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Expression of the *ras*-related *rab3a* gene in insulinoma-derived cell lines

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This study was designed to search for the expression of the small-molecular-weight GTP-binding protein *rab3a* in endocrine pancreatic cell lines. Total RNA was isolated from five different cell lines (RINm5F, RIN 104836, β-TC1, HIT-15, and INRI-G9) and from whole rat brain. The expression of *rab3a* was analyzed by Northern blots. Similar as in brain two transcripts of 1300 and 1800 bp were detected in RIN-cells at low stringency conditions with the predominant signal at 1300 bp. At high stringency the stronger signal was at 1800 bp. When a 300 bp *Pst*I fragment derived from the coding region of *rab3a* was utilized as probe the 1800 bp signal was predominant under each condition. Only a faint band at 1800 bp occurred in preparations from βTC1-cells and no signal at all was found in HIT-15 and INRI-G9-cells. In conclusion, *rab3a* is expressed in rat insulin-releasing insulinoma-derived RIN-cells with a specific 1800 bp transcription product.

rab3a; Clonal cell; RINm5F; βTC1; HIT-15; INRI-G9

1. INTRODUCTION

A broad range of cellular functions in eukaryotic cells are regulated by proteins that undergo a cycle of guanosine 5′-triphosphate (GTP)-binding and hydrolysis [1,2]. Recent work on secretory mutants of yeast [3] and permeabilized regulated secretory cells has provided more insight into exocytosis at the molecular level [4,5]. One common theme emerging is that a variety of small GTP-binding proteins, related to the oncogene proteins of the *ras*-family, have a role in many steps of intracellular membrane traffic [1,6,7] including regulated exocytosis [8–10]. It fits well into this concept that previous experiments demonstrated that guanine nucleotides induced insulin secretion in the clonal cell line RINm5F from endocrine pancreas [11] and that this particular cell line expresses several GTP-binding proteins which were so far only described by their molecular weights and isoelectric points [12]. Today, it seems likely that GTP-binding proteins are involved in every step along the secretory pathway, and there may be enough distinct GTP-binding proteins in a cell that each step may have its own specific G-protein, as proposed by Bourne [13].

One of the most carefully studied classes of these molecules consists of small monomeric proteins that show clear sequence homology to the proteins encoded by the *ras* oncogenes.

Genes exhibiting fundamental sequence homology to *ras* genes have been identified in a variety of eukaryotic organisms [14]. In mammals, the enlarged *ras* gene family also includes *ral* and R-*ras* genes, the *rho* genes and the *rab* genes [1,2,15]. It was of special interest that, at variance with all other *ras* and *ras*-like genes tested so far, one member of the *rab* family, *rab3*, showed a tissue specificity [16]. It was reported that the expression of a major 1.8 kb transcript is restricted to brain tissue [16].

The current study was undertaken to search for the expression of *rab3a* in insulinoma-derived B-cell lines and a glucagonoma cell line. It was based on the findings that rab3a is localized on synaptic vesicles, the secretory organelles of the synapse that store and release neurotransmitters [6,17] and is also expressed in endocrine tissue such as the adrenal medulla [17]. We were particularly interested in the possible expression of *rab3a* in insulin-releasing cells since an important role in regulated secretion for this and other *rab* gene products is under discussion [18].

2. EXPERIMENTAL

2.1. *Materials*

CDTA, formamide, formaldehyde, 50× Denhardt's and sonicated herring sperm DNA (10 mg/ml) and RNA markers were obtained from Sigma, Deisenhofen, Germany and LiCl from Merck, Darmstadt, Germany. RNA isolation kits and Nuctrap Probe Purification Columns were purchased from Stratagene, La Jolla, USA. Multiprime labeling system, Hybond N membrane and [α-^{32}P]dCTP (specific activity 110 TBq/mmol) were from Amersham, Braunschweig, Germany. For autoradiography the gels were exposed to X-OMAT X-ray film (Kodak) at −80°C.

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Abbreviations: CDTA; 1,2-diaminocyclohexane-*N,N,N′,N′*-tetraacetic acid; 50× Denhardt's, 1% bovine serum albumin/1% ficoll/1% polyvinylpyrrolidone; SDS, sodium dodecyl sulfate; 20×SSC, 3 M sodium chloride/0.3 M sodium citrate; bp, base pairs.

2.2. *RNA isolation*

Cells were grown under conditions as described by Praz et al. [19]. RINm5F- and RIN 104638- from rat and βTC1-cells from mouse insulinoma were kept in DMEM, 11 mM glucose, 10% horse serum, 2.5% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. INRI G9- (hamster glucagon-producing tumor cells) and HIT-15-cells (hamster insulinoma) were kept in RPMI 1640, 11 mM glucose, 10% fetal bovine serum, penicillin, and streptomycin. All cell culture chemicals were from Gibco, Eggenstein, Germany. Cells were split 1:3 after 4–6 days and media were changed after two days. Total RNA was isolated from cells according to Birnboim [20]. Whole brain from adult rats was frozen in liquid nitrogen after dissection and total RNA was isolated according to Chomzcynski and Sacchi [21] utilizing RNA isolation kits.

2.3. *Northern analysis*

A 2100 bp *Eco*RI fragment of the cDNA from rat *rab3a* (a kind gift from Drs. Zahraoui and Tavitian, Paris) and a 300 bp *Pst*I fragment of the coding region of rat *rab3a* were radioactively labeled with [α-^{32}P]dCTP utilizing random priming procedure (Amersham) according to the suppliers protocol and were purified with Nuctrap Probe Purification Columns. Total RNA was fractionated on 1% agarose gels containing 2.2 M formaldehyde [22], transfered to Hybon N membrane and immobilized by UV crosslinking. Hybridizations were performed under conditions of low and high stringency (50% formamide, 5 × Denhardt's s, 5 × SSC, and 0.1 mg/ml sonicated herring sperm DNA at 42° and 55°C, respectively) using 2–3 × 10^6 cpm of the corresponding labeled cDNA fragment. Final washings were performed in 0.1 × SSC at 42°C (low stringency) or 0.1 × SSC, 0.1% SDS at 70°C (high stringency). RNA quantity and integrity was verified by reversibly staining the Hybond N membranes with Methylene blue prior to hybridization [23]. Migration positions of the signals were calculated as compared to RNA markers.

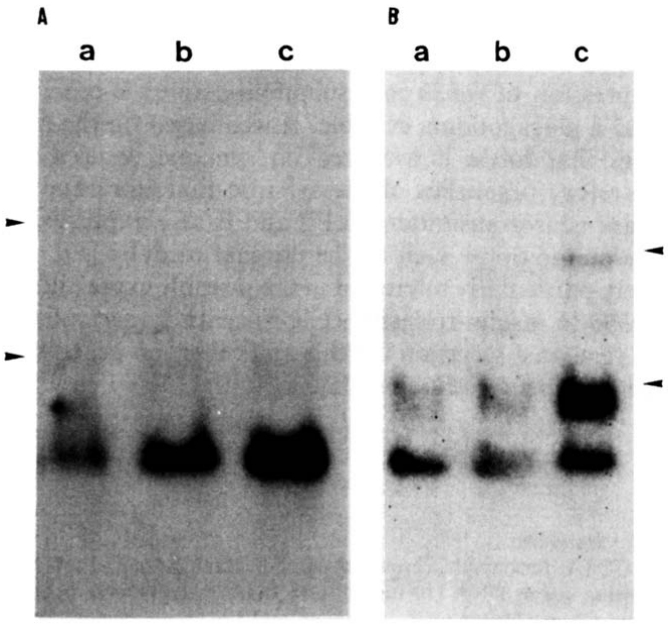

Fig. 1. Northern blot analysis of *rab3a* expression in RIN cells utilizing a 2100 bp Eco RI fragment of the rat *rab3a* cDNA. Hybridization with rat brain total RNA (lane c of both panels) served as control. Left panel (A) shows hybridization under low (42°C) and right panel (B) under high (70°C) stringency conditions (a, RINm5F-; b, RIN 104836 cells). Gels were exposed to X-ray film for 12 h (A) or 3 days (B). Arrows indicate migration positions of rRNAs.

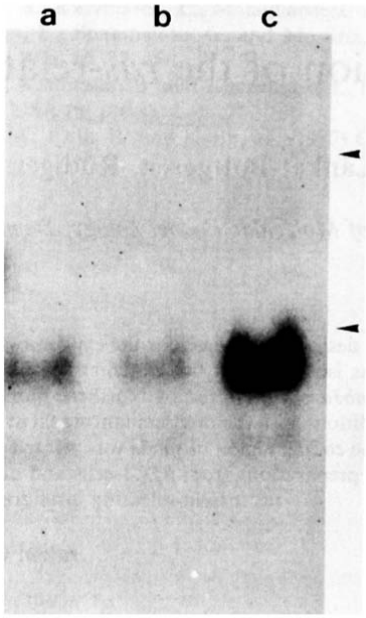

Fig. 2. Northern blot analysis of *rab3a* expression in RIN cells utilizing a 300 bp *Pst*I fragment from the coding region of the rat *rab3a* cDNA. The demonstrated result was obtained under low stringency conditions (a, RINm5F-; b, RIN 104836 cells; c, rat brain (control), 3 days exposure of gels to X-ray film). Arrows indicate migration positions of rRNAs.

3. RESULTS

Total RNA from the indicated cell lines and whole rat brain was isolated. In Northern blot analysis a 2100 bp *Eco*RI fragment of rat *rab3a* cDNA [16] was used as radioactively labeled probe. Two transcripts of 1300 and 1800 bp were detected in brain under conditions of low stringency (hybridization and final washing at 42°C) which corroborates previous findings [16]. A similar result was obtained in our studies with RINm5F- and RIN104836-cells, although the intensity of the transcript signals was somewhat weaker as compared to RNA from brain after identical exposure (Fig. 1). However, under conditions of high stringency (hybridization at 55°C and final washing at 70°C) the distribution of the intensities of the two transcripts changed. In both experiments, analyzing brain or RIN-cell RNA, the stronger signal was now associated with the 1800 bp transcript.

To further investigate the nature of the two transcripts we utilized a 300 bp *Pst*I fragment which was prepared from the coding region of the rat *rab3a* cDNA as labeled probe. Using this smaller fragment we found the 1800 bp signal predominantly even under conditions of low stringency in brain and RIN-cells (Fig. 2). The smaller transcript of 1300 bp was no more detected at all at high stringency conditions (data not shown).

Fig. 3 demonstrates that HIT-15 and INRI G9 cells which were obtained from hamster tumors revealed no expression of rab3a whereas a faint band at 1800 bp was

seen in mouse βTC1-cells after a prolonged exposure time (14 days) as compared to the signal in the RIN-cells which was already strong after only three days of exposure.

4. DISCUSSION

Low molecular weight GTP-binding proteins are strong candidates for regulators of membrane traffic [1,2]. Of main interest in this context are the *rab* gene products, among which *rab3a* deserves attention. These proteins exhibit striking similarities with the yeast *ras*-like proteins YPT1 and SEC4p which are accepted regulatory elements in the secretory pathway of *Saccharomyces cerevisiae* [24,25]. It is thought that they control the movement of the secretory vesicles and/or regulate the fusion of these vesicles with cellular membrane compartments [3,24,25]. Recently, it was shown that *rab3a* dissociates quantitatively from the vesicle membrane after Ca^{2+}-dependent exocytosis [6]. These observations make it intriguing to speculate on a role of *rab3a* in insulin secretion. However, up to now it was unknown whether *rab3a* is expressed in endocrine pancreatic cells. At first sight, this seemed even doubtful since an organ-specific brain-restricted expression of *rab3a* was previously reported [16]. Recently, it was demonstrated that *rab3* is also expressed in endocrine chromaffine cells [17] but was suggested to be exclusively localized to synaptic vesicles [17]. We report now that *rab3a* is expressed in insulinoma-derived cell lines from rat and, to a lesser extent, mouse tumors. The used probes did not hybridize with material from hamster insulinoma or glucagonoma which might indicate a certain species specificity.

Two transcripts were detected whereas our data indicate that the smaller transcript of 1300 bp is most likely a result of cross-hybridization with a mRNA species with high homology to rab3a rather than a specific transcript. The specific 1800 bp transcript found in RIN cells corresponds to an 1800 bp brain transcript under high stringency conditions.

It was proposed earlier that *rab3a* is exclusively localized to synaptic vesicles [17]. We know, furthermore, that although the synapse with its associated presynaptic specializations is a neuron-specific structure, synaptic vesicle-specific proteins are also expressed in endocrine cells that do not form synapses. Studies carried out on the pituitary and the adrenal medulla, and on cell lines derived from these tissues, have shown that in endocrine cells these proteins are localized in the membranes of a population of microvesicles distinct from peptide-containing secretory granules [27]. In this context, it is noteworthy that RINm5F cells were shown to exhibit such microvesicles [28] which are organelles of a pathway distinct from the typical regulated insulin secretory pathway. They undergo like synaptic vesicles exocytosis, endocytosis, and recycling [29]. Therefore, considering this data the expression of *rab3a* in insulin-

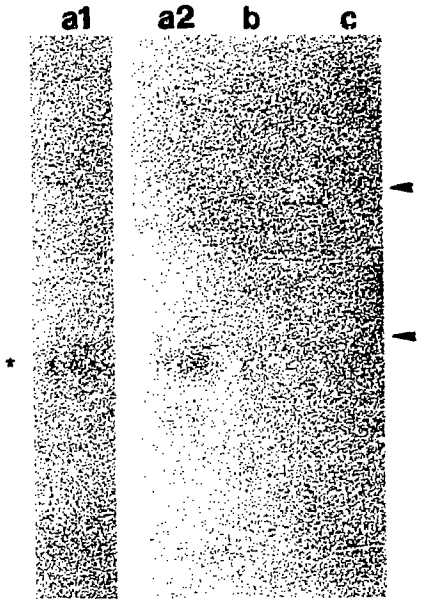

Fig. 3. Northern blot results for the analysis of material from βTC1 (a1, 70°C; a2, 42°C), INRI-G9 (b), and HIT-15 (c) cells. Gels were exposed for 14 days to X-ray film. Arrows indicate migration positions of rRNAs. The bands are indicated by an asterisk.

secreting endocrine cells does not contradict the concept of an exclusive localization of this GTP-binding protein to synaptic vesicles but, on the other hand, makes a key role for *rab3a* in the regulation of the membrane events during insulin secretion unlikely.

Furthermore, although the clonal cell lines RINm5F, RIN104836 and βTC1 are widespreadly utilized models for islet B-cell stimulus secretion coupling [11,19,26] our data do not prove that rab3a is expressed in normally differentiated B-cells. It is possible that the tumor cells share a closer relationship to neuroendocrine cells than regular B-cells. Supportive evidence for this assumption comes from studies of our laboratory which showed that RINm5F cells express the preprotachykinin-I gene in contrast to normal pancreatic B-cells [30]. A similar case was reported for the expression of tyrosine hydroxylase, a neuronal enzyme, in pancreatic B-cells [31]. Furthermore, it has been recently found that a selective induction of neuroendocrine cytodifferentiation occurs in RINm5F cells after sodium butyrate treatment [32].

In conclusion, the expression of *rab3a* in insulinoma cells rather reflects the relationship to neuroendocrine cells than adds clues to understand the late molecular events during insulin exocytosis. However, the presently utilized cell model is an easy to handle tool to further study the expression of *rab3a*.

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