is rapidly reversed by insulin administration [20] (Fig. 1).

In adipose tissue, insulin in vivo has been shown to stimulate the synthesis of hexokinase II via the formation of new RNA [11,21,22]. Moreover, insulin stimulates the activity of glucose-6-P-dehydrogenase, phosphofructose kinase, and pyruvate kinase [11,21,22]; these effects are blocked by the administration of actinomycin D. It has been reported that insulin in vitro increases lipoprotein lipase activity in 3T3-L_1 fibroblasts [23] in part by nuclear regulation.

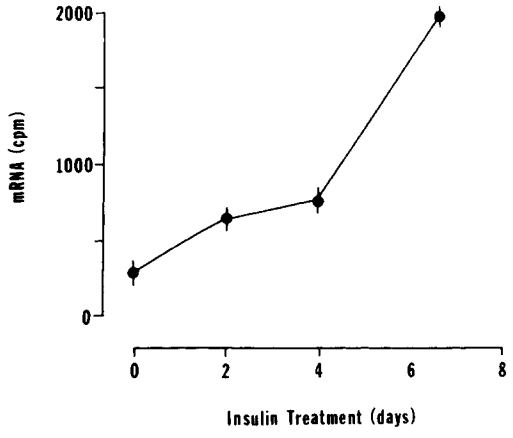

Fig. 1. Effect of insulin injections on pancreatic amylase mRNA levels from diabetic rats. Adapted from Korc et al [20].

In mammary glands, insulin in vitro activates RNA synthesis, stimulates RNA polymerase activity, and stimulates the phosphorylation of histone and nonhistone proteins [24–27] and increases mRNA levels [28].

BINDING SITES FOR INSULIN ON NUCLEAR ENVELOPES AND PLASMA MEMBRANES

Specific binding sites for insulin on purified liver plasma membranes were first demonstrated by Freychet et al [29] and have now been described in many cell types [30]. The major characteristics of insulin binding to these receptors are listed in Table II. In addition to these cell surface binding sites, other insulin binding sites have also been described on intracellular structures, including nuclei [31–36] and nuclear membranes [37–39], smooth and rough endoplasmic recticulum [31,34], and Golgi apparatus [40].

Specific binding sites for insulin on purified rat liver nuclei free of other cellular components were first described by Horvat and coworkers [31] and then confirmed both in our laboratory [32,34] and that of Goidl [33]. In addition, specific nuclear binding sites have been detected in thyroid nuclei [35,36]. The major site of insulin binding to the nucleus is the nuclear envelope [37–39]. When whole nuclei are incubated with native insulin followed by an immunofluorescence procedure, fluorescence is detected only on the nuclear surface [38]. Further, when nuclei are first incubated with ^{125}I-labeled insulin and then subfractionated, most of the specific hormone binding is seen with the nuclear membrane fractions [37]. In addition, when nuclei are incubated with high concentrations of detergent to remove both layers of the nuclear envelope, binding is either reduced or eliminated. Finally, insulin does not bind directly to DNA or histones, but does bind directly to purified nuclear membranes [37,38] (Figs. 2–4).

TABLE I. Influence of Insulin on mRNA Levels in Liver and Other tissues [14–17,20,28]

Liver
1. albumin
2. TAT (tyrosine aminotransferase)
3. fatty acid synthetase
4. α_{2u} globulin

Pancreas
1. amylase

Mammary gland
1. casein

TABLE II. Characteristics of Insulin Binding to Cellular Membranes

	Plasma	Nuclear
High salt optimum	yes	no
Alkaline pH optimum	yes	no
Negative cooperativity	yes	no
Antireceptor antibody	yes	no
Low temperature stability	yes	no
Insulin degradation	yes	no
Regulation by exogenous insulin	yes	yes

The binding of insulin to nuclear membranes, like its binding to plasma membranes, fulfills the requirements of a hormone receptor. It is rapid, reversible, of high affinity, and hormone-specific (Fig. 2). Two insulin analogues with decreased biological potencies, proinsulin and desoctapeptide insulin, were less effective in both nuclear and plasma membranes. The characteristics of insulin binding to the nuclear membrane differ, however, in a number of respects from the characteristics of insulin binding to plasma membrane (Table I). Studies of insulin binding to liver plasma membranes have revealed two classes of binding sites [30]. In our studies of insulin binding sites to nuclear membranes prepared by the methods of Kashnig and Kasper [41], two orders of binding sites were seen but with lower affinities (K_d 5.6 nM, 65 nM) than that seen on the plasma membrane (K_d 0.5 nM, 10 nM) (Fig. 3). Both plasma membranes and nuclear membranes, however, have similar total insulin binding capacities (ca. 2 nmol/mg protein). Horvat [38], studying nuclear membranes

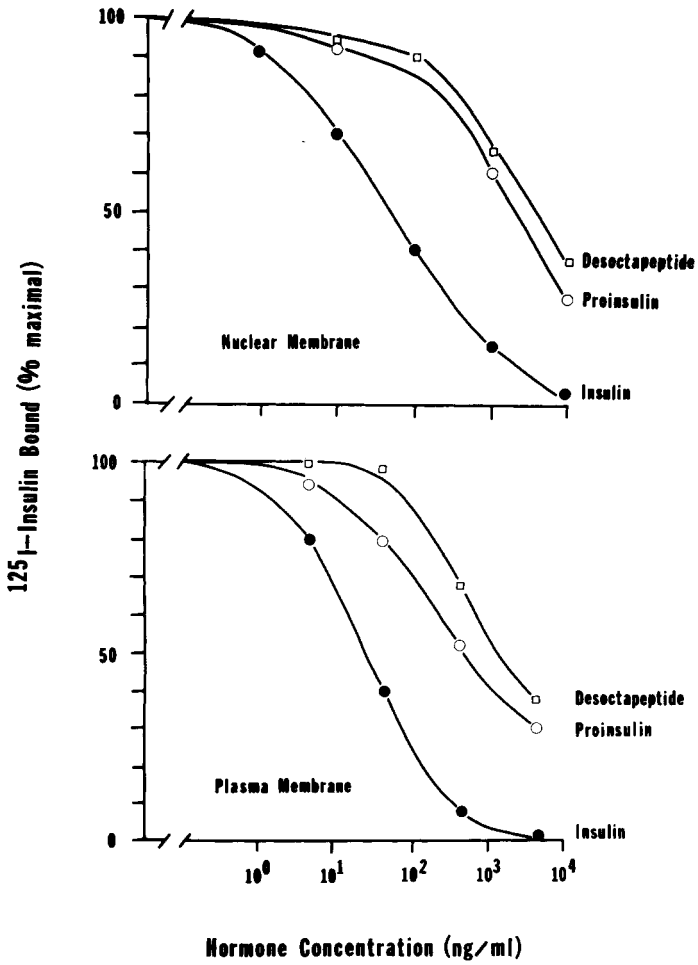

Fig. 2. Inhibition of ^{125}I-insulin binding to nuclear (top) and plasma membranes (bottom) by native insulin, proinsulin, and desoctapeptide insulin.

prepared by RNAase and DNAase digestion, reported only one class of binding sites for insulin on nuclear membranes having a K_d of 3 nm. In liver and other tissues, the binding of insulin to plasma membranes has three distinctive characteristics [30,37,38]: a sharp pH optimum of 8.0, enhanced binding in the presence of high concentrations of NaCl, and an enhanced dissociation of labeled insulin in the presence of unlabeled insulin which may be due to negative cooperativity. When the characteristics of insulin binding in nuclear membranes were examined, we found that the pH optimum was between 7.0 and 7.5, that there was no enhanced binding in the presence of NaCl, and that addition of unlabeled insulin did not enhance the dissociation rate of labeled insulin (Fig. 4). Moreover, Horvat did not find any indication of negative cooperativity of insulin binding to nuclear membranes [38].

In the serum of patients with severe insulin resistance and acanthosis nigricans, there are antibodies to the plasma membrane insulin receptor, and preincubation of plasma membranes with these antibodies blocks the subsequent binding of insulin (Fig. 5) [39]. This inhibition of binding by these antibodies can be demonstrated with insulin receptors from a variety of species and tissues. These antibodies, however, do

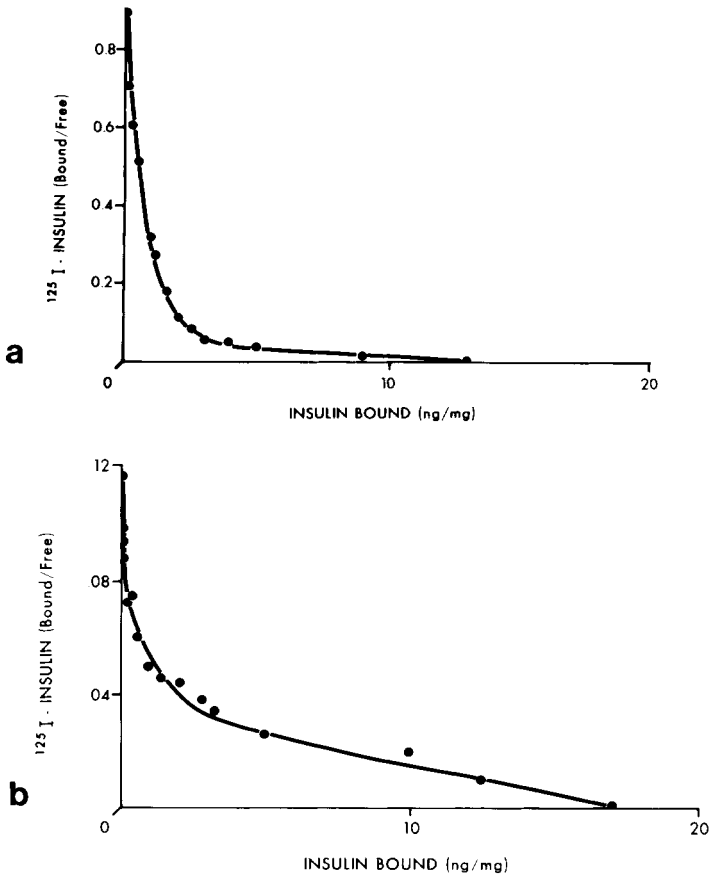

Fig. 3. Scatchard plot of insulin binding to plasma membranes (a) and to nuclear membranes (b). From Vigneri et al [37].

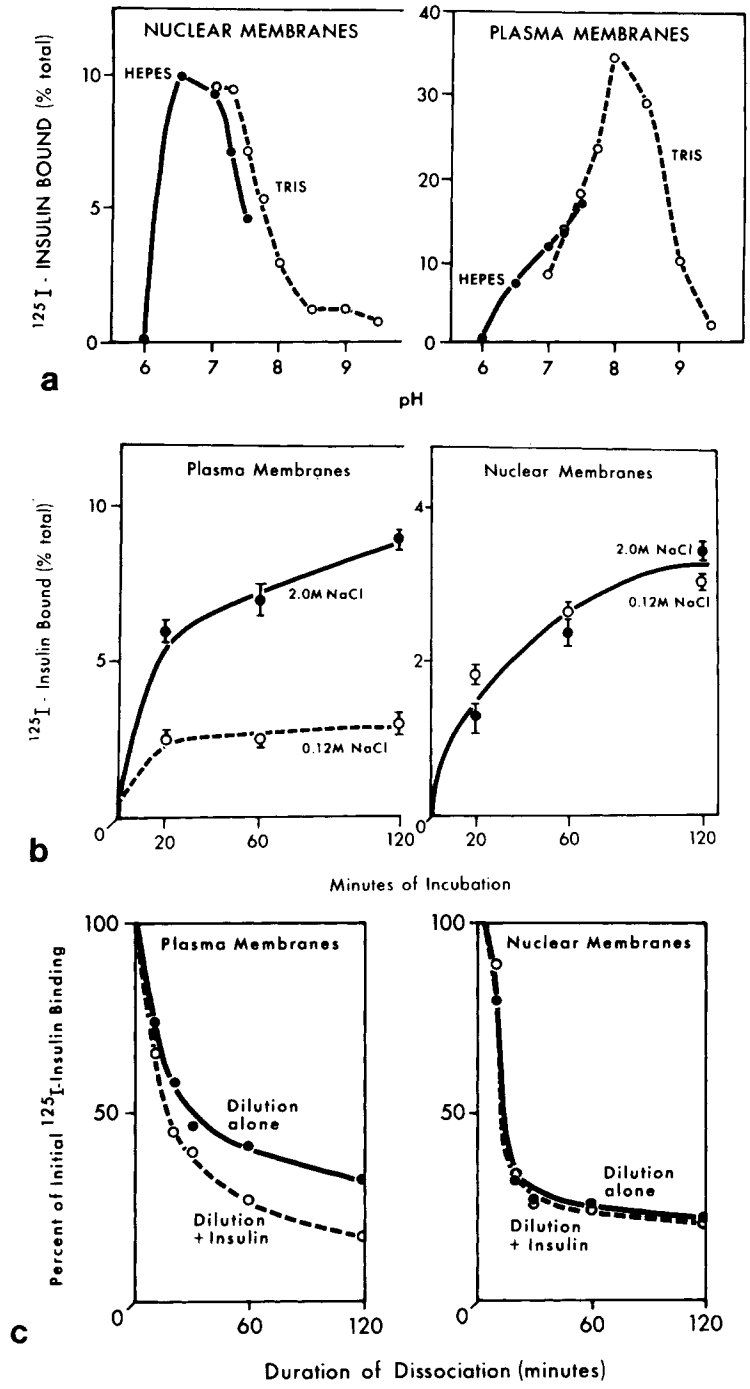

Fig. 4. (a) Effect of pH on the specific binding of ^{125}I-insulin to plasma membranes and nuclear membranes. (b) Effect of high concentrations of NaCl on the binding of insulin to plasma membranes and nuclear membranes. (c) Lack of effect of unlabeled insulin to enhance the dissociation of ^{125}I-insulin from nuclear membranes (absence of negative cooperativity) as compared to plasma membranes. From Vigneri et al [37].

not bind to receptors for other hormones, such as glucagon, and growth hormone [39]. When we preincubated this antiserum with nuclei, there was little inhibition of the subsequent binding of labeled hormone [39]. This finding suggested that the insulin binding sites in the nuclear envelope are proteins separate fom the insulin binding sites on the plasma membrane. Another possibility is that they are the same binding site, but that the different milieu of the nuclear membrane significantly alters the characteristics of insulin binding. For instance, the lipid composition of nuclear membranes, especially the cholesterol content [42], is markedly different from that of plasma membranes. Since the lipid environment of the plasma membrane causes alterations in insulin binding [42], there is little support for the latter hypothesis.

In contrast to the above results, Bergeron et al [40] have not been able to detect specific insulin binding sites on rat liver nuclei and have suggested that insulin binding to nuclei and nuclear envelopes is the result of the contamination of these preparations with plasma membranes. Several lines of evidence, however, make this possibility very unlikely. First, the characteristics of insulin binding to nuclear membranes are different from those for plasma membranes (Table I). Second, marker enzymes of the plasma membrane, such as 5′nucleotidase and (Na^+,K^+)ATPase, are very low in nuclei and nuclear membranes [31–33,37,41]. Third, plasma membranes, but not nuclear membranes, readily bind glucagon, a hormone that acts at the plasma membrane [37].

STRUCTURE OF THE NUCLEAR ENVELOPE

The nuclear envelope is a bilayered membrane structure that separates the nucleoplasm and cytoplasm of eukaryotic cells. The outer nuclear envelope is associated with the endoplasmic reticulum and the inner nuclear membrane is intimately associated with the peripheral heterochromatin [43,44] (Fig. 6). It is known that small molecules can freely enter into and exit from the nucleus [44,45]. There is, however, evidence that the translocation of RNA and other macromolecules to and from the nucleus may proceed via a more complicated mechanism [44]. Thus it is likely that the nuclear envelope has more than a passive role in nuclear cytoplasmic interactions.

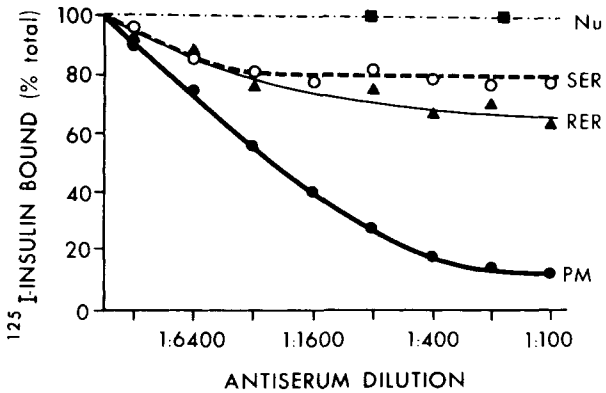

Fig. 5. The effect of preincubation with an antiserum to the plasma membrane insulin receptor on the subsequent specific binding of ^{125}I-labeled insulin to isolated nuclei (Nu), rough (RER) and smooth (SER) endoplasmic reticulum, and plasma membranes (PM). Adapted from Goldfine et al [39].

Pores filled with a unique structure termed the nuclear pore complex are dispersed throughout the nuclear envelope (Fig. 6). The nuclear pore complex has a double annulus, each annulus having eight peripheral subunits of approximately 250 Å in diameter [43,44]. In addition, there is a central granule between the double annuli (Fig. 6). The nuclear pore complex is attached to both the inner and outer nuclear envelope, but the nuclear pore complex is not covered on its inner surface by heterochromatin. Further, it has been postulated that both the central granule and

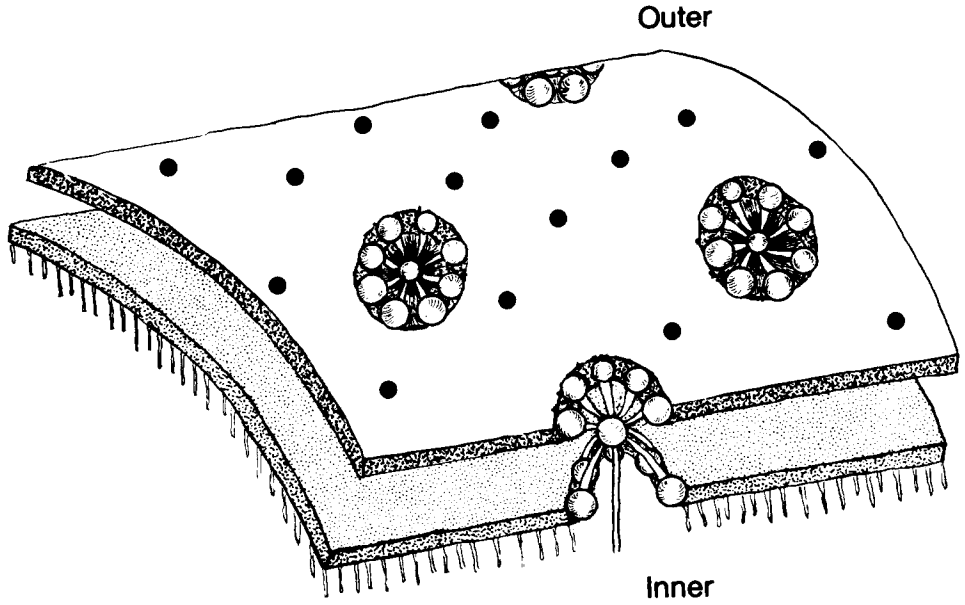

Fig. 6. Schematic drawing of the nuclear envelope. Nuclear pores can be seen joining the two layers of the nuclear envelope.

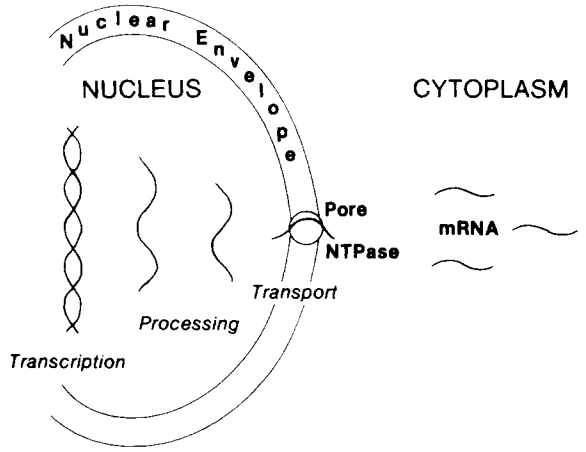

Fig. 7. Major sites of mRNA processing.

peripheral subunits are hollow tubes. Thus the nuclear pore complex could play a role in the nuclear cytoplasmic translocation of mRNA.

RELATIONSHIP BETWEEN mRNA EFFLUX AND NUCLEAR MEMBRANE NTPase

In order to understand the mechanism of mRNA transport from the nucleus, rats have been injected with radiolabeled orotic acid or uridine and the efflux of labeled RNA from isolated nuclei into a surrogate cytoplasm studied [46–49]. Most investigators have employed nuclei from liver. Many criteria suggest that the nature of the transported RNA (including size, base and poly(A)content, activity in directing protein synthesis, incorporation into polysomes, and inclusion into specific RNP particles) under the conditions studied is mRNA [46–48].

RNA transport in vitro involves both intranuclear RNA processing and subsequent efflux (Fig. 7). A source of high energy phosphate is necessary for transport, but not processing [46–48]. One high energy phosphate bond is hydrolyzed to transport one nucleotide of mRNA. Studies indicate that the high energy phosphate specificity is not highly selective since ATP, UPT, CTP, and GTP are all effective [46–48].

There is considerable evidence that a nuclear triphosphatase (NTPase) provides the energy for the transport of mRNA. For instance, the activation energy for RNA transport is 13 kcal/mol and for NTPase activity is 13.3–13.8 kcal/mol [47]. Further, the affinities of ATP for both NTPase activity and facilitated RNA transport are similar. Also, cyclic AMP stimulates both functions whereas NaF inhibits them [46–48]. Finally, trypsin treatment of nuclei inactivates both functions [48]. Histocytochemical studies from our laboratory indicate that this enyzme is located throughout the nuclear envelope [47]. Others, however, have suggested that this enzyme resides in the nuclear pore [48].

INSULIN ACTION IN ISOLATED NUCLEI AND NUCLEAR ENVELOPES

In view of the influence of insulin on mRNA levels in liver, several studies have been carried out in vitro with isolated nuclei and nuclear envelopes. Schumm and Webb [50] measured mRNA transport from liver nuclei of normal rats prelabeled 30 min in vivo with [^{14}C]orotic acid and found that the direct addition of insulin in vitro to their nuclei markedly enhanced mRNA transport [50]. In these studies, however, higher than physiological levels of insulin were needed (100 nM). We have modified their methods by both eliminating liver cytosol and using diabetic rats. We now find that in vitro insulin as low as 1 pM can stimulate mRNA efflux (Fig. 8).

In light of the observations that insulin may directly stimulate nuclear mRNA efflux and that nuclear membrane NTPase activity is necessary for this function, we investigated whether insulin directly influenced nuclear membrane NTPase activity. Highly purified nuclear membranes were prepared by the method of Monneron [51]. In these membranes we found that basal nuclear membrane NTPase activity was higher in liver of normal rats than in liver from hypoinsulinemic diabetic rats [52]. Moreover, the direct addition of insulin to purified nuclear envelopes of liver from diabetic rats stimulated NTPase activity. An effect was detectable at 1 pM and maximal effects were seen at 10–100 pM (Fig. 9). Other studies indicated that insulin increased the V_{max} of the enzyme [52].

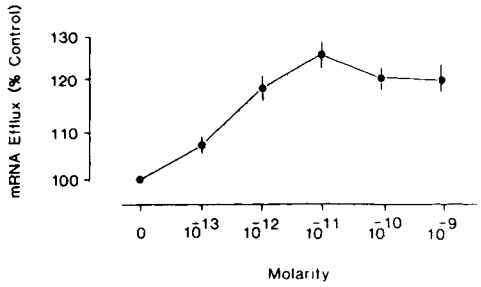

Fig. 8. Effect of insulin on stimulation of [^{14}C]RNA release from isolated liver nuclei obtained from diabetic rats.

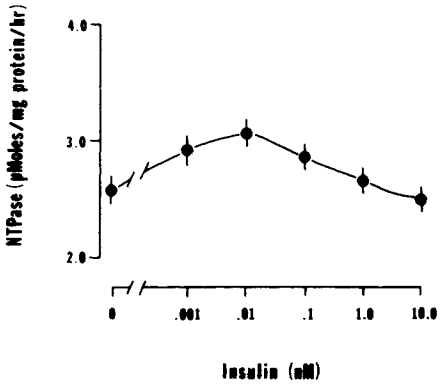

Fig. 9. Effect of insulin on stimulation of NTPase activity in liver nuclear envelopes from diabetic rats. Nuclear membranes, 100 μg protein/ml, were incubated with 1 mM [γ-^{32}P]ATP and insulin for 10 min at 37°C and the hydrolysed ^{32}P measured [47].

CONCLUSION

The nuclear envelope contains specific high affinity binding sites for insulin. Moreover, the nuclear envelope and its pore complex play a major role in the transport of mRNA from the nucleus. Recent studies indicate that insulin directly stimulates the release of mRNA from isolated nuclei. In addition, insulin directly stimulates nuclear envelope NTPase, the enzyme that regulates mRNA efflux. These observations raise the possiblity, therefore, that insulin may regulate nuclear functions by acting at the nuclear surface.

ACKNOWLEDGMENTS

This research was supported by NIH grant no. AM26667 and the Elise Stern Haas Research Fund, Harold Brunn Institute, Mount Zion Hospital and Medical Center.

REFERENCES

1. Gey GO, Thalhimer W: J Am Med Assoc 82:1609, 1924.
2. Smith GL, Temin HM: J Cell Physiol 84:181, 1974.

3. Rechler MM, Podskalny JM, Goldfine ID, Wells CA: J Clin Endocrinol Metab 39:512, 1974.
4. Chochinov RH, Daughaday WH: Diabetes 25:994, 1976.
5. Bucher NLR, Weir GC: Metab Clin Exp 25:1423, 1976.
6. Price JB Jr: Metab Clin Exp 25:1427, 1976.
7. Richman RA, Claus TH, Pilkis SJ, Friedman DL: Proc Natl Acad Sci USA 73:3589, 1976.
8. Koontz JW, Iwahashi M: Science 211:947, 1980.
9. Steiner DF: Vitam Horm 24:1, 1966.
10. Morgan CR, Bonner J: Proc Natl Acad Sci USA 65:1077, 1970.
11. Krahl ME: Annu Rev Physiol 36:331, 1974.
12. Weber G: Isr J Med Sci 8:325, 1972.
13. Steiner DF, King J: J Biol Chem 239:1292, 1964.
14. Hill RE, Lee K-L, Kenny FT: J Biol Chem 256:1510, 1981.
15. Peavy DE, Taylor JM, Jefferson LS: Proc Natl Acad Sci USA 75:5879, 1978.
16. Pry TA, Porter JW: Biochem Biophys Res Commun 100:1002, 1981.
17. Roy AK, Chatterjee B, Prasad MSK, Unakar JJ: J Biol Chem 225:11614, 1980.
18. Korc M, Iwamoto Y, Sankaran H, Williams JA, Goldfine ID: Am J Physiol 240 (Gastrointest Liver Physol 3):G56, 1981.
19. Söling HD, Unger KO: Eur J Clin Invest 2:199, 1972.
20. Korc M, Owerbach D, Quinto C, Rutter WJ: Science 213:351, 1981.
21. Hansen RJ, Pilkis SJ, Krahl ME: Endocrinol 81:1397, 1967.
22. Hansen RJ, Pilkis SJ: Endocrinol 86:57, 1970.
23. Spooner PM, Chernick SS, Garrison MM, Scow RO: J Biol Chem 254:10021, 1979.
24. Stockdale FE, Topper YJ: Proc Natl Acad Sci USA 56:1283, 1966.
25. Topper YJ, Friedberg SH, Okta T: Dev Biol (Suppl)4:101, 1970.
26. Turkington RW: Endocrinol 82:540, 1968.
27. Terry PM, Banerjee MR, Lui RM: Proc Natl AcadSci USA 74:2441, 1977.
28. Bolander FF Jr, Nicholas KR, Van Wyk JJ, Topper YJ: Proc Natl Acad Sci USA 78:5682, 1981.
29. Freychet P, Roth J, Neville DM Jr: Proc Natl Acad Sci USA 68:1833, 1971.
30. Goldfine ID: In Symthies JR, Bradley RJ (eds): "Receptors in Pharmacology." New York:Marcel Dekker, 1978, p 335.
31. Horvat A, Li E, Katsoyannis PG: Biochim Biophys Acta 382:609, 1976.
32. Goldfine ID, Smith GJ: Proc Natl Acad Sci USA 73:1427, 1976.
33. Goidl JA: Biochemistry 18:3674, 1979.
34. Vigneri R, Pliam NB, Cohen DC, Pezzino V, Wong KY, Goldfine I: J Biol Chem 253:8192, 1978.
35. Brisson-Lougarre A, Blum CJ: CR Acad Sci (D) (Paris) 289:129, 1979.
36. Brisson-Lougarre A, Blum CJ: CR Acad Sci (D) (Paris) 290:889, 1980.
37. Vigneri R, Goldfine ID, Wong KY, Smith GJ, Pezzino V: J Biol Chem 253:2098, 1978.
38. Horvat A: J Cell Physiol 97:37, 1978.
39. Goldfine ID, Vigneri R, Cohen D, Pliam NB: Nature 269:698, 1977.
40. Bergeron JJM, Evans WH, Geschwind II: J Cell Biol 59:771, 1973.
41. Kashnig DM, Kasper CB: J Biol Chem 244:3786, 1969.
42. Goldfine ID: Life Sci 23:2639, 1978.
43. Franke WW: In Bourne GH, Danielli JF (eds): "International Review of Cytology." New York: Academic Press, 1974, p 71.
44. Harris JR, Agutter PS: In Maddy AH (ed): "Biochemical Analysis of Membranes." New York: John Wiley, 1976, p 132.
45. Feldherr CM: Adv Cell Molec Biol 2:273, 1972.
46. Schumm DE, Webb TE: J Biol Chem 253:8513, 1978.
47. Clawson GA, James J, Woo CH, Friend DS, Moody D, Smuckler EA: Biochem 19:2756, 1980.
48. Agutter PS, McCaldin B, McArdle HJ: Biochem J 182:811, 1979.
49. Ishikawa K, Sato-Odani S, Ogata K: Biochim Biophys Acta 521:650, 1978.
50. Schumm DE, Webb TE: J Biol Chem 253:8513, 1978.
51. Monneron A, Blobel G, Palade GE: J Cell Biol 55:104, 1972.
52. Purrello F, Vigneri R, Clawson GA, Goldfine ID: Science 216:1005, 1982.