The endoproteolytic processing of proproteins in the secretory pathway depends on the expression of selected members of a family of subtilisin-like endopeptidases known as the prohormone convertases (PCs). The main PC family members expressed in mammalian neuroendocrine cells are PC2 and PC1/3. The differential processing of proglucagon in pancreatic ß-cells and intestinal L cells leads to production of distinct hormonal products with opposing physiological effects from the same precursor. Here we describe the establishment and characterization of a novel ß-cell line (ßTC-ΔPC2) derived from PC2 homozygous null animals. The ßTC-ΔPC2 cells are shown to be similar to the well-characterized ßTC1–6 cell line in both morphology and overall gene expression. However, the absence of PC2 activity in ßTC-ΔPC2 leads to a complete block in the production of mature glucagon. Surprisingly, ßTC-ΔPC2 cells are able to efficiently cleave the interdomain site in proglucagon (KR 70–71). Further analysis reveals that ßTC-ΔPC2 cells, unlike ßTC1–6 cells, express low levels of PC1/3 that lead to the generation of glicentin as well as low amounts of oxyntomodulin, GLP-1, truncated GLP-1, and N-terminally extended GLP-2. We conclude that ßTC-ΔPC2 cells provide additional evidence for PC2 as the major convertase in ß-cells leading to mature glucagon production and provide a robust model for further analysis of the mechanisms of proprotein processing by the prohormone convertases.

Since the discovery of propeptide processing in the production of mature insulin (1), endoproteolytic cleavage of cellular proteins to generate biologically active products has become recognized as a widespread and fundamental regulatory mechanism of the proteome. This type of processing occurs in multiple subcellular compartments, including the cytoplasm, various organelles, and on membrane surfaces (2–6). Consequent to initial findings that proinsulin processing occurs in the regulated secretory pathway, multiple small peptide hormones and neuropeptides are now recognized as being derived from larger precursors by similar processing in post-Golgi compartments (7–13). Early studies of these processing events led to the recognition that peptide cleavage usually occurs C-terminal to dibasic amino acid motifs, followed by removal of the C-terminal basic amino acids by carboxypeptidases, such as carboxypeptidase E (14). Following the discovery of the yeast calcium-dependent subtilisin-like endopeptidase kexin (Kex2p), which acts on dibasic motifs in the ß mating factor and killer toxin precursors in the distal yeast secretory pathway (15–17), the cloning and characterization of related mammalian enzymes soon followed (18–20). These proteases have been designated subtilisin-like prohormone or proprotein convertases (SCPs, or more simply PCs)1 in recognition of their role in processing, not only prohormones, but a wide variety of other precursor proteins that traverse the secretory pathway. Although the mammalian PC family is now quite large, consisting of seven members with varying basic residue specificities, those mainly responsible for precursor processing in the regulated secretory pathway are now believed to be PC3 (also called PC1, and here referred to as PC1/3), PC2 (13), and to a lesser extent PC5/6A (21–23).

The PCs are all calcium-dependent serine endopeptidases with acidic pH optima consistent with their function in the calcium-rich acidic secretory granules of neuroendocrine cells. A recent solution of the crystal structure of the PC furin confirms their structural relationship to the subtilase family and provides more detailed insight into the structural basis of substrate recognition (24). Like subtilisin, these proteases become active by autocatalytic cleavage of an N-terminal propeptide, which is required for folding of the proenzymes (10, 25). A downstream domain of about 150 amino acids, called the P- or Homo B-domain (8, 9), is also required for folding and activity. This domain plays a regulatory role, influencing both the calcium dependence and pH optima (26). The variable C-terminal regions of the PCs are less conserved and play a role in their subcellular localization (10, 27).

Although PC1/3 and PC2 share many biochemical and functional characteristics, subtle structural differences must account for observed differences in their recognition of distinct dibasic amino acid motifs within precursors (8, 9, 13). This functional refinement between PC1/3 and PC2 becomes strikingly apparent in the processing of proglucagon, a multifunctional precursor that contains multiple cleavage sites recognized with varying efficiencies by these two convertases (28–

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1 The abbreviations used are: PC, prohormone convertase; GLP-1, -2, glucagon-like peptides 1 and 2; Tricine, N-[2-hydroxy-1,1-bis(hydroxy-methyl)ethyl]glycine; RT, reverse transcription; MCA, 7-methoxy coumarin; MOPS, 4-morpholinoepanesulfonic acid; SSPE, saline/sodium phosphate/EDTA.
31). In this way proglucagon can be processed in different cells to yield unique mixtures of products with differing functions. Thus, in the pancreatic α-cell abundant expression of PC2 is associated with the nearly exclusive production of glucagon (28, 30), whereas in intestinal L cells processing, mediated mainly by PC1/3, leads instead to the production of glucagon-like peptides 1 and 2 (GLP-1 and GLP-2) (29, 31).

We describe the development and characterization of a transformed pancreatic α-cell line from PC2 null mice (32), which has provided the opportunity to study proglucagon processing in a pure population of α-cells in vitro in the absence of PC2 activity.

MATERIALS AND METHODS

Reagents—The human 7B2 CT peptide, 1–31 amino acids, was a kind gift from Dr. Iris Lindberg of Louisiana State University Medical Center, New Orleans, LA. The rabbit anti-GLP-1 antibody was a gift from Dr. Jens J. Holst of the Panum Institute, University of Copenhagen, Denmark. All the cell culture reagents and superscript first strand synthesis system for RT-PCR were purchased from Invitrogen. The TaqDNA polymerase and protein A-agarose were from Roche Diagnostics.

The RNeasy kit for total RNA extraction was purchased from Qiagen. The Prime-It II kit, sonicated salmon sperm DNA, and Duro-Blot membrane were from Stratagene. The fluorogenic substrate, pyrGlu-Arg-Thr-Lys-Arg-MCA (36), in the absence or presence of the PC2 inhibitory peptide, human 7B2 CT peptide (1–31 amino acids) (37), at a final concentration of 10 μM. The assay mixture was incubated for 18 h at 37 °C, and the amount of MCA liberated from the substrate as a result of enzymatic cleavage in each sample was measured following quenching with 1 M acetic acid as described before (37).

Northern Blotting—Total RNA from each cell type was extracted following Qiagen’s protocol. Equal amounts of total RNA were size-fractionated in 1% agarose gels containing 0.2M MOPS (pH 7.0) and 2.2 mM formaldehyde and transferred onto Duralon membrane. It was baked at 80 °C for 2 h under vacuum to immobilize RNA. Membrane was pre-hybridized at 42 °C for 2 h in a solution containing 50% formamide, 6× SSPE, 0.5% SDS, 5× Denhardt’s solution and 0.1 mg/ml sonicated salmon sperm DNA and hybridized in the same solution overnight at 42 °C in the presence of radiolabeled, denatured cDNA probe (specific activity, 106 dpm/μg) in a hybridization oven. Membrane was washed in 2× SSC at 50 °C for 30 min (2× 15 min) followed by 0.2× SSC plus 0.1% SDS at 60 °C for 45 min before exposure to film. In some cases, more stringent washes were performed using 0.1× SSC plus 0.1% SDS at 65 °C. For removal of hybridization probe to re-probe with a different cDNA, membrane was placed in a boiling stripping solution of 0.1× SSPE with 0.5% SDS for at least 10 min and rinsed with 1× SSPE before prehybridization. The cDNAs, used as hybridization probes, were synthesized by RT-PCR using specific sense and antisense primers for each gene.

Microarray Analysis—αTC1–6 and αTC-ΔPC2 cells were processed for microarray analysis as described previously (38). Affymetrix set 430 microarrays, which assess over 34,000 mouse genes, were used. Analysis was performed in duplicate, and values were averaged.

RESULTS

Establishing an α-Cell Line Lacking Catalytically Active PC2—To obtain a neuroendocrine cell line devoid of active PC2 and to better define the contribution of PC2 in the processing of proglucagon in the pancreatic islets, an α-cell line lacking PC2 activity was produced. The Glu2-TAg6 transgenic animal is known to produce pancreatic glucagonomas at a high frequency through expression of the SV40 large T antigen from the rat glucagon promoter (32, 39). Mating Glu2-TAg6 animals to the previously described PC2 null animals (32) yielded animals that were PC2 homozygous null and carrying the Glu-TAg transgene (see “Materials and Methods”). A single cell line was isolated from the resulting pancreatic α-cell tumors that was characterized further. This cell line has been designated αTC-ΔPC2.

Under light microscopic analysis, αTC-ΔPC2 cells exhibit morphological features similar to those of the well characterized αTC1–6 cell line (see Fig. 1, A and B). Furthermore, immunoelectron microscopic analysis demonstrates that, like αTC1–6 cells, αTC-ΔPC2 cells contain numerous secretory granules with (pro)glucagon-like positive immunoreactivity (see Fig. 1, C and D).

αTC-ΔPC2 Cells Express a Set of Genes Similar to αTC1–6 Cells and Unlike βTC-3 Cells—To further characterize the new cell line, the expression of several pancreatic islet cell-specific...
transcripts were defined by RT-PCR. Like αTC–1–6 cells, αTC–ΔPC2 cells express proglucagon at high levels, confirming their similarity to normal α-cells (Fig. 2). Like αTC–1–6 and unlike the β-cell line βTC-3, αTC–ΔPC2 shows no expression of proinsulin 1 or proinsulin 2. Because the PC2 null allele produces a transcript but no catalytically active PC2 (32) and the RT-PCR primers used in our analysis exclude the exon 3 region of PC2, all three cell lines produce a PC2 transcript that is of the same size. Also, we have not seen detectable levels of prosomatostatin in either αTC–1–6 or αTC–ΔPC2 cells (data not shown). Therefore, like the well characterized αTC–1–6 α-cell line, the new αTC–ΔPC2 cells express transcripts known to be found in α-cells (proglucagon and PC2) and fail to express transcripts known not to be expressed in α-cells (proinsulin 1 and 2 and prosomatostatin), suggesting that the new cell line has maintained its α-cell phenotype through cell line derivation.

To confirm that the PC2 transcript found in the αTC–ΔPC2 cells corresponds to the null allele, Northern blotting analysis was undertaken. The transcript from the null allele is 144 bp shorter than the wild-type transcript. Blotting for PC2 in βTC3, αTC–1–6, and αTC–ΔPC2 cells demonstrates that, as expected and seen in Fig. 2, all three cells express PC2. Furthermore, the transcript for the PC2 null allele demonstrates a slightly increased mobility consistent with its shorter transcript size (Fig. 3A). Anterior pituitary derived AtT20 cells and nonendocrine COS-7 cells, used as controls, do not express detectable levels of PC2. Also, consistent with data from Fig. 2, both α-cell lines express high levels of proglucagon, unlike βTC-3 cells.

αTC–ΔPC2 Cells Lack Enzymatically Active PC2—Previous characterizations of islets from PC2 homozygous null mice have demonstrated that the null allele leads to the production of a truncated, inactive proPC2 protein of ~72 kDa (32). This protein is not secreted and therefore must be degraded intracellularly (32). To confirm the production of this inactive PC2 peptide, immunoblotting was carried out on cell extracts from βTC-3, αTC–1–6, and αTC–ΔPC2 cells. Both βTC-3 and αTC–1–6 cells show high levels of mature PC2 and smaller levels of proPC2 (75 kDa) as seen previously (28) (see Fig. 3B). The αTC–ΔPC2 cells, however, produce a faster migrating, truncated proPC2 protein, along with lesser amounts of degraded or incorrectly processed peptides, none of which correspond to wild-type active PC2. Interestingly, blots of BiP levels show no significant differences between the PC2 null line and several other neuroendocrine cell lines, indicative of a lack of endoplasmic reticulum stress (see Fig. 3C).

Biochemical analysis of culture media from αTC–1–6, αTC–ΔPC2, and βTC-3 cell lines for PC2 enzyme activity demonstrates high levels of activity in both αTC–1–6 and βTC-3 (Fig. 4). As expected, this activity is inhibited by the PC2-specific inhibitor, TB2 CT peptide (36, 40, 41). On the other hand, αTC–ΔPC2 culture media demonstrated no specific PC2 activity, similar to negative control media from NIH-3T3 cells. These results indicate that the αTC–ΔPC2 cell line maintains an α-cell phenotype in the absence of active PC2 and therefore provides an appropriate model of α-cell function for the study of propeptide processing in the absence of PC2 activity.

Proglucagon Processing in αTC–ΔPC2 cells—The endocrine cell-specific processing of proglucagon is a highly regulated and complex reaction leading to the production of differing mixtures of bioactive peptide hormones, depending on cell type and complement of expressed convertases (28–31). Analysis of cellular protein extracts by immunoblotting for proglucagon and glucagon-containing peptide products confirm that αTC–1–6 cells efficiently process proglucagon to mature glucagon along with considerable amounts of glicentin and glicentin-related polypeptide-glucagon intermediates (see Fig. 5A). In contrast, αTC–ΔPC2 cells produce no mature glucagon, but instead accumulate large amounts of glicentin. This differs significantly from the nearly complete block in proglucagon processing seen in pancreatic islets of PC2 null animals (42), suggesting the presence in αTC–ΔPC2 cells of another PC not normally expressed. Pulse-chase analysis of secreted proglucagon-derived peptides confirm that glicentin is a major secreted product along with significant amounts of proglucagon in αTC–ΔPC2 cells at both 45 min and 3 h post-labeling (see Fig. 5B). Significantly, mature glucagon is again not detected. Unlike cellular extracts however, the secreted protein contains a small but significant amount of oxyntomodulin from the αTC–ΔPC2 cells, indicating late or very inefficient processing at the dibasic site Lys31–Arg32. Therefore, the partial processing of the interdomain cleavage site and Lys31–Arg32 led to production of glicentin and oxyntomodulin, but not mature glucagon, from the N-terminal domain of the proglucagon in αTC–ΔPC2 cells.

To investigate potential processing of the C-terminal domain of the proglucagon in the αTC–ΔPC2 cells, GLP-1 production was assessed. Using antisera to GLP-1, we demonstrate the
presence of both GLP-1(1–37) and truncated GLP-1 (both 7–37 and 7–36 NH₂) at low levels in media from αTC-ΔPC2 cells (see Fig. 5C). In the same cell extracts we detect GLP-1(1–37) but no truncated forms (see Fig. 5C), indicating late processing at the monobasic site, Arg77. Also, we show the generation of N-terminally extended GLP-2(111–158) at low levels in αTC-ΔPC2 cells using anti GLP-2 antibody (see Fig. 5D). αTC-1 cells by contrast secrete mature glucagon with significant amounts of glicentin and glicentin-related polypeptide-glucagon and small amounts of oxyntomodulin (see Fig. 5B), as well as GLP-1 and extended GLP-2, but no truncated GLP-1 fragments (see Fig. 5, C and D).

The above results thus demonstrate that efficient early cleavage at the interdomain KR motif (residues 70 and 71) to produce glicentin and later minor cleavages at the dibasic Lys31-Arg32 and Arg109-Arg110 and monobasic Arg77 to generate oxyntomodulin, and the N-terminally extended forms of GLP-1 and GLP-2 and tGLP-1 occur in the absence of PC2 activity in the αTC-ΔPC2 cells. These processing events are also consistent with the probable expression of another convertase in the αTC-ΔPC2 cells. Because PC1/3 has been shown to be capable of cleaving at these sites (29, 31), we investigated the possibility that PC1/3 is expressed in these cells. Northern blot analysis of total RNA from αTC1–6, αTC-ΔPC2, and βTC-3 cells indicates that αTC-ΔPC2 cells show low, but detectable, levels of PC1/3, whereas αTC1–6 cells show no indication of PC1/3 expression (Fig. 6A). As expected, αT20 and βTC-3 cells show robust expression of PC1/3 (19, 20, 26). As negative controls RNA from NIH-3T3, COS-7, and Chinese hamster ovary cells show no PC1/3 expression. To confirm the presence of PC1/3 protein in the αTC-ΔPC2 cells, immunoblots for PC1/3 were carried out on protein extracts from the above four cell lines (see Fig. 6B). Confirming previous results (28) and the above Northern blot, αTC1–6 showed no PC1/3 expression, whereas βTC-3 and AtT20 cells showed high levels of mature PC1/3. Consistent with the low but detectable levels of PC1/3 transcript in αTC-ΔPC2, protein extracts from these cells show low levels of mature PC1/3 protein. It is thus likely that PC1/3 also accounts for the low level of cleavage of synthetic substrate seen in αTC-ΔPC2 cell media (see Fig. 4) and both the observed interdomain cleavage, as well as the later products of proglucagon (see Fig. 5).

Microarray Comparison of αTC1–6 and αTC-ΔPC2 Cell Lines—Because of the unexpected finding of expression of PC1/3 in the αTC-ΔPC2 cells, we carried out a microarray gene expression analysis, as previously described (43), to examine a broader range of α-cell-specific markers. Salient findings are summarized in Table I. The results confirm the expression of PC1/3 at low levels in αTC-ΔPC2 cells, but not in the αTC1–6 cell line. However, many other α-cell-specific markers, including Arx, Brain-4, c-Maf, and, of course, proglucagon, did not differ significantly. The same was true for other neuroendocrine markers common to both α- and β-cells, such as 7B2, chromogranin B, PC2, and proSAAS. β-Cell markers were also largely absent except for PC1/3 and, surprisingly, a low but
significant level of PDX-1 expression, which may correlate with the observed elevation of islet amyloid polypeptide (amylin) gene expression. Assessment of PC5/6 transcripts by both RT-PCR and microarray analysis showed negligible expression in both cell types (data not shown).

**DISCUSSION**

The differential processing of proglucagon in pancreatic α cells to glucagon and in intestinal L cells to GLP-1 and GLP-2 has been a major area of investigation (28–31). Although the PC family of convertases is large and expressed in multiple tissues, the primary enzymes responsible for neuroendocrine proprotein processing are PC1/3 and PC2 (13). PC2 null animals demonstrate profound defects in proglucagon processing in pancreatic α-cells, producing only very small amounts of glicentin and no detectable mature glucagon (32, 42). This is associated with mild chronic hypoglycemia accompanied by a dramatic increase in α-cell mass in the islets. Although PC2 is expressed in many other neuroendocrine cells in the body, proof that the lack of active glucagon brings about the above phenotypes has been provided in experiments where exogenous glucagon was delivered via long term, slow release osmotic pumps. Replacement of glucagon in these animals led to normalization of both blood glucose levels and islet α-cell mass (34). However, because of potential interactions between different islet cell types, partially overlapping expression of convertases in the different cell types, and difficulty in obtaining purified α-cells from animal models, many questions regarding the function of the PC2 null α-cells...
were unanswered. To address these issues we have derived an α-cell line from PC2 null animals that has allowed the investigation of glucagon processing in isolated α-cells in the absence of PC2 catalytic activity. The characterized cells have been designated αTC-ΔPC2 cells.

Morphologically, αTC-ΔPC2 cells resemble the previously described αTC1–6 cells with a characteristic epithelioid shape and containing numerous secretory granules with (pro)glucagon-like immunoreactivity (32). Our results indicate that these cells express appropriate RNA and protein markers for α-cells while lacking PC2 activity. Genome-wide expression analysis also provides a detailed analysis of cellular state (43), and our findings comparing the expression of islet amyloid polypeptide.

Identical to the previously characterized islets from PC2 null animals, αTC-ΔPC2 cells produce a form of PC2 that is not processed and demonstrates no enzymatic activity (see Figs. 3 and 4). The expression of the PC2 transcript and production of the protein for this inactive enzyme at levels comparable to that of PC2 in wild-type α-cells (αTC1–6), however, further confirm the α-cell nature of these cells. Moreover, although the truncated PC2 protein is degraded intracellularly, there is no indication of endoplasmic reticulum stress. BiP levels are not altered (see Fig. 3C).

Analysis of glucagon processing in αTC-ΔPC2 cell extracts demonstrates a complete lack of mature glucagon production, as expected if PC2 is necessary for one or more of the required processing reactions (see Figs. 5 and 7). The major product, however, is not glucagon, but rather glicentin, suggesting the ability of the cells to catalyze the cleavage reaction at the dibasic site at amino acids 70–71 (KR). Analysis of glucagon processing in intact islets from PC2 null animals demonstrates a much more limited ability of the primary tissues to carry out this reaction (42) Pulse-chase experiments analyzing secreted proteins from the αTC-ΔPC2 cells further suggest that an additional protease is present in these cells that is able to cleave at the dibasic sites, Arg31-Lys32 and Arg109-Arg110, to produce small amounts of oxyntomodulin and both the N-terminally extended forms of GLP-1 and GLP-2. Also, this additional protease could process the monobasic Arg77 site to generate low amounts of tGLP-1 (nonamidated and amidated peptides). Northern blotting analysis demonstrates (see Fig. 6) that the αTC-ΔPC2 line shows weak, but detectable, levels of PC1/3 transcript compared with other cell lines that are known to express PC1/3 (AtT20 and βTC-3 cells). This is further confirmed by microarray analysis (see Table I).

In both normal α-cells and the αTC1–6 cell line, expression of PC1/3 is undetectable, similar to negative control cells (NIH-3T3, COS-7, and Chinese hamster ovary cells). These findings therefore suggest that the strong initial processing at the 70–71 dibasic motif and the less efficient processing at the 31–32, 109–110, and 77 sites in αTC-ΔPC2 cells could be carried out by the small amounts of PC1/3 present in this cell line. The finding of small amounts of truncated GLP-1 (see Fig. 5C) is also consistent with this notion (31).

That PC1/3 is normally involved in the L cell processing of proglucagon to GLP-1 and GLP-2 is borne out by recent studies on mice lacking PC1/3 (44). Intestinal extracts from these mice contain mainly intact proglucagon and only small amounts of glicentin (44, 45). However, there is no defect in proglucagon processing in the pancreas of these animals, and the ultrastructure of the α-granules is completely normal, in contrast to the PC2 null α-cells (46). Studies of proglucagon processing in a variety of neuroendocrine cells having differing levels of both PC1/3 and/or PC2 indicate that the 70–71 site is highly sensitive to cleavage (28–31). It is cleaved first in both α and L cells, indicating that both enzymes can readily recognize this site. The low levels of PC1/3 in αTC-ΔPC2 cells, however, produce only low levels of the normal L cell products, including oxyntomodulin, GLP-1, extended GLP-2, and tGLP-1 (see Fig. 7).

The lack of detectable expression of PC5/6, another possible

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**Table I**

Selected results of mouse expression set 430 microarray analysis of αTC1–6 and αTC-ΔPC2

| Source Transcript Signal ΔPC2 Detectiona ΔPC2 | TC1–6 Detection | ΔPC2 vs. TC1–6 | Fold change | ΔPC2 vs. TC1–6 | ChangeΔPC2 vs. TC1–6 | Probe set |
|----------------------------------------------|----------------|----------------|------------|---------------|----------------------|-----------|
| α-Cells                                      |                |                |            |               |                      |           |
| Arx                                          | 1,447          | P              | 760        | P             | Less than 2         | NC        |
| Arx                                          | 604            | P              | 561        | P             | Less than 2         | NC        |
| Brain-4                                      | 1,530          | P              | 1,428      | P             | Less than 2         | NC        |
| Brain-4                                      | 582            | P              | 605        | P             | Less than 2         | NC        |
| c-Maf                                        | 599            | P              | 912        | P             | Less than 2         | NC        |
| Proglucagon                                  | 11,816         | P              | 15,725     | P             | Less than 2         | NC        |
| α- and β-Cells                               |                |                |            |               |                      |           |
| 7B2 protein                                  | 6,644          | P              | 7,358      | P             | Less than 2         | NC        |
| Chromogranin B                               | 15,757         | P              | 11,340     | P             | Less than 2         | NC        |
| Nkx2-2                                       | 5,020          | P              | 4,143      | P             | Less than 2         | NC        |
| Pax6                                         | 3,453          | P              | 1,688      | P             | Less than 2         | NC        |
| Pax6                                         | 870            | P              | 473        | P             | Less than 2         | NC        |
| PC2                                          | 11,536         | P              | 11,682     | P             | Less than 2         | NC        |
| ProSAAS                                       | 7,450          | P              | 5,633      | P             | Less than 2         | NC        |
| β-Cells                                      |                |                |            |               |                      |           |
| Glucokinase                                  | 21             | A              | 21         | A             | Less than 2         | NC        |
| Glut-2                                       | 44             | A              | 43         | A             | Less than 2         | NC        |
| HBH                                          | 44             | A              | 44         | A             | Less than 2         | NC        |
| Proinsulin I                                 | 212            | A              | 157        | A             | Less than 2         | NC        |
| Proinsulin II                                | 180            | A              | 41         | A             | 4                     | NC        |
| IAPP                                         | 1,975          | P              | 130        | P             | 15                    | I         |
| Pax6                                         | 8              | A              | 4          | A             | Less than 2         | NC        |
| PC1/PC3                                      | 893            | P              | 39         | A             | 23                    | I         |
| PDX1                                         | 511            | P              | 18         | A             | 28                    | I         |
| PDX1                                         | 68             | A              | 6          | A             | 11                    | I         |

*a* P, present; A, absent.

ΔNC, no significant change detected; I, increased.

* Islet amyloid polypeptide.

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FIG. 7. A scheme to show the differences in proglucagon processing between αTC1–6 and αTC-APC2 cells.

The scheme includes, at the top, a diagram of proglucagon molecule with its various subsegments, and at the bottom, a flow chart depicting proglucagon processing in αTC1–6 and αTC-APC2 by PC2 and PC1/3, respectively. The letters K and R represent the amino acids Lys and Arg, respectively. The arrows at the top of the diagram indicate the potential cleavage sites for PC2 and/or PC1/3.

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