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Metabolic control of insulin gene expression and biosynthesis

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The regulation of insulin production by pancreatic β cells is mediated by a number of circulating nutrients and hormones, of which glucose is the predominant physiological stimulus. Over short periods of 1–2 h glucose regulates insulin biosynthesis mainly at the level of translation (Permutt & Kipnis, 1972). This is in keeping with the requirement to rapidly replenish insulin stores, secreted in response to elevated circulating glucose levels following food intake. In addition to the acute effect on translation, glucose also affects insulin mRNA production and stability over longer periods (Brundstedt & Chan, 1982; Nielsen *et al.* 1985; Welsh *et al.* 1985). Here we will describe some recent experiments, which have increased our understanding of how these transcriptional and translational processes respond to external stimuli.

THE METABOLIC CONTROL OF INSULIN GENE EXPRESSION

There is substantial evidence that expression of the insulin gene is regulated by metabolic stimuli. Indirect evidence was provided by the observation that Actinomycin D, an inhibitor of RNA synthesis, inhibited the late stage of glucose-stimulated insulin biosynthesis in isolated rat pancreatic islets (Permutt & Kipnis, 1972). Advances in recombinant DNA technology then permitted the direct quantification of insulin mRNA using hybridization methods. Thus, starvation of rats for 4 d led to a reduction in insulin mRNA levels. Normal mRNA levels could be restored by refeeding or injecting the animals with glucose (Giddings *et al.* 1981). Further studies showed that increased glucose concentrations had an effect on insulin mRNA levels (Brundstedt & Chan, 1982), and on the rate of transcription of the insulin gene (Nielsen *et al.* 1985) in isolated pancreatic islets. The effect of glucose on insulin gene expression involved a simultaneous stimulation of transcription and inhibition of insulin mRNA degradation (Welsh *et al.* 1985). Glucose-mediated effects on insulin gene transcription were subsequently shown to be mimicked by cAMP (Nielsen *et al.* 1985; Hammonds *et al.* 1987; Philippe &

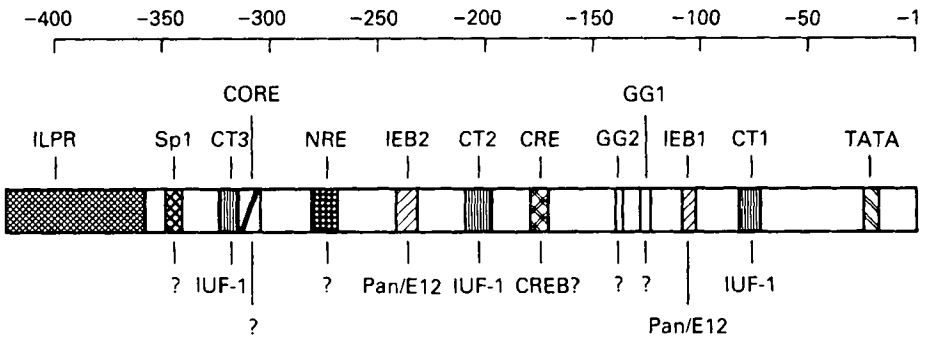

Fig. 1. Organization of regulatory sequences in the human insulin gene. The name for each sequence motif is given above, and the corresponding DNA binding protein below the schematic of the 5′ region of the insulin gene. Although it is too early to form a comprehensive picture of insulin gene regulation, it is likely that the process involves the combinatorial action of several distinct positively and negatively acting regulatory sites as indicated in this scheme. For further details, see Clark & Docherty (1992).

Missotten, 1990*a*). Thus, cAMP-dependent phosphorylation may be implicated in the control of insulin gene expression. Possible substrates for phosphorylation might include transcription factors which interact with DNA regulatory sequences within the insulin gene.

DNA sequences involved in the regulation of insulin gene transcription are located within approximately 350 base pairs upstream of the transcription start site (Walker *et al.* 1983; Edlund *et al.* 1985). Most of the studies have been performed on the rat I, rat II (there are two insulin genes in the rat) and human insulin genes. The arrangement of regulatory elements in the human insulin gene is illustrated in Fig. 1. Duplication of the insulin genes in the rat (Soares *et al.* 1985) may have altered the selective pressures operating on transcriptional regulation. Thus, although the three genes are highly homologous in the 5′ region and most of the identified regulatory sites are common to all three genes, the utilization of these sites may differ between genes. It should also be noted that these sequences in the human gene represent, for the most part, protein-binding sites (Boam & Docherty, 1989; Boam *et al.* 1990), and that some of the functional experiments have yet to be completed. Much of what follows on the properties of these sequences is, therefore, by inference, from known properties of related sequences in the rat I gene.

In the rat I insulin gene mutation of either the IEB1 or IEB2 motifs (see Fig. 1) reduced transcription by roughly 85% whilst mutation of both sequences essentially abolished transcription. Thus, the IEB motifs are critical elements of the enhancer, with other elements being important to a lesser extent (Karlsson *et al.* 1989). The IEB motifs bind a transcription factor which is present in a wide variety of cells. cDNAs encoding this factor have been cloned and sequenced (Nelson *et al.* 1990; Shibasaki *et al.* 1990; Walker *et al.* 1990). Designated Pan, this factor is identical to a factor (E12/E47) involved in the control of immunoglobulin gene expression. Pan is a member of a family of transcription factors which contain a dimerization domain composed of two α helices joined by a loop structure, i.e. helix loop helix (HLH) domain. HLH proteins can be divided into those that are tissue-specific and those that are present in many cell types.

Heterodimers form between ubiquitous and tissue-specific HLH proteins, e.g. MYOD and E12/E47 in muscle cells (Murre *et al.* 1989). One might conclude that a β cell-specific HLH protein has yet to be described, and that this protein will dimerize with Pan to play a major role in controlling insulin gene expression, and possibly also in determining islet cell development.

The three CT motifs (see Fig. 1) which are located adjacent to the IEB motifs and at an upstream site (–313) bind a β cell-specific factor. Designated IUF-1 (Boam & Docherty, 1989), this factor is probably different to Isl-1 (Karlsson *et al.* 1990; Scott *et al.* 1991). Paradoxically, the CT motifs, which contain no enhancer activity on their own, are the only sequences to bind a β cell-specific factor. The CT boxes, however, will potentiate the activity of the IEB motifs (Karlsson *et al.* 1989). An upstream negative regulatory element (NRE), which functions in β cells, is located 258 base pairs upstream of the transcription start site in the human, and probably the rat II, insulin genes. Its activity is modulated by the presence of an adjacent positive-acting element (A. R. Clark and K. Docherty, unpublished results). Although not definitively mapped, the positive element is likely to include the enhancer core and CT3 motifs. Other sequences which have yet to be assigned a function include the GG1 and GG2 motifs, the Sp1 motif, and the insulin-linked polymorphic region (ILPR), the latter of which is present only in the human insulin gene.

The current findings are compatible with the view that the process of insulin gene regulation involves the combinatorial action of several distinct positively and negatively acting regulatory sites. Identified regulatory sites bind several proteins, not all of which are restricted to insulin-secreting cells, and not all of which are necessarily involved in the regulation of transcription. It is clear that the IEB motifs represent the dominant tissue-specific regulatory sequences, with the other indicated motifs (see Fig. 1) involved in: (1) maintaining the gene in a transcriptionally inactive state in non-β cells, (2) programming the activity of gene expression at particular stages of development, (3) mediating the transcriptional response of the insulin gene to metabolic stimuli. How these sequences respond to nutritional stimulation is not well understood, but some potential mechanisms have been investigated.

The cAMP-response element (CRE) (see Fig. 1) is likely to be involved in the response of the insulin gene to cAMP. Mutagenesis of this sequence at critically important nucleotides abolishes the response of the insulin promoter to cAMP (Philippe & Missotten, 1990*a*). Furthermore, this sequence binds a ubiquitous factor which exhibits a molecular size of 43 000 (Philippe & Missotten, 1990*a*), the approximate size of the CRE-binding protein (CREB). However, it has recently been demonstrated that sequences in addition to those containing the CRE responded to glucose when transfected into rat islets (German, *et al.* 1990). These sequences have yet to be mapped. Interestingly, a reduced response to glucose was observed in the presence of the Ca^{2+}-channel blocker verapamil (German *et al.* 1990). This suggests that Ca^{2+}-dependent second messenger systems may play a role in modulating the activity of the insulin gene. Finally, the observation that the insulin gene is coordinately regulated with the gene for glucokinase (*EC* 2.7.1.2) may be important in understanding how these genes respond to external stimuli. A marked parallel decrease in mRNA levels for insulin and glucokinase was observed in exercise-trained rats (Koranyi *et al.* 1991). Similar transcription factors, reponsive to metabolic changes in the β cell may, therefore, interact with regulatory sequences in both genes.

THE TRANSLATIONAL CONTROL OF INSULIN BIOSYNTHESIS

Glucose metabolism appears to be essential for its effect on insulin biosynthesis. However, the mechanisms linking glucose metabolism and increased translation of insulin mRNA are not well understood. In one study, theophylline, an inhibitor of cAMP degradation, increased the rate of initiation of insulin mRNA in isolated islets (Welsh *et al.* 1987). This implicated cAMP in the stimulation of insulin biosynthesis. However, in the hamster β cell line, HIT T15, neither forskolin nor IBMX affected glucose-stimulated insulin biosynthesis (Gold *et al.* 1988), suggesting that cAMP was not a stimulator of insulin biosynthesis in these cells. In the same study, dexamethasone inhibited insulin biosynthesis in HIT T15 cells, but this effect is likely to result from the ability of dexamethasone to destabilize insulin mRNA (Philippe & Missotten, 1990*b*). Changes in intracellular Ca^{2+} are unlikely to be involved, since Ca^{2+} depletion does not alter basal or glucose-stimulated insulin biosynthesis (Guest *et al.* 1989). Thus, the extent to which insulin secretion (Ashcroft, 1980) and biosynthesis are regulated in parallel remains to be established.

The effect of glucose on protein biosynthesis in β cells is not restricted to insulin. Thus, glucose stimulated the biosynthesis of growth hormone in isolated pancreatic islets from transgenic mice carrying a metallothionein–growth hormone gene fusion (Welsh *et al.* 1986*a*). Glucose also stimulated the biosynthesis of a number, but not all, of the proteins present in the insulin secretory granule. Chromogranin A and the insulin-granule membrane protein SGM110, were stimulated by glucose, but the biosynthesis of carboxypeptidase H was unaffected (Grimaldi *et al.* 1987; Guest *et al.* 1989). In fact the majority of insulin granule constituents are coordinately regulated in response to glucose (Guest *et al.* 1991).

There are several examples of translational control of eukaryotic protein biosynthesis, where the mechanisms at least in part, have been characterized. These may provide insights into the differential translational response to stimuli in β cells. Translation of the yeast transcription factor GCN4 is stimulated in response to amino acid starvation. The 5′ region of the GCN4 mRNA contains four open reading frames (ORF), only one of which will generate a functional protein. The selection of a particular ORF is dependent on the amino acid content of the yeast culture media (Hinnesbusch, 1988). Similarly, translation of yeast CPA1 mRNA, which encodes a subunit of an enzyme involved in arginine biosynthesis is repressed by arginine. This repression involves translation of an upstream ORF of the CPA1 mRNA, which encodes a twenty-five amino acid peptide. Another example is provided by the effect of iron on the translation of ferritin and transferrin receptor mRNA. Both mRNA contain common sequence motifs which can form stem loop structures. A specific factor will bind to this stem loop structure or Fe-response element (IRE) at times of low cellular Fe, blocking initiation of translation of ferritin mRNA, or increasing stability of transferrin receptor mRNA (Rouault *et al.* 1990).

Thus, translational control can be mediated through selective use of upstream ORF, or through interaction of a factor with stem loop structures within the 5′ or 3′ untranslated regions of the mRNA. It is of interest, therefore, that the rate of initiation of insulin mRNA, as shown by the transfer of cytoplasmic RNA to a fraction co-sedimenting with ribosomes, is stimulated by glucose (Welsh *et al.* 1986*b*). Examination of the sequence of the mRNA encoding insulin demonstrates that there are no upstream ORF, but application of appropriate algorithms capable of predicting RNA structures indicates that a stem loop structure exists in the 5′ untranslated region. On the basis that the stem

loop structure may represent the binding site for a specific protein or translation factor, we investigated the binding of proteins to various sequences corresponding to regions of the insulin mRNA 5′ untranslated region. Two discrete binding sites were localized (Knight & Docherty, 1991). The role of proteins binding at these sites in glucose-stimulated initiation of translation is presently being assessed.

In addition to its effect on the rate of initiation of insulin mRNA, it was also shown that glucose stimulated a movement of pre-initiated mRNA from a soluble to a membrane-associated pool (Itoh & Okamoto, 1980; Welsh *et al.* 1986*b*). Presumably this involves a signal recognition particle (SRP)-mediated arrest of translation; possibly with resultant stacking of ribosomes on the mRNA (Wolin & Walter, 1988). There appears to be some specificity in this process, since it is clear that of all the proteins translocated across the endoplasmic reticulum in β cells, the majority are not affected by glucose in a similar manner (Guest *et al.* 1991). This tempts speculation that the sequence encoding the signal peptide or the signal peptide itself may be important in the translational response to glucose.

Finally, evidence was also presented that glucose stimulated the rate of elongation of preproinsulin on pre-initiated mRNA. At low concentrations cycloheximide will slow down polypeptide chain elongation such that initiation of translation will become rate-limiting. Thus, the observation that cycloheximide at appropriate low concentrations had no effect on glucose-stimulated proinsulin biosynthesis in isolated rat islets, suggested that glucose affected the rate of proinsulin polypeptide chain elongation (Welsh *et al.* 1986*b*; Itoh, 1990).

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