Original Article

Probe-Independent and Direct Quantification of Insulin mRNA and Growth Hormone mRNA in Enriched Cell Preparations

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Task division in multicellular organisms ensures that differentiated cell types produce cell-specific proteins that fulfill tasks for the whole organism. In some cases, the encoded mRNA species is so abundant that it represents a sizeable fraction of total mRNA in the cell. In this study, we have used a probe- and primer-free technique to quantify such abundant mRNA species in order to assess regulatory effects of in vitro and in vivo conditions. As a first example, we were able to quantify the regulation of proinsulin mRNA abundance in β-cells by food intake or by the glucose concentration in tissue culture. The second example of application of this technique is the effect of corticosteroids on growth hormone mRNA in enriched somatrotrophs. It is anticipated that other examples exist in which measurement of very abundant mRNAs in dedicated cells will help to understand biological processes, monitor disease states, or assist biotechnological manufacturing procedures. ***Diabetes*** **55:3214–3220, 2006**

Now that increasing numbers of genomes have been sequenced, a major goal in biology is to understand how specialized cells use parts of the genome to produce the proteins required for their function (1). Questions related to the expression and function of networks of interacting genes in a complex biological process can now be addressed on a genome-spanning basis, using mRNA expression microarrays (2), analysis of the proteome (3), or phenotypic changes caused by systematic mRNA silencing (4). In the process of performing microchannel electrophoresis (5) quality controls for microarray mRNA expression analysis, we noticed that some transcripts are expressed at such a high level that microarrays are not quantitative because of probe saturation (6). Moreover, we observed that such transcripts can be quantified accurately and directly, i.e., without participation of primers and probes, but only using microchannel electrophoresis, an often-used separation technique for low amounts of cellular RNA (5). The potential of the cRNA detection method is illustrated in this study in two cases: *1*) regulation of pancreatic islet β-cell insulin mRNA expression level by food intake (7), by the glycemia (8), or by glucose concentration in tissue culture (9–13) and *2*) regulation of pituitary somatothroph growth hormone mRNA abundance by corticosteroids. These specific cases were chosen because of preexisting knowledge in the literature, our expertise to enrich the responsible mRNA-producing cells from a mixture of hormone-producing cells, and the potential biomedical relevance of this regulation.

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AUC, area under the curve; DMEM, Dulbecco's modified Eagle's medium; FACS, fluorescence-activated cell sorter.

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RESEARCH DESIGN AND METHODS

Preparation of tissues and purified cells. All experiments based on laboratory animals were approved by committees for animal welfare at Katholieke Universiteit Leuven, the Vrije Universiteit Brussel, and the Université Catholique de Louvain. Control mouse and rat tissues (liver, skeletal muscle, brain, and seminal vesicles) were hand dissected from male Wistar rats (± 3 months of age) and male random-fed or overnight-fasted C57Bl6 mice (8–10 weeks of age), rinsed in PBS, frozen in liquid nitrogen, and stored at −80°C. Islets of Langerhans were collagenase isolated from the rat or mouse pancreas after injecting collagenase solution in the pancreatic duct. Digestion of mouse pancreata was 3 min at 37°C, after which islets and acini were separated at 0°C by three rounds of sedimentation (1*g*) and handpicking under a dissection microscope. For further purification, rat islets were dissociated using trypsin/DNase into single cells (14) and sorted into purified non–β-cell and β-cell fractions via autofluorescence-activated cell sorting (15). Purity of β-cells (>90% insulin-positive cells) and non-β-cells (>75% glucagons-positive cells) was assessed via immunocytochemistry (15). Male Wistar rat pituitary somatotrophs and lactotrophs were purified according to cell size/density from trypsin/DNase-digested anterior lobes using a 0.3–2.4% BSA gradient in Dulbecco's modified Eagle's medium (DMEM) to separate the cells as previously described (16), yielding fractions enriched in somatotrophs and lactotrophs (purity 70 and 65%, respectively).

Cell and tissue culture. Rat islets were precultured for 1 week in RPMI-1640 medium containing 10 mmol/l glucose and 5 g/l BSA (17). The medium was renewed after 1 day and then every other day thereafter. The islets were then cultured overnight in RPMI-1640 medium supplemented with different glucose concentrations (2–30 mmol/l). Islet β-cells, as well as pituitary somatotrophs and lactotrophs, were reaggregated on a gyratory shaker at 37°C before tissue culture. Islet β-cells were cultured for 72 h in serum-free Ham's F10 medium containing 1% BSA at different glucose concentrations (18). Somatotrophs and lactotrophs were cultured for 5 days in serum-free medium based on a mixture of Ham's F12 and DMEM (19) supplemented with 50 pmol/l T_3, either with or without 20 nmol/l dexamethasone. Rat INS-1 832/13 cells were obtained from

Dr. C.B. Newgard (Duke University, Durham, NC) and were grown in RPMI-1640 medium supplemented with 11 mmol/l D-glucose (20). MIN6-cells were obtained from Dr. Eiji Yamato (Division of Stem Cell Regulation Research, Osaka University Medical School, Osaka, Japan) and cultured in DMEM containing 25 mmol/l glucose and 15% heat-inactivated FCS (21).

RNA extraction. Total RNA from mouse and rat tissues (liver, muscle, brain, and seminal vesicles), rat pituitary, and rat pancreatic α- and β-cells was extracted using TRIzol Reagent according to the manufacturer's protocol (Gibco, Carlsbad, CA), followed by a clean-up procedure with RNeasy columns (Qiagen, Cologne, Germany). Total RNA from rat and mouse islets, as well as mouse acinar tissues and purified somatotrophs and lactotrophs, was extracted using Absolutely RNA microprep (Stratagene, CA). Total RNA from MIN6 and INS-1 cells was extracted using the RNeasy mini columns (Qiagen) according to manufacturer's protocol. The total RNA quantity and quality was determined using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) and the 2100 Bioanalyzer (Agilent, Waldbronn, Germany), respectively. Total RNA profiles of all tested samples were similar, with sharp 18S and 28S rRNA peaks on a flat baseline. PolyA-mRNA was extracted starting from total RNA using the Oligotex mRNA mini kit (Qiagen) according to the manufacturer's protocol.

mRNA expression analysis via microchannel electrophoresis. Microchannel electrophoresis of abundance of major mRNA species in total cellular mRNA was performed using commercially available precast gels (nanochips; Agilent) that were run on the Agilent 2100 Bioanalyzer. Analysis of extracts was performed in the context of preparing Affymetrix (Santa Clara, CA) microarray experiments. Briefly, total cellular mRNA was reverse transcribed into cDNA (SuperScript Choice System; Invitrogen, Carlsbad, CA) using oligo-dT primers and a T7 RNA polymerase promoter site. In all cases, the cDNA was in vitro transcribed and biotin labeled for further microarray analysis using a commercially available kit (Affymetrix IVT labeling kit) and its precursor, the Bioarray high-yield RNA transcript labeling kit (Enzo, Farmingdale, NY) for the rat tissues and β-cells/non-β-cells. The obtained cRNA profile of rat and mouse pancreatic cell, brain, liver, muscle, and rat pituitary preparations and mouse seminal vesicle preparations was analyzed on the Bioanalyzer using ~200 ng cRNA. The relative abundance of major cRNA species was expressed as percentage of the total area under the curve (AUC) under the cRNA profile. On basis of the molecular standards used in the same assay (RNA 6000 Ladder; Ambion, Austin, TX), the transcript length of the major species could be calculated.

Quantitative RT-PCR insulin and growth hormone mRNA expression analysis. cDNA of rat islets, β-cells, non-β-cells, and INS-1 cells was prepared using the Reverse-IT first-strand synthesis kit (Abgene, Epsom, U.K.) using random decamers according to the manufacturer's protocol. The quantitative PCR was performed using the RotorGene3000 (Corbett Research, Sydney, Australia) and the Absolute QPCR-mix (Abgene) according to the protocol. Specific primers and dual-labeled probe for insulin had the following nucleotide sequence: 5′-ATCTTCAGACCTTGGCACTGGA-3′ (forward), 5′-GTAGAG GGAGCAGATGCTGGT-3′ (reverse), and 5′-FAM-ATCCACAATGCCACGCTTC TGCCG-TAMRA-3′ (probe) (Proligo, Boulder, CO).

Quantitative RT-PCR for growth hormone mRNA was performed as described previously (22) by use of the TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA). Specific intron-spanning primers and labeled probe (Eurogentec, Seraing, Belgium) for detection of growth hormone mRNA had following nucleotide sequence: 5′-CTCGGACCGCGTCTAT GAGA-3′ (forward) B:5′-TGAGGATCTGCCCAATACGG-3′ (reverse), and 5′-FAM-CCGTCTTCCAGCTCCTGCATCAGAG-TAMRA-3′ (probe).

Specific primers and minor groove binding probe for β-actin were ordered using a TaqMan gene expression assay from Applied Biosystems. The ΔΔCt method was used for relative quantification, since there was no difference in efficiency between insulin and actin mRNA or growth hormone and actin mRNA in the validation experiment. The sample with the highest ΔCt value was chosen as calibrator.

Construction of insulin cRNA standards. Mouse islet cDNA was produced from total RNA with the RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany) using oligo-dT primers in a 20-μl reaction. One microliter of a 10-times–diluted cDNA was then used in a 25-μl PCR with modified primers (5′-GGATCCTAATACGACTCACTATAGGGAGGCCAG CTATA ATCAGAGACCA-3′ forward) (5′-TTTTTTTTTTTTTTTCATTATAAAA CTCTTTTTT TTGG TGC-3′ reverse) specific for mouse insulin one mRNA. The forward and reverse primer contained a T7-promoter sequence and an oligo-dT sequence, respectively (underlined) (23). One microgram of the PCR product was next used in a 20-μl in vitro transcription reaction (AmpliScribe T7 Transcription kit; EpiCentre Biotechnologies, Madison, WI), and the cRNA concentration was measured in duplicate using the NanoDrop ND-1000 spectrophotometer. The number of molecules present in the mixture was calculated with the equation:

$$N = \frac{C}{MW} \times N_A$$

where N is the number of molecules per microliter, C is the cRNA concentration in grams per microliter, MW is the molecular weight of the cRNA in grams per mole, and N_A is the Avogadro constant. A threefold dilution series was prepared, of which the standard with the highest concentration contained 1×10^{12} insulin cRNA molecules. Standards, either pure or spiked into pancreatic acinar cell cRNA, were analyzed on the 2100 BioAnalyzer (Agilent Technologies, Waldbronn, Germany), and the absolute AUC of the insulin peaks was measured and plotted versus the number of insulin cRNA molecules.

Statistical analysis. Results are expressed as means ± SD or SE of experiments with at least three independent biological replicates. Statistical significance was assessed by a two-way unpaired Student's t test. A P value <0.05, corrected by the Bonferroni method in case of multiple comparisons, was considered to be statistically significant.

RESULTS

Presence of major mRNA species in pancreatic exocrine and endocrine cells. When we prepared cRNA from purified islets of Langershans (endocrine) and isolated acini (exocrine) from whole collagenase digests of mouse pancreas, we noticed (Fig. 1) that the microchannel electrophoresis profiles were reproducible for the cell type and different between cell types. In mouse acini, three major complexes (composed of one to three peaks) were detected. The most abundant cRNA (length 950 nt) represented 13.4 ± 0.5% of all expressed cRNA. Two other cRNA fractions (estimated size 1,660 and 2,100 nt) each contribute to >5% of total cRNA. Freshly isolated islets of Langerhans, handpicked from the same collagenase pancreas digestion, contained very similar cRNA species, eluting between 34 and 44 s, although their relative abundance was at least 10 times lower than in purified acini. In contrast, the islets contained a predominant cRNA (estimated length 549 ± 9 nt) that was detectable in purified acini but at a >200-fold lower level than in islets. The major mouse islet cRNA species was about equally abundant in rat and mouse islets (Fig. 2*A–B*) but ~30-fold less abundant in mouse or rat insulinoma cells (Fig. 2*C–D*). The high expression in whole islets can be ascribed to insulin-producing β-cells as illustrated by cRNA profiles of fluorescence-activated cell sorter (FACS)-purified β-cell and non-β-cell preparations (Fig. 1*E* and *F*). In contrast, purified non-β-cells contained a specific abundant transcript with estimated length of 1,485 ± 42 nucleotides. The pancreas specificity of these profiles was illustrated by the observation that none of the major cRNA species were detected in cRNA profiles of total liver, skeletal muscle, and brain from mice or rats (online appendix Fig. 1 [available at http://diabetes.diabetesjournals.org]). We noticed that the obtained relative abundance of a major cRNA species was reproducible for a given cell type or condition. For instance, the abundance of the major cRNA in purified β-cells (means ± SD 20.1 ± 0.3%, $n = 3$) had a coefficient of variation of only 1.5%. Since variation in the abundance of this cRNA in non-β-cells was much more important (0.9 ± 0.7%, $n = 4$, range 0.3–1.8%), we hypothesized that β-cell contamination in the non-β-cells was responsible for this signal. Microarray analysis of expression of specific β-cell markers (e.g., GLUT2, PC1, and GAD1) confirmed this notion (data not shown); therefore, we concluded that the major cRNA species in islets is β-cell specific. Conversely, the major cRNA species in non-β-cells was present with a reproducible abundance (6.1 ± 0.6%, $n = 4$, range 5.5–6.7%), whereas cRNA with the same length was just detectable as a second minor peak in whole islets or FACS-sorted β-cells.

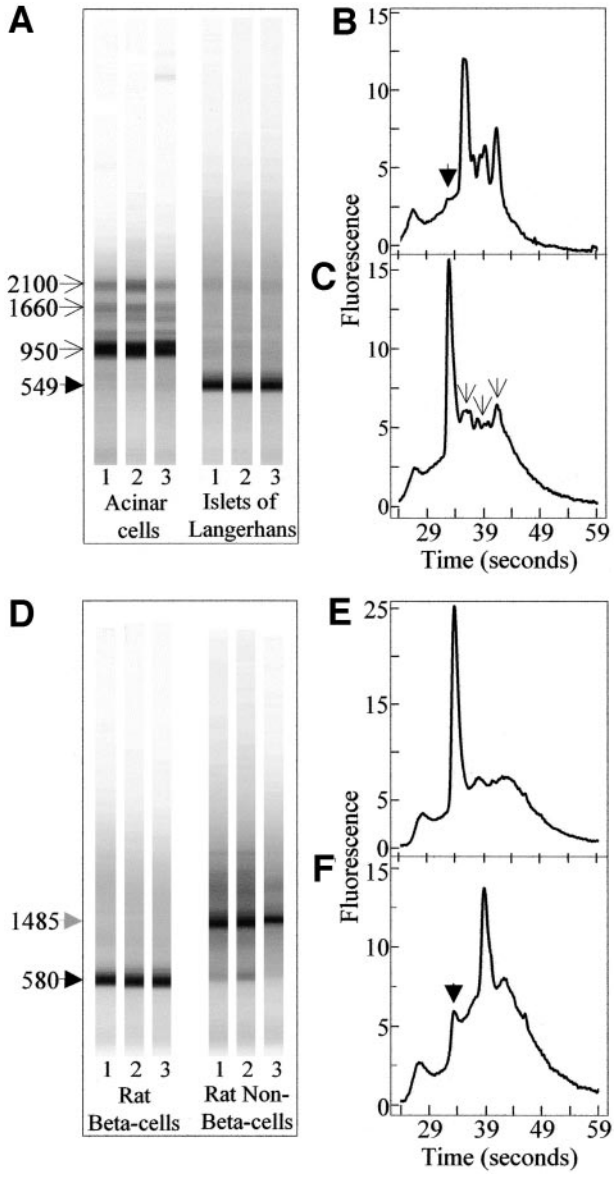

FIG. 1. Microchannel electrophoresis of cRNA profiles from pancreatic cell populations. Imaginary gels (*A* and *D*) and cRNA profiles (*B*, *C*, *E*, and *F*) from total cellular cRNA prepared from primary pancreatic cell preparations. *Lanes 1–3* represent independent biological replicates of the same cell type. Male C57Bl6 mouse pancreas (*A–C*) was digested by collagenase (3 min, 37°C) and separated into acini and islets of Langerhans immediately afterward. Rat collagenase-isolated islets (*D* and *F*) were dissociated into single cells and further purified via FACS into β-cells and non-β-cells (15). Estimated abundance of the major cRNA species in acinar cells (length 950 nt) was 13.4 ± 0.5%. The same cRNA species were detected in whole islets (open arrow), but the abundance was 10 times lower. Whole islets and purified β-cells express a major cRNA species with estimated size of 550 nt (mouse) and 497 nt (rat) and a relative abundance of 20.1 ± 0.3% (rat-purified β-cells) and 12.5 ± 1.1% (mouse islets) of total cRNA. Please note that cRNA of the same length (closed arrow) is also present in acinar cells (abundance ~0.1%) and in non-β-cells (abundance 0.9 ± 0.7%). The major cRNA species in non-β-cells (abundance 6.1 ± 0.6%) has an avarage transcript length of 1,485 nt.

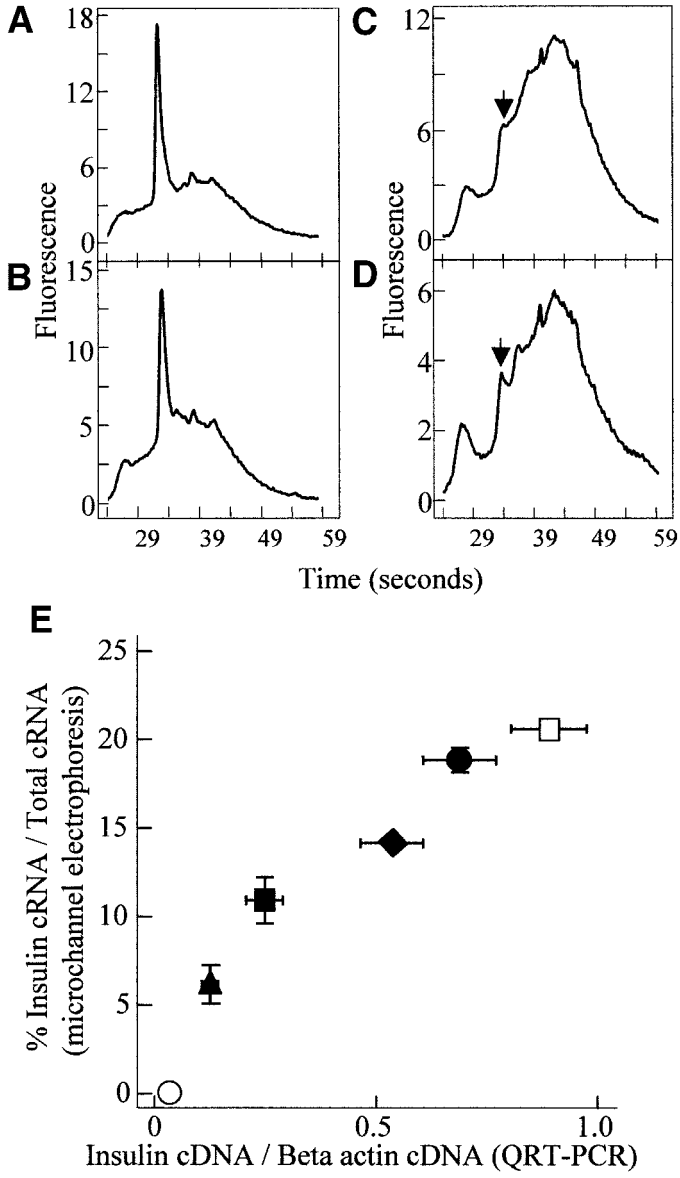

FIG. 2. Major difference in the abundance of the major islet cRNA species in primary islets versus insulin-producing cell lines. Shown are representative microchannel electrophoresis analysis of cRNA profiles (n = 3) of rat (*A*) and mouse (*B*) islets and glucose-responsive β-cell lines INS-1 (*C*) and MIN6 (*D*). The 10- to 100-fold higher abundance of the major cRNA species in primary islets is paralleled by a similar difference in cellular insulin content (data not shown). *E*: Comparison of insulin cRNA quantification via the microchannel electrophoresis method and real-time RT-PCR in samples from rat pancreatic cell preparations and cell lines. The relative abundance of insulin cRNA (% of total cRNA) detected via the microchannel electrophoresis method correlates well (R = 0.93) with real-time RT-PCR data (normalized for β-actin). Data represent means ± SE of at least three replicates. ○, INS1-cells; □, FACS-purified rat β-cells; ▲, ■, ●, and ♦, rat islets cultured at 2, 5, 10, or 30 mmol/l glucose, respectivley.

Validation of the microchannel electrophoresis cRNA quantification via real-time RT-PCR. The overall abundance, as well as cell specificity of the expression of the major cRNA species in rodent pancreatic β-cells, suggested that the material corresponded to proinsulin mRNA. This hypothesis was further supported by the predicted length (497 ± 12 nt rat and 549 ± 9 nt in mouse), which is ~60–90 nt larger than the predicted length without poly A-tails in public database (460–469 nt for mouse; see NM_008386 and NM_008387) and similar to the measured length of rat insulin mRNA in Northern blots (7). The full length of preproglucagon mRNA in rat pancrease was estimated by Northern blots to be 1.4 Kb long, which is similar to the currently estimated size of 1,485 nt (24). To further document the identity of the detected major cRNA species of β-cells, we next compared the result of microchannel electrophoresis quantification to the out-

come of real-time RT-PCR on the same samples. Figure 2*E* shows the correlation analysis of 20 different rat endocrine pancreatic cRNA samples (19 primary samples and 3 samples from INS-1 cells) that were analyzed in parallel with the two different methods. It was thus observed (Fig. 2*E*) that the relative abundance of insulin cRNA (% of total cRNA) as predicted by the microchannel cRNA electrophoresis method correlates well (R = 0.93) with the observed real-time RT-PCR insulin expression data, normalized for β-actin.

Linear range and detection limit of the microchannel electrophoresis method. To investigate the sensitivity and linear range of the microchannel electrophoresis quantification method of major mRNA species, we prepared a set of insulin 1 cRNA standards that were measured as such or spiked into cRNA samples prepared from mouse pancreatic acinar cells. As seen in Fig. 3, virtually the same relationship is observed, indicating that the Bioanalyzer technique is linear over a large concentration range and that the addition of insulin cRNA in a mixture of different cellular cRNA species does not alter (favor or interfere) with its detection. With the used protocol, we estimate the detection limit of insulin cRNA at 10^{10} molecules. By loading variable amounts of cRNA from the same sample onto the system (Fig. 4*A*), it could be shown that the insulin–to–total cellular cRNA ratio is a characteristic of the studied tissue (mouse islets in this case) and not dependent upon small variations in loading. Indeed, whereas the AUC of the internal standard RNA (spike running at 22 s) is constant, the AUC of the insulin cRNA (peak with running time 33 s), and that of total cRNA, is directly linear with the amount of loaded material. It could be argued that the preparation of cRNA, using reverse transcriptase and in vitro transcription, artificially creates major cRNA species that does not exist as mRNA in normal tissue. Therefore, we examined the abundacy and elution time of a major mRNA species following a more "direct" protocol in which the cellular mRNA was enriched from total RNA extract via oligo-dT columns and run on the Bioanalyzer (online appendix Fig. 2). As at least 100 μg total RNA was needed in this protocol (at least 10,000 islet equivalents), we performed this analysis on RNA from seminal vesicles, a tissue that also expresses a major mRNA species, detectable either directly as mRNA or as cRNA (online appendix Fig 2*B* vs. *C*). Thus, we conclude that the used protocol in the main document does not selectively enrich abundant or small RNA species but allows estimation of the ratio of that transcript species over the total mRNA expressed in a sensitive and reliable manner.

Regulation of insulin mRNA abundance by glucose and food intake. To further illustrate the quantitative power of microchannel cRNA electrophoresis in studying a biological process, we evaluated with this new technique the well-known effect of food intake in vivo (7) and of D-glucose during tissue culture (9–11) upon insulin mRNA abundance in rodent islets or FACS-purified β-cells. The ex vivo approach on freshly collagenase-isolated islets from overnight-fed and -fasted animals is shown in Fig. 5*D*. Confirming previous observations based on Northern blots (7), the relative insulin cRNA abundance decreased by 20% in overnight-fasted animals (P = 0.01). In agreement with the literature (8,11), it seems likely that this food intake–dependent regulation is related to periods of glucose stimulation. Indeed, the insulin cRNA abundance in rat FACS-purified β-cells can be maintained during 3 days of

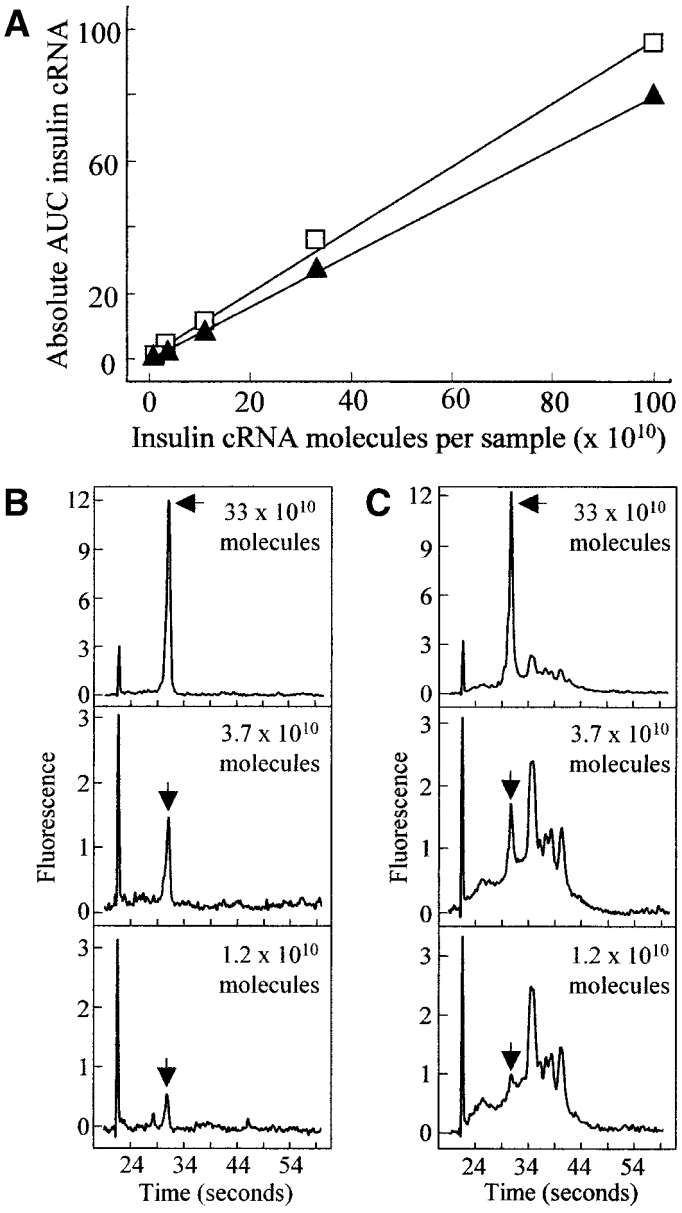

FIG. 3. Linearity and detection limit of microchannel electrophoresis insulin mRNA quantification. *A*: Standards of insulin 1 cRNA either alone (□) or spiked into acinar cRNA extract (▲) were loaded onto the Bioanalyzer, and the AUC of the peak eluting at second was calculated. R^2 of each of the two lines = 0.999. Representative elution profiles are shown for low, intermediate, and high insulin cRNA standards alone (*B*) or cRNA spiked into acinar cRNA (*C*).

tissue culture close to the ex vivo level of expression, when the culture glucose concentration is 10 mmol/l, while up to 60% loss of expression occurs during culture in 5 mmol/l glucose (Fig. 5*A* and *B*). This effect was not a particular result of the chosen medium, nor an artifact of β-cell purification, because it was reproduced in whole islets of Langerhans that were cultured in RPMI medium (Fig. 5*C*). Thus, we were able to reproduce with this technique, using two different preparations of β-cells and two time points (18 and 72 h), that glucose concentrations in the physiological range have a powerful effect on the insulin cRNA copy number per cell. Culture conditions in hyperglycemic conditions, both with the FACS-purified β-cells and the whole islets (Fig 5*B* and *C*, respectively), show a biphasic effect of glucose, as the relative insulin

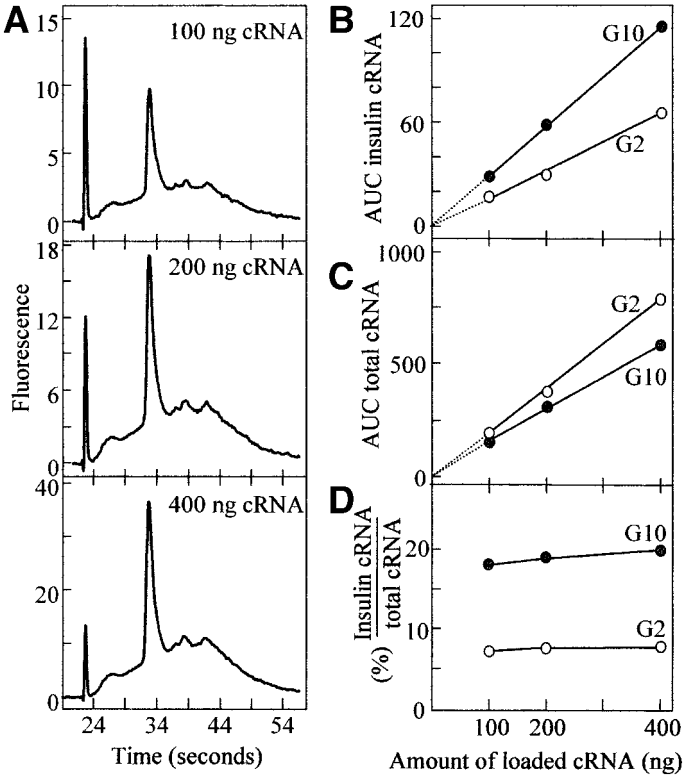

FIG. 4. Microchannel electrophoresis measurement of relative insulin cRNA/total cRNA abundance: independence of amount of loaded cRNA. *A*: cRNA profiles of three different quantities of cRNA (100–400 ng) prepared from rat islets of Langerhans that were cultured for 18 h at 10 mmol/l glucose. $AUC_{insulin}$ (*B*) and AUC_{total} (*C*) increase linearly with the amount of loaded material, but the $AUC_{insulin}$-to-AUC_{total} ratio (*D*) is constant for the tested experimental condition. Therefore, the stimulatory effect of glucose on the relative insulin mRNA abundance (threefold between 2 and 10 mmol/l glucose; see also Fig. 5) is detected, irrespective of the amount of loaded material.

cRNA abundance is decreased compared with 10 mmol/l glucose. Fig. 4 shows that the experimental results cannot be attributed to variations in the amount of loaded cRNA but are related to the glucose concentrations during culture. Thus, the first example underlines the quantitative power of this technique, allowing the quantification of expression of a particular transcript as a proportion of all expressed cRNA molecules.

Regulation of growth hormone mRNA abundance by dexamethazone. Because the microchannel cRNA electrophoresis could quantify, in a probe-independent manner, major mRNA species that are expressed in pancreatic cell populations, we next assessed its applicability to the abundance of growth hormone, the most abundant transcript in somatotrophs from the pituitary (Fig. 6). Like the pancreatic islet of Langerhans, the mammalian pituitary is a complex hormone-secreting tissue composed of several different cell types. Since the profiles of whole pituitary microchannel cRNA electrophoresis revealed abundant cRNA "species" with a broad peak (Fig. 6*A–B*), some degree of molecular heterogeneity was suspected. We therefore separated dispersed anterior pituitary cells into fractions, one enriched in prolactin-producing lactotrophs and another in growth hormone–producing somatotrophs, and determined whether specific microchannel electrophoresis cRNA expression profiles existed (Fig. 6*C–D*). Like in the case of FACS-purified islet cell populations, albumin-gradient–separated pituitary lactotrophs and somatotrophs produced slightly different cRNA profiles, notably a cell type–specific major cRNA species (Fig. 6*C–D*) with a relative abundance of up to 4% of total mRNA. The most abundant cRNA species in the lactotroph-enriched fraction was larger (estimated length 1,070 ± 21 nt) than the most abundant major species detected in the somatotroph-enriched cell population (887 ± 17 nt). These lengths correlate well to the estimated full sizes of rat prolactin mRNA (1,016 nt including a 20-nt poly-A tail; see NM_012629) and growth hormone mRNA (790 bp without poly-A tail; see V01237). Furthermore, we noticed that during a 5-day aggregate cell culture of the purified cell populations, there was a specific effect of 20 nmol/l dexamethasone upon growth hormone mRNA abundance (Fig. 6*E–F*), as assessed by the microchannel electrophoresis method (Fig. 6*E*) and via real-time RT-PCR (Fig. 6*F*). Interestingly, the somatotroph-enriched cell population also contained a second, less abundant cRNA species (estimated length 1,022 ± 27 nt); this species is likely to reflect proopiomelanocortin cRNA (predicted length 967 nt, including a 29-nt poly A-tail; see NM_139326) because its abundance, assessed via the microchannel electrophoresis method and real-time RT-PCR, was downregulated (with 35 and 55%, respectively) by dexamethasone in aggregate cell culture (data not shown).

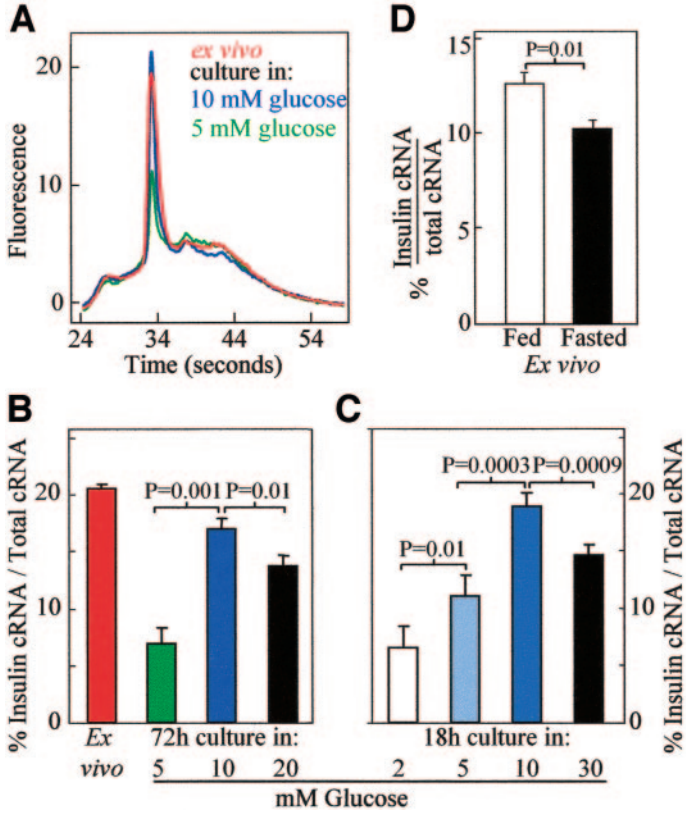

FIG. 5. Effect of extracellular glucose and feeding/fasting on insulin cRNA abundance in islet β-cells. *A*: Microchannel electrophoresis cRNA profiles from a representative experiment comparing freshly isolated rat β-cells (red) versus β-cells cultured for 72 h in either 5 mmol/l (green) or 10 mmol/l (blue) glucose. *B–C*: Insulin mRNA is regulated by glucose in a bimodal manner with maximal relative abundance in β-cells (*C*) or islets (*D*) cultured at 10 mmol/l glucose. *D*: Comparison of the insulin mRNA abundance of islets in the fed and fasted state. Data are means ± SD of three to four independent biological replicates.

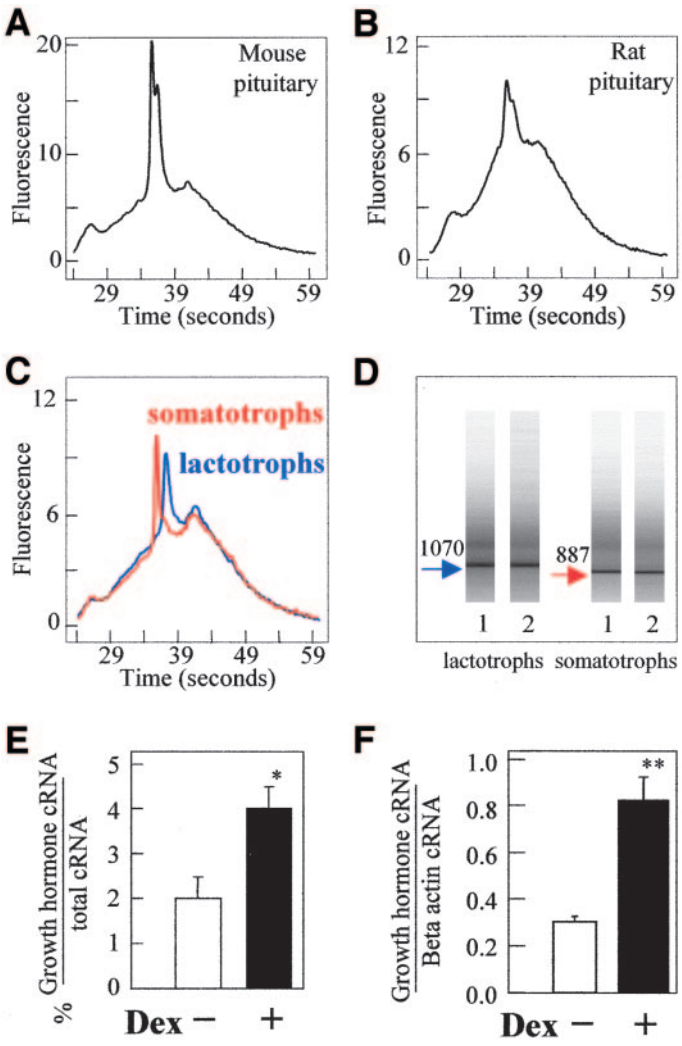

FIG. 6. Microchannel electrophoresis profiles of cRNA prepared from male Wistar rat pituitary preparations. *A–B*: Ex vivo detection of major pituitary cRNA species (estimated length 900 nt) appears to be the merger of at least two different expressed major cRNA species. Image from gels (*D*) and representative profiles (*C*) from cultured male pituitary cell populations, enriched in lactrotropic and somatotropic cells by unit gravity gradient sedimentation. Major cRNA species in these pituitary subpopulations (up to 4% of total cRNA) have distinguishable size (respectively 1,070 ± 21 and 887 ± 17 nt, $n = 4$). *C–D*: The influence of 20 nmol/l dexamethasone during 5-day cell culture (indicated as Dex) on the abundance of the major cRNA species in somatotrophs was assessed by the microchannel electrophoresis method; the major cRNA species is identified as growth hormone mRNA on basis of length and dexamethasone-regulated abundance as measured via real-time RT-PCR (*F*). Data are means ± SD of 3–4 experiments. Statistical significance of difference between control and dexamethasone cultures was calculated using the two-tailed unpaired Student's *t* test. *$P = 0.02$, **$P < 0.01$.

DISCUSSION

Microchannel electrophoresis was introduced ~7 years ago to quantify total (ribosomal) RNA in low numbers of cells (5) and has been used to measure PCR-amplified cDNA fragments (25). The technique has been used in a widespread manner to control the quality of total RNA extracted from cells or tissues. Data in this article illustrate that the microchannel cRNA electrophoresis method is a quantitative and reproducible technique to directly measure the relative abundance of one or several major mRNA species that are present in the total cellular mRNA pool. Experiments with mouse seminal vesicles allowed direct comparison between major RNA species in mRNA and cRNA profiles. From this comparison, we consider it unlikely that major RNA species detected in cRNA profiles are artificial, e.g., by favoring reverse transcription or in vitro transcription of small RNA transcripts. The generated data with this novel technique, both for insulin and growth hormone, correlate reasonably well with results obtained by real-time RT-PCR experiments. Generally, PCR data are normalized for a "housekeeping gene" like actin or glyceraldehyde-3-phosphate dehydrogenase with the potential drawback of artifacts generated by small changes in expression level of the housekeeping gene. On the contrary, the microchannel cRNA electrophoresis can give extra information as it expresses the percentage of the total messenger RNA pool a cell type dedicates to the expression of a particular transcript. In other words, instead of showing the ratio between two specific transcripts, the technique quantifies the fraction of the transcipt over all mRNA in the cell. The profiles should, therefore, predict a basis for translational activity in the examined tissue. We thus saw a remarkable similarity between the SDS-PAGE profile of ^{35}S-methionine–labeled acini on the one hand (Fig. 2 in ref 26) and the cRNA profile of such cells (Fig. 1*A*). Furthermore, the biphasic effect of culture glucose on insulin mRNA abundance β-cells (Fig. 5) correlates well with the biphasic effect of culture glucose on the maximal rates of cellular proinsulin biosynthesis (11,18).

The chance to observe major mRNA species depends on the intrinsic properties of the cell type and the purity. It was estimated before, based on hybridization kinetics between mRNA and cDNA, that the most abundant class of mRNA species in liver (27) and heart (28) have a copy number of 20.000–30.000 per cell, which may be insufficient to be noticed as major cRNA species with the current technique at the whole organ or tissue level. Our data indicate that 20% of the mRNA mass in β-cells represents proinsulin mRNA. It is known that rat β-cells contain ~0.75 pg total RNA per cell (29,30). When it is assumed that between 2 and 4% of this material represents mRNA, the results of the present method indicate that a differentiated adult β-cell contains 100.000–200.000 insulin mRNA molecules, which is close to the previously estimated number obtained via real-time RT-PCR (12). Knowing that the cDNA-cRNA enriches/amplifies ~50-fold and that one β-cell contains ~10^5 insulin mRNA molecules, the prediction is that the technique works by putting extract representing about one islet on the column, which is precisely what we have observed. This means that only the most abundant mRNA species, such as those present in pancreas or peptide-secreting cells of the pituitary, can be quantified via the microchannel cRNA electrophoresis method.

In conclusion, we show in this article that microchannel cRNA electrophoresis is a reproducible and accurate method to directly quantify very abundant transcripts in specialized endocrine cells. Because no antibody, probe, or primer is involved, we believe the described technique can be generalized to other cell types (wild-type or engineered cells) that are specialized in producing large quantities of a given transcript species. Specific new examples of its usefulness are expected for the bioindustry in controlling recombinant secretory protein expression, as well as for developmental studies of pituitary and pancreatic cells.

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