Processing and release of human proinsulin-cleavage products into culture media by different engineered non-endocrine cells: a specific assessment by capillary electrophoresis

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Abstract

The aim of this study was to compare the metabolic pathway to mature insulin through the intermediate forms (32–33 split, 65–66 split, des31,32 and des64,65) in human or murine cells engineered for the release of wild-type human proinsulin and in a genetically mutated one, in the search for a new approach for an insulin-dependent diabetes mellitus cure by gene therapy.

Primary human fibroblasts, myoblasts and stabilized cell lines (HepG2 and NIH3T3) were transduced either with a retroviral vector coding for wild-type proinsulin or for a genetically mutated one, carrying cleavage sites sensitive to furin. The pattern of all the proinsulin cleavage products released into the cell culture supernatants was analyzed by capillary electrophoresis.

All the cells transduced with the wild-type gene released intact proinsulin. HepG2 released a considerable amount of 65–66 split and des64,65, while primary myoblasts released all the intermediate forms and a limited amount of mature insulin. All the cells transduced with a furin-sensitive proinsulin gene released a higher amount of mature insulin (23–59% conversion yield) than the cells expressing wild-type proinsulin, whereas the total insulin was nearly constant. Only primary cells released all the cleavage products.

Screening a wide variety of non-endocrine cells has revealed a large difference in the processing and release of immature and mature insulin forms, pointing to human hepatic cells as the most efficacious. Capillary electrophoresis provided on-line and in a single run a complete overview of the proinsulin metabolic pathway in different cells.


Introduction

In the search for a gene therapy-based cure for diabetes (Kolodka et al. 1995, Levine 1997), many different non-endocrine cells have been genetically modified by introduction of the human pre-proinsulin gene (HPI), thus obtaining synthesis of the prohormone (Groskreutz et al. 1994). In non-endocrine cells, furin, a Golgi-anchored enzyme (Groskreutz et al. 1994, Vollenweider et al. 1995) whose concentration is cell line specific, exerts a proteolytic activity like PC2 and PC3 (endocrine cell-restricted endoproteases) recognizing a cleavage site composed of the tetra-basic amino acid sequence Arg-x-x-Lys/Arg-x-x-Arg-x-x (Fig. 1) (Rhodes & Halban 1987, Vollenweider et al. 1992, Halban 1994, Hutton 1994, Kahn & Halban 1997) whose accumulation can vary in relation to pathological conditions (Sizonenko et al. 1993, Ward et al. 1987, Yoshioka et al. 1988, Mykkanen et al. 1997).

In order to verify the proinsulin metabolism in non-endocrine cells transduced both with the wild-type human proinsulin gene (HPI) and with modified HPI, a specific method able to quantify all the conversion products is necessary. RIA gives the ‘total immunoreactive insulin’
(IRI) because of its cross-reaction with the proinsulin-like molecules, hI and C-peptide. This property leads to overestimation of the mature insulin concentration, particularly when proinsulin and intermediates are increased (Temple et al. 1989, 1990, Crowther & Gray 1996). IRMA- (Mohamed-Ali et al. 1996) and ELISA-based assays (Tao & Kennedy 1997) can only detect analytes for which the specific antibodies have been raised, so more versatile analytical procedures based on HPLC (Nagi et al. 1990, Vollenweider et al. 1992, Ostrega et al. 1995), mass-spectrometry (MS) (Stocklin et al. 1997) or capillary electrophoresis (CE) (Tao & Kennedy 1997, Tao et al. 1998) have been developed.

A new, interesting tool for protein quantification is represented by CE (Nielsen et al. 1989, Chen & Sternberg 1994, Nashabeh et al. 1994), which has been shown to separate complex protein mixtures with remarkable resolving power by exploiting the differences in charge/mass ratio of the analytes (Oda et al. 1997, Bergmann et al. 1998). Its advantages can be summarized as: on-line detection of different kinds of molecules in the same run, low reagent volume consumption, low sample requirements, and the possibility of automation.

The aim of this study was to make a comparison of the hPI metabolism in different non-endocrine stabilized or primary cell lines transfected both with the wild-type and

![Figure 1](https://www.endocrinology.org/download/proinsulin-cleavage-by-non-endocrine-cells.png)
with the modified gene constructs. To reach this goal hPI, hI and the split and des intermediate forms released into the cell supernatants were purified by solid phase extraction and directly quantified by capillary zone electrophoresis (CZE) coupled with UV absorbance (Arcelloni et al. 1998).

Materials and Methods

Standard proteins

hI was purchased from Boehringer (Mannheim, Germany) and the lyophilized powder reconstituted with bi-distilled water to a final concentration of 1-6 mmol/l. hPI, des31,32, des64,65, 32–66 split were kindly donated by Eli Lilly and Company (Indianapolis, IN, USA) and reconstituted with water to a final concentration of 20 μmol/l. Aliquots were stored at −20 °C.

Retroviral vector and cell transduction

The insertion of the EcoR1/Hga1343 bp fragment of the wild-type hPI into the EcoR1/Hpa1 sites of retroviral vector L-X-SFCM, derived from the Moloney murine leukemia virus backbone, was described previously (Falqui et al. 1999). For cell marking and selection, the L-X-SFCM vector coding for the truncated human low-affinity nerve growth factor receptor (ΔLNGFR), was employed (Mavilio et al. 1994). Subsequently, two mutations were introduced by PCR technique into the wild hPI cDNA, to obtain the permanent synthesis of a proinsulin molecule with modified cleavage sites (Falqui et al. 1999). The modified hPI cDNA (FurHPI) was then inserted into the LX-SFCM retroviral vector. The generation of infective supernatants with high titers of viral particles was obtained in packaging cells, as previously described (Mavilio et al. 1994, Falqui et al. 1999). Infective supernatants were harvested from confluent packaging cells and overlaid for 24 h onto target cells in the presence of polybrene (8 μg/ml). After 1 week, the percentage of retrovirally transduced cells was evaluated by flow cytometry for de novo surface expression of ΔLNGFR.

Cell culture and media collections

Primary human fibroblasts (PHF) and HepG2 were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (GIBCO Life Technologies, Paisley, Scotland) supplemented with 10% (v/v) fetal calf serum (FCS) (Sigma, St Louis, MO, USA), whereas murine fibroblasts (NIH3T3) were cultured in DMEM supplemented with 10% (v/v) newborn calf serum and primary rat hepatocytes (PRH) in Medium 199 (GIBCO) supplemented with growth factors. Primary human myoblasts (PHM) were cultured in RPMI medium containing 20% FCS. For CZE analysis, culture media were harvested after 24 or 48 h of incubation. Cells were counted and hPI, hI and intermediates accumulation in culture media was expressed as pmol/24 h per 10^6 ΔLNGFR-positive cells.

CZE

All reagents were of analytical grade. Diethylenetriamine (DETA), N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS), trifluoroacetic acid and acetonitrile were from Fluka (Buchs, Switzerland). The analyses were carried out with slight modifications of the previously published procedure (Arcelloni et al. 1998) to allow resolution of all the proinsulin cleavage products. A P/ACE 5010 equipped with UV detector and computer controlled (Beckman, Palo Alto, CA, USA) was used. All the proteins were separated in an uncoated fused silica capillary (50 μm i.d. × 27 cm total, 20 cm to the detector) eluted with a running buffer composed of TAPS 100 mmol/l, pH 11.5, DETA 17.8 mmol/l and methanol (45:45:10 v/v/v), at a constant voltage of +9kV (about 18 μA). The detector was set at 200 nm and the capillary thermostatted at 20 °C.

Purification procedure

Cell supernatants (20-30 ml) were purified as described (Arcelloni et al. 1998). Briefly, after a solid-phase extraction with Sep-Pak Vac 6 ml, C_{18} 500 mg cartridges (Waters, Milford, MA, USA), the fraction containing the proteins of interest was concentrated under vacuum and ultrafiltrated (Centricon-50, 50 000 Mₙ cut-off; Amicon Division, Beverly, MA, USA). Aliquots of the ultrafiltrates were injected (5–10 s, corresponding to 9.8–19.5 nl) at pressure mode 3·4 × 10⁻³ MPa.

hI and hPI routine assay

RIA Determination of IRI was carried out by RIA (Insulin RIA Kit; Incstar Corporation, Stillwater, MN, USA). The standard curve concentration ranged from 0 to 600 pmol/l. ¹²⁵I-insulin, antibody and anti-insulin serum were incubated overnight at 4 °C. Precipitating complex was added, vortexed, centrifuged and the supernatant discarded. Radiiodine in the precipitates was counted for 60 s using a gamma scintillation counter. The polyclonal antibodies used cross-react 100% with bovine and porcine insulin and 30–80% with proinsulin-like molecules.

hI IRMA The determination of mature insulin was done with a BI-INSULIN IRMA kit (Sano-Diagnostic Pasteur, Paris, France), based on a monoclonal antibody. The analytical range was 1·4–3000 pmol/l and the
cross-reactivity was 100% with porcine and bovine insulin and 65–66 split or des64,65 proinsulin, while <0.0001% with hPI and 32–33 split and des31,32.

ELISA Two non-isotopic microplate assays (Dako, Glostrup, Denmark) for total hPI and intact hPI (IhPI) proinsulin were used. The first assay detects unprocessed hPI and cross-reacts (65–99%) with all intermediate forms of hPI (32–33 split, des31,32; 65–66 split and des64,65) while the IhPI assay detects the unprocessed molecule along with 65–66 split and des64,65. Antibodies used in the kits give a cross-reactivity of 0·1% with insulin and C-peptide. The standard analytical range was 0–100 pmol/l and the detection limit <0·2 pmol/l for both (as provided by the manufacturer).

Results

Validation of CZE analysis showed a calculated recovery rate for each protein from solid-phase extraction of 85 ± 14% (s.d.) and a sensitivity limit of the CZE analysis of 2 fmol injected, corresponding to 0·5 nmol/l in the supernatant. The response was linear in the 0·1–10 μmol/l range corresponding to 2–200 nmol/l in the supernatants. With respect to the already published method (Arcelloni et al. 1998), the separation has now been optimized to include also 32–33 split. In Fig. 2 is reported the electrophoretic separation of the six standard proteins. Figure 3 shows the electropherograms obtained by analysis of the supernatants from non-transfected PHM cells, PHM-HPI and PHM-FurHPI cells. The abundant peak at 6·4 min (just before hl) is unrelated to cell metabolism, being present in all the fresh culture media employed for cells growth (data not shown). By adding increasing amounts of standard hl to wild-type supernatant, however, its resolution from the big peak migrating just before was never hindered. The major peaks at 7·4, 7·8 and 10·0 min, together with numerous other minor peaks were present in both transfected cells but not in the non-transfected lines. Although their identification is not available yet, they are probably related to proinsulin production by these cells and can be also used for monitoring cell transfection.

The identification of the (pro)insulin-related proteins within the rather complex electrophoretic profiles was accomplished by an extensive post-analytical review with the System Gold software (Beckman version 8.1). First a comparison with a non-transfected supernatant spiked with the pure standard and extracted as the unknown samples was done (external calibration). The good reproducibility of the migration time (Mt) and the presence of
some important peaks taken as ‘references’ for peak assignment ensured the accuracy of the procedure. The identification of the separated components, however, was always confirmed by co-injection of the samples with one pure standard at a time. The increase of only one peak with respect to the surrounding ones was always well evidenced and confirmed the previous assignment. No peaks at the Mt of the proteins under investigation were found in the CZE profile of the non-transfected cells.

**Proinsulin metabolism in transduced cell supernatants**

hPI was produced by all the HPI cell lines investigated, at a rate ranging from 0·6 ± 0·2 (s.d.) to 1·9 ± 1·2 pmol/24 h per 10⁶ cells, while mature insulin (as expected) was never identified in these cells, except for a small amount (less than 1·0% of the total (pro)insulin-related proteins) in PHM–HPI cells (B in Fig. 3; Table 1). Looking at the sum of all the (pro)insulin-related proteins, PHM–HPI displayed the highest production efficiency. The metabolism of HepG2 cells seems to be more prone toward maturation products like 65–66 split and des64,65 (35 and 46% respectively) while in PHF culture media the major components were intact hPI and des31,32 (50 and 35% respectively) and NIH3T3 released a high amount (68% of all the (pro)insulin-related proteins) of 32–33 split. When cells were genetically modified to express the FurHPI, mature hI was found to accumulate in the culture media of all the FurHPI cell lines at a rate ranging from 0·6 ± 0·1 pmol/24 h per 10⁶ cells in PRH and NIH3T3 cells to 2·4 ± 0·3 pmol/24 h per 10⁶ cells in the HepG2 (Table 1). The conversion of intermediates to hI was decreased in the order HepG2>NIH3T3>PRH>PHF>PHM (59, 46, 32, 26 and 23% respectively). PHF and PHM showed in the media the presence of all the intermediate forms of hPI to hI maturation (C in Fig. 3), while in HepG2 cells only accumulation of des64,65 (34%) was found. Conversely, PRH released into the supernatant only a large amount of des31,32 (68%) in addition to mature hI (32%). Traces of unprocessed hPI were observed in all cells except PRH (Table 1).

**Immunological assays**

In non-transduced cells, RIA and hI IRMA values were always under the detection limit (data not shown). Comparison of results obtained by CZE with RIA and hI IRMA determinations are reported in Table 2. The sum of
all (pro)insulin-related proteins calculated by CZE was in good agreement with the RIA quantification, while IRMA value appeared overestimated with respect to the mature hI quantification by CZE.

Some of the samples were tested also with two ELISA kits developed for total and intact hPI determination on human plasma. The tests carried out directly on the cell culture media yielded an hPI overestimation of 130–600% (data not shown). This was corrected neither by 1:8000 dilution with a human serum pool, nor by purification of the supernatant or by the procedure described for CZE.

Discussion

The gene therapy approach to insulin-dependent diabetes mellitus requires the development of a retroviral vector-based system able to induce the permanent production, in a regulated way, of mature insulin in endocrine (Clark et al. 1997) or non-endocrine (Falqui et al. 1999) cell lines. To evaluate the metabolic activity of transfected cells, however, an accurate method able to detect the residual hPI, mature hI and all the other related cleavage products is necessary. The determination of hI and intact hPI (Deberg et al. 1998, Houssa et al. 1998) without interference from the intermediates is now possible thanks to the availability of RIA and ELISA tests for plasma or serum based on the use of specific monoclonal antibodies. However, the quantification of each single split- or des- form remains still laborious. Some groups successfully employ HPLC to separate hPI, hI and the intermediate forms and RIA to quantify the proteins in the collected fractions (Nagi et al. 1990, Vollenweider et al. 1992, Ostrega et al. 1995). Alternatively, cells are labeled with [3H]leucine, [35S]cysteine or [35S]methionine, immunoprecipitated with anti-insulin antibodies and the radioactivity associated with each peak evaluated directly on the collected.

Table 1 CE analysis of supernatants of retrovirally transduced cells. Values are means ± s.d. pmol/24 h per 10^6 cells of three different cell incubations. Each purified sample was injected into the CZE five times

<table>
<thead>
<tr>
<th>HPI cells</th>
<th>hPI</th>
<th>32–33split</th>
<th>des31,32</th>
<th>65–66split</th>
<th>des64,65</th>
<th>hI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHM</td>
<td>1.9 ± 1.0</td>
<td>0.6 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>1.6 ± 1.0</td>
<td>0.5</td>
<td>5.8 ± 1.0</td>
</tr>
<tr>
<td>PHF</td>
<td>1.8 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>1.6 ± 0.6</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>3.6 ± 1.2</td>
</tr>
<tr>
<td>NIH3T3</td>
<td>0.6 ± 0.2</td>
<td>1.3 ± 0.9</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.9 ± 0.9</td>
</tr>
<tr>
<td>HepG2</td>
<td>0.6 ± 0.3</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 0.6</td>
<td>n.d.</td>
<td>3.7 ± 0.9</td>
</tr>
</tbody>
</table>

FurHPI cells

<table>
<thead>
<tr>
<th></th>
<th>hPI</th>
<th>32–33split</th>
<th>des31,32</th>
<th>65–66split</th>
<th>des64,65</th>
<th>hI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHM</td>
<td>1.3 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>0.6 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>1.6 ± 0.5</td>
<td>6.8 ± 1.0</td>
</tr>
<tr>
<td>PHF</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.9 ± 0.6</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.3</td>
<td>4.6 ± 1.0</td>
</tr>
<tr>
<td>PRH</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.3 ± 0.5</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.6 ± 1.0</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td>NIH3T3</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.6 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>HepG2</td>
<td>0.2 ± 0.02</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.4 ± 0.3</td>
<td>2.4 ± 0.3</td>
<td>4.1 ± 1.3</td>
<td></td>
</tr>
</tbody>
</table>

n.d. under the detection limit.

Table 2 Comparison between CZE, RIA and IRMA quantification of total (pro)insulin related-proteins and mature hI. Values are means ± s.d. pmol/24 h 10^6 cells of three determinations

<table>
<thead>
<tr>
<th>HPI cells</th>
<th>Total (pro)insulin-related proteins</th>
<th>Mature hI</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIA</td>
<td>CZE</td>
<td>IRMA</td>
</tr>
<tr>
<td>PHM</td>
<td>6.0 ± 1.5</td>
<td>5.8 ± 1.0</td>
</tr>
<tr>
<td>PHF</td>
<td>2.3 ± 0.7</td>
<td>3.6 ± 1.2</td>
</tr>
<tr>
<td>NIH3T3</td>
<td>2.2 ± 1.6</td>
<td>1.9 ± 0.9</td>
</tr>
<tr>
<td>HepG2</td>
<td>3.8 ± 1.3</td>
<td>3.7 ± 1.6</td>
</tr>
</tbody>
</table>

FurHPI cells

<table>
<thead>
<tr>
<th></th>
<th>Total (pro)insulin-related proteins</th>
<th>Mature hI</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIA</td>
<td>CZE</td>
<td>IRMA</td>
</tr>
<tr>
<td>PHM</td>
<td>4.1 ± 1.2</td>
<td>6.8 ± 1.0</td>
</tr>
<tr>
<td>PHF</td>
<td>5.0 ± 2.3</td>
<td>4.6 ± 1.0</td>
</tr>
<tr>
<td>PRH</td>
<td>1.3 ± 0.2</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td>NIH3T3</td>
<td>1.5 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>HepG2</td>
<td>4.5 ± 1.4</td>
<td>4.1 ± 1.3</td>
</tr>
</tbody>
</table>

n.d. under the detection limit.

n.a. quantification not available.
fractions (Neerman-Arbez et al. 1993, Sizonenko et al. 1993, Kaufmann et al. 1997). Although these remain the only methods providing the required sensitivity for the determination in plasma of all the proinsulin intermediates, the approach is based on two or more steps and on the use of labeled compounds. Recently, an isotope dilution MS (ID-MS) method was proposed for insulin analysis using labeled compounds. Recently, an isotope dilution determination in plasma of all the proinsulin intermediates, only methods providing the required sensitivity for the cross-reactivity of 65–66 split and/or des64,65 in the IRMA assay is of minor importance because in the beta cells’ secretory pathway these intermediates are negligible. Otherwise, use of an IRMA assay may lead to an overestimated hI quantification when dealing with non-endocrine cells having an hI cleavage metabolism different from that of beta cells, or when other factors (insulin-like growth factor, degradation products) may interfere. The hPI quantification by ELISA on cell supernatant failed to provide reliable results, probably because of a matrix effect generated by the culture media components or by the reagents employed in the solid-phase extraction. To minimize the source of misreading, a more specific sample pre-treatment (precipitation step) is probably necessary (Kjems et al. 1993).

The first finding of this study with respect to our previous investigation (Falqui et al. 1999) is that although the gene construct inserted into the cells was the same, the hPI conversion pathway appeared different from the primary to the stabilized cell lines, maybe due to the combined action of endogenous endoproteases (Vollenweider et al. 1992) or modified enzyme-to-substrate ratio. The quasi-quantitative pattern of the products released into the medium by transfected cells may vary in relation to the gene constructs and constitutive or co-transfected PC3, PC2, furin (Vollenweider et al. 1992, 1995, Yanagita et al. 1993, Groskreutz et al. 1994, Falqui et al. 1999) or SPC4 (subtilisin-related proprotein convertases) conversion enzymes (Smeekens et al. 1992), and a spontaneous production of hPI derivatives was noted also after subcutaneous administration of hPI in vivo (Peavy et al. 1985).

A very low amount of mature hI was found only in the supernatant of PHM–HPI which comprised also all the intermediate forms and showed the highest production rate on the sum of (pro)insulin related proteins. The 32–33 split and/or des31,32 deriving from furin cleavage of wild-type hPI at the B chain/C-peptide junction (Hutton 1994) were detected in different percentages among the tested cells. Intermediates 65–66 split and/or des64,65, known to have an increase in receptor binding and biological potency in vitro and in vivo as compared with intact hI (Peavy et al. 1985), were released only by PHM–HPI and HepG2–HPI, maybe by the co-action of another widely expressed enzyme (PACE4) (Hutton 1994). The expression system used here did not fully reproduce the conditions within normal beta cells because of the lack of the specific endoproteases and of the regulated secretory pathway, but the processing conditions occurring in non-endocrine transfected cells could favor the action of furin, which has a neutral pH optimum instead of the more acid one required for PC2 and PC3 action (Smeekens et al. 1992).

The efficacy of double-mutated hPI gene transduction and of the prohormone processing was confirmed by the high amount of mature hI released into the supernatant by all the transfected cells. In addition to mature hI, all the four intermediates together with the precursor hPI were found in PHM and PHF and the conversion rate to hI (23–29% respectively) was similar to that of PRH (32%) but lower than in the other tested cells (46–59%). In HepG2 cells, hI maturation occurred the most efficient way (59%), probably related to the highest constitutive furin content (Smeekens et al. 1992, Yanagita et al. 1993), although the unprocessed hPI was still present and the distribution (percentage) of the processing intermediates was different with respect to that found in the primary cells. However, an incomplete post-translational processing of hPI to mature hI was also observed in engineered insulinoma cell lines when proinsulin was expressed at very high levels (Clark et al. 1997).

On the basis of the results obtained we can now better explain the previously demonstrated efficacy of the in vivo transplantation of primary human cells (Falqui et al. 1999). Moreover, we showed that some of the tested cell lines, although unable to quantitatively process hPI to mature hI, released a considerable amount of 65–66 split and/or des64,65, whose activity should be taken into account for a possible therapeutic utilization. These cells seem to be a promising model to develop a regulated insulin-release machinery, being highly expressing lines with efficient maturation of hPI into hI and all the processing intermediates, showing low proliferation and in vitro and in vivo stability of their phenotypic characteristics. Future clinical application of engineered cells requires a regulated release of insulin. Since non-endocrine cells do not possess the mechanisms for regulated (exocitotic) secretion, a conceivable approach is to regulate the level of hormone synthesis by expressing the gene under the transcriptional control

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of glucose-inducible regulatory sequences (Lu et al. 1998). Alternatively, an inducible release of insulin from engineered cells has been recently obtained by engineering a fusion protein, FKBPI2-insulin, that accumulates as aggregates in the endoplasmic reticulum. Rapid release of insulin can be stimulated by a drug that induces protein desegregation (Rivera et al. 2000).

In conclusion, the method proposed proved appropriate for the screening of the metabolic products of human and rodent cells engineered for the non-regulated constitutive released of mature insulin.

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