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Functional properties of proximal tubule cell lines derived from transgenic mice harboring L-pyruvate kinase-SV40 (T) antigen hybrid gene

R. Lacave[1], M. Bens[2], N. Cartier[3], V. Vallet[3], S. Robine[4], E. Pringault[4], A. Kahn[3] and A. Vandewalle[2,*]

[1]Laboratoire Hospitalo-Universitaire d'Histologie et de Biologie Tumorale, Hôpital Tenon, 75020 Paris, France
[2]Unité 246, Institut National de la Santé et de la Recherche Médicale (INSERM), UER Xavier Bichat, 16 rue Henri Huchard, 75018 Paris, France
[3]Unité 129, INSERM, Institut Cochin de Génétique Moléculaire, Génétique et Pathologie Moléculaires, Faculté Cochin, 75014 Paris, France
[4]Unité de Biologie des Membranes, Département de Biologie Moléculaire, Institut Pasteur, Paris, France

*Author for correspondence

SUMMARY

This study describes the functional characterization of two cell lines derived from the proximal convoluted (PKSV-PCT cells) and proximal straight (PKSV-PR) tubules microdissected out from kidneys of transgenic mice harboring the simian virus 40 (SV40) large T and small t antigens placed under the control of the rat L-type pyruvate kinase (L-PK) 5′ regulatory sequence. Both cell lines exhibited cellular cyclic AMP stimulated by parathormone (PTH) and calcitonin (CT) and a sodium-dependent glucose transporter. Uptake of the fluid-phase marker [^{3}H]inulin showed that both cell lines grown on filters exhibited biphasic apical and basolateral endocytic rates. Results from Northern blot analysis indicate that the expression of the T antigen gene (Tag) is dependent on the concentration of D-glucose in the medium and show that the L-PK construct has maintained its capacity for up- or down-regulation by carbohydrates. Replacement of D-glucose by neoglucogenic substrates (lactate, oxaloacetate) blunted the expression of Tag transcripts and induced arrest of cell growth. Compared to cell grown in D-glucose-enriched medium, the hormonal sensitivities to PTH and CT and the sodium-dependent glucose uptake were unchanged whereas quiescent cells exhibited increased hydrolase content. Thus the proximal function has been preserved in these cultured cells derived from tissue-specific targeted oncogenesis in transgenic mice. As the expression of Tag transcripts is controlled by D-glucose, the structural and physiological characteristics of these cell lines can be studied in either quiescent or active growth conditions.

Key words: cAMP, glucose transport, hydrolase, SV40, proximal tubule cells

INTRODUCTION

The proximal tubule of the mammalian kidney is composed of polarized epithelial cells endowed with specific ion transport capacities and biochemical functions distinct from those of cells in the more distal segments of the renal tubule (for reviews, see Schmidt and Guder, 1976; Jacobson, 1981; Morel, 1981).

Primary cultures of proximal cells retain most of their properties during the first two or three weeks in culture (Chung et al., 1982; Sakhrani et al., 1984; Ronco et al., 1990), but usually loose their differentiated functions when they are sub-cultured. Renal proximal tubule-like established cell lines, such as LLC-PK$_1$ and OK cells, have been used to analyze glucose and ion-transport regulation (Rabito and Ausiello, 1980; Misfeldt and Sanders, 1981; Caverzasio et al., 1986) and to study processes controlling membrane protein sorting (Louvard, 1980). However, the precise origin of these cells remains unclear, since LLC-PK$_1$ cells, for example, have both proximal and distal tubule cell functions (Sanders et al., 1983; Gstraunthaler et al., 1985).

We established two lines of proximal cells derived from the convoluted (PKSV-PCT cells) and straight (PKSV-PR cells) portions of the proximal tubule from kidney of L-PK/Tag1 transgenic mice carrying the L-pyruvate kinase (L-PK)-SV40 (Tag) antigen hybrid gene (Cartier et al., 1992). When grown in D-glucose-enriched medium, cells exhibit the morphological features of the parental proximal cells and remained fully polarized (companion article). In this study the specific hormonal sensitivities, the glucose transport properties and endocytic capacities, considered as hallmarks of the renal proximal tubule phenotype, were analyzed in the two PKSV-PCT and PKSV-PR cell lines that we have established. Like that observed in vivo, where the L-PK gene is activated by carbohydrate-rich diet (Vaulont et al., 1986; Decaux et al., 1989), the expression of Tag was dependent on the concentration of D-glucose in the culture medium, suggesting that the SV40 transcription

remained under the control of the L-PK promoter in vitro. The capacity almost completely to extinguish SV40 Tag transcripts and stop cell proliferation by removing D-glucose was used to study the relationship between cell growth and differentiation in these models of renal proximal epithelial cultured cells obtained by targeted oncogenesis.

MATERIALS AND METHODS

Cell cultures

PKSV-PCT and PKSV-PR cells were routinely cultured in a modified culture medium (CM: DMEM:Ham's F_{12} (1:1, v/v, 30 nM sodium selenate, 5 μg/ml transferin, 2 mM glutamine, 50 nM dexamethasone, 1 nM triiodothyronine, 10 nM epidermal growth factor, 2% fetal calf serum, 20 mM HEPES, pH 7.4) supplemented with 5 μg/ml insulin and 20 mM D-glucose at 37°C in 5% CO_2/95% air atmosphere. In some sets of experiments, sub-confluent PKSV-PCT and PKSV-PR cells grown in modified culture medium (CM) supplemented with 10 or 20 mM D-glucose and 5 μg/ml insulin were further cultivated for various times in this medium or in CM devoid of D-glucose and/or insulin, depending on the experimental procedure. D-glucose was then replaced by 10 mM lactate and 2 mM oxaloacetate in D-glucose-free CM. All studies were performed between the 15th and 50th passages.

Intracellular cAMP content

The effects of various polypeptide hormones and compounds on the cellular cyclic AMP (cAMP) content were assayed as previously described (Vandewalle et al., 1989). Cell monolayers from 12-well trays were incubated with 1 ml DMEM with or without parathyroid hormone (PTH: 10^{-7}M, 1-34 synthetic fragment from bovine parathormone, Beckman, France), calcitonin (CT: 100 ng/ml, Sigma) desmopressin (dDAVP: 10^{-6}M, Ferring Pharmaceuticals, Sweden) for 5 min at 37°C. The reaction was stopped by rapidly removing the medium and adding 1 ml ice-cold 95% ethanol 5% acid formic solution. The supernatants were evaporated to dryness and cAMP was determined using the Pasteur radioimmunoassay kit (no. 79830, Institut Pasteur, France). Protein content was measured by the Lowry et al. (1951) method using bovine serum albumin (BSA) as the standard.

Glucose uptake studies

The Na^+-dependent uptake of α-methyl glucose into confluent cells grown on 12-well trays was determined as previously described (Vandewalle et al., 1989). Cells were rinsed twice in modified Kreb's Ringer solution (KRS: 140 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 10 mM HEPES-KOH, pH 7.4) and incubated at 37°C for 10 min with 1 ml KRS containing 0.5 mM [*methyl*-α-^{14}C]glucose (spec. act. 258 mCi/mmol, NEN, France). Additional incubations were performed in parallel using the same buffer in which choline (140 mM) was substituted for sodium chloride or in which KRS contained 0.5 mM phlorizin. The reaction was stopped by adding 1 ml ice-cold stop-solution (130 mM NaCl, 10 mM Na_2HAsO_4, 10 mM HEPES-KOH, pH 7.4). The cells were rinsed and dissolved in 1 M NaOH. Radioactivity was counted and the protein content was measured according to the Lowry method.

Enzymatic studies

Enzyme activities were measured in the two established proximal renal cell lines grown in the presence or absence of D-glucose. γ-Glutamyl transpeptidase (γ-GT), aminopeptidase N (APN), dipeptidylpeptidase IV (DPP IV), and phosphoenol pyruvate carboxykinase (PEPCK) were measured as previously described (Vandewalle et al., 1989).

Measurements of [^{3}H]inulin uptake and release

The endocytic capacity of the cells was estimated by the rates of uptake of [^{3}H]inulin, used as a fluid-phase marker, by confluent cells grown on permeable filters (Transwell-COL). The procedure was similar to that described by von Bondorff et al. (1985) with slight modifications. Briefly, confluent monolayers were rinsed in CM containing 0.2% BSA. The inside (for apical uptake) and outside (for basolateral uptake) of the culture chambers were then filled with 300 ml and 1.2 ml, respectively, of the same medium containing the same concentration (1 μCi/ml) of [^{3}H]inulin (spec. act. 350 mCi/g solid material, NEN, France; 5 mCi dissolved in 5 ml phosphate buffered saline solution (PBS)). After incubation (37°C and 4°C) for various periods of time, the marker solution was removed and the culture chambers were brought rapidly to 0°C. They were rinsed five times with 0.5 (inside the chamber) and 1.5 ml (outside of the chamber) ice-cold PBS containing 0.1 mg/ml unlabelled inulin. After drying (2 hours at room temperature) the filters were removed and the radioactivity was extracted with 1 ml of 1 M NaOH for 2 hours at room temperature and counted in a liquid scintillation counter (Wallac, Wallac Oy, Finland). As a control, similar uptake studies were performed at 4°C and with filters without cells which were pre-incubated in CM for 24 hours. For each experiment, the number of cells was determined on separate filters by counting the cells with hemocytometer after the addition of 1 ml trypsin-EDTA on the top of the filters.

The rate of [^{3}H]inulin release was also measured in confluent PKSV-PCT cells grown on permeable filters. Cell monolayers were incubated in similar conditions as described above: the insides or outsides of the culture chambers were filled with CM containing 0.2% BSA and [^{3}H]inulin (2 μCi/ml) at 37°C for 3 hours. Efflux studies were performed at 37°C. Filters were rapidly rinsed 4 times with 0.5 ml (inside the chamber) and 1.5 ml (outside the chamber) ice-cold CM. Thereafter, cells were layered with 0.5 ml (inside the chamber) and 1.5 ml (outside the chamber) prewarmed CM medium. Samples (50 μl for inside and 100 μl for outside) of supernatents were taken at various times (15 to 60 min) following CM addition and radioactivity was measured by scintillation counting. The zero-time intracellular [^{3}H]inulin content was measured in cells rinsed five times with ice-cold PBS as described above. Results are expressed as percentage of the 0-time intracellular [^{3}H]inulin content.

Morphometric analysis

Cell height, membrane surface area and cell volume were estimated morphometrically on enlarged micrographs (× 11,200) from electron micrographs (× 3000) of transverse sections of confluent cells cut perpendicular to the filters. Ultrathin sections were cut from transversally orientated confluent monolayers as previously described (Vandewalle et al., 1989) and viewed under a Philips electron microscope (EM 410). Calculations were performed by using the technique described by von Bondorff et al. (1985), with slight modifications. For each individual cell analyzed, the lengths of apical, lateral and basal membranes were determined from digitized image drawn on a digitizer grid master graphic tablet (Apple) recorded to a computer (Apple II).

Northern blot analysis

RNA was extracted as previously described (Genton et al., 1987). The integrity of all RNA preparations stained with ethidium bromide was analyzed by electrophoresis in agarose/formaldehyde submarine minigels. Aliquots (10-20 μg) of total RNA were size-fractionated in 20% formaldehyde/1.5% agarose gels, transferred to nylon screens (Gene Screen Plus) and subjected to Northern

blot analysis. The cDNA plasmid probe was labelled by a multiprime labelling kit (Amersham) with [α-^{32}P]dCTP to a specific activity of > 5 × 10^7 cts/min per/mg DNA, according to the manufacturer's instructions. The nylon filters were preincubated in prehybridization buffer for 2-4 h at 42°C. The multiprime labelled probe(s) was denatured at 100°C for 5 min, cooled and added to the hybridization buffer. The RNA blots were hybridized for 12-18 hours at 42°C, washed extensively, dried, wrapped in Saran Wrap, and exposed to X-ray film at −80°C for 6-24 hours using intensifying screens. The Tag probe was a 2.7 kb *Bam*HI-*Bam*HI fragment. The human poly(A)-binding protein probe (pAbp) was the 2.9 kb complete cDNA (Grange et al., 1987).

Immunofluorescence studies

The apical membrane expression of APN was analyzed by indirect immunofluorescence using a polyclonal antibody directed against APN (3.01.01.75 antibody, kindly provided by Dr S. Maroux). The immunocytochemical procedure was identical to that described in the companion article (Cartier et al., 1993).

RESULTS

Hormonal sensitivities and glucose transport properties of proximal cell lines

Both PKSV-PCT and PKSV-PR cells grown in D-glucose-enriched medium were parathormone (PTH) sensitive and showed sodium-dependent glucose transport, which are considered to be distinguishing characteristics of proximal cells (Jacobson, 1981; Morel, 1981). As shown in Table 1, PTH increased cAMP production above the basal value by 9-fold in PKSV-PCT and by 4-fold in PKSV-PR cells. dD-AVP, which acts on more distal segments of the renal tubule (Morel, 1981), had no significant effect on cAMP production (Table 1). However, the cAMP content was also enhanced by calcitonin by 22- and 16-fold in PKSV-PCT and PKSV-PR cells, respectively. The cAMP production induced by calcitonin is in agreement with the findings of Chabardes et al. (1977), who showed that this hormone stimulated adenylate cyclase activity in microdissected proximal tubules from mouse kidney.

Table 1. cAMP content and α-methyl glucose uptake of confluent PKSV cells grown in the presence of insulin and glucose

	PKSV-PCT cells	PKSV-PR cells
	cAMP (pmol . 5 min^{-1}. mg $protein^{-1}$)	
Basal	8.2 ± 13.6	24.9 ± 2.1
dD-AVP (10^{-6} M)	13.9 ± 4.0	38.7 ± 12.9
PTH (10^{-7} M)	74.1 ± 17.4	100.1 ± 42.8
CT (100 ng/ml)	182.9 ± 16.4	385.2 ±112.9
	α-MG (nmol . 10 min^{-1}. mg $protein^{-1}$)	
Sodium	8.1 ± 1.2	3.1 ± 2.
Choline	1.5 ± 0.5	0.8 ± 1.0
Sodium + phlorizin	1.1 ± 0.2	0.8 ± 1.2

cAMP was measured in confluent cells in the absence (Basal) or presence of dD-arginine vasopressin (dD-AVP) parathormone (PTH), or calcitonin (CT). Glucose transport was measured by α-methyl glucose (α-MG) uptake on confluent cells in the presence of 140 mM sodium chloride (Sodium) or choline chloride (Choline) with 0.5 mM substrate. The specificity of the sodium-dependent glucose uptake was assessed by α-MG uptake performed in the presence of 140 mM sodium chloride plus 0.5 mM phlorizin (sodium + phlorizin). Values are the means ± s.e.m. from 3 to 5 separate passages, performed in duplicate for each test condition.

Table 1 also shows the uptake of a-methyl glucose by PKSV-PCT and PKSV-PR cells. In keeping with the known properties of the glucose transporter present in the luminal membranes of proximal tubule cells (Jacobson, 1981), this uptake was inhibited by 74-81% after addition of phlorizin or replacement of NaCl by choline. The rates of uptake in PKSV-PCT was 2.6-fold higher in PKSV-PCT than PKSV-PR cells. The rates of sodium-dependent glucose uptake were equivalent to (for PKSV-PR cells) or twice as high as (for PKSV-PCT cells) that measured in similar conditions of incubation in primary cultures of rabbit kidney proximal cells (Vandewalle et al., 1989). Thus, these results indicate the presence of a sodium-coupled glucose cotransport system in both cell lines, which appeared twice as effective in cells derived from the convoluted portion than from the Pars recta of the proximal tubule.

Endocytic capacity of proximal renal cell lines

Proximal tubule cells exhibit high apical endocytic capacity in vivo (Nielsen et al., 1987, 1989). PKSV cells also presented an endocytotic capacity. Fig. 1 illustrates the uptakes of [^{3}H]inulin, used as a marker of fluid phase (von Bondorff et al., 1985), over a period of 2 hours at 37°C and 4°C from apical (Fig. 1A,B) and basolateral (Fig. 1C,D) sides. In both cell lines the uptake from the apical and basolateral sides of the cells was biphasic at 37°C. Corrected by the values obtained at 4°C, the rates of apical fluid accumulation were 7- to 22-fold higher, respectively, before (PKSV-PCT: 30.8 × 10^{-7} nl/min per cell; PKSV-PR: 23.8 × 10^{-7} nl/min per cell, n = 3) than after the 30 min incubation time (PKSV-PCT: 1.7 × 10^{-7} nl/min per cell; PKSV-PCT: 3.3 × 10^{-7} nl/min per cell, n = 3). In contrast, the differences in the rate of [^{3}H]inulin uptake from the basolateral side were less marked before (PKSV-PCT: 27.6 × 10^{-7} nl/min per cell; PKSV-PR: 32.4 × 10^{-7} nl/min per cell, n = 3) than after 30 min incubation (PKSV-PCT: 14.6 × 10^{-7} nl/min per cell; PKSV-PR: 17.3 × 10^{-7} nl/min per cell, n = 3). Results from morphometric analysis (Table 2) indicate that the ratio of basolateral to apical membrane surface area is 1.2 and 2 for PKSV-PCT and PKSV-PR cells, respectively. Thus, normalized by the respective membrane surface area, apical endocytic rates are almost equivalent to basolateral endocytic rates (Table 2).

The release of inulin from the apical and basolateral side of the cells was estimated by [^{3}H]inulin efflux measurements, expressed as percentage of the initial uptake, following incubation of confluent PKSV-PCT cells with [^{3}H]inulin applied at the apical or the basolateral side of the cells. Fig. 2 illustrates the results obtained when cells were loaded with [^{3}H]inulin from the apical side. The efflux of the fluid-phase marker was 3-fold higher from the basolateral side than from the apical side of the cells. Identical results were obtained when cells were loaded from the basolateral side (not shown). Thus, no differences were observed in the percentages of apical and basolateral [^{3}H]inulin effluxes after the cells have been loaded from either the apical or the basolateral side. No irreversible intracellular accumulation was observed, and after 60 min 79% of the

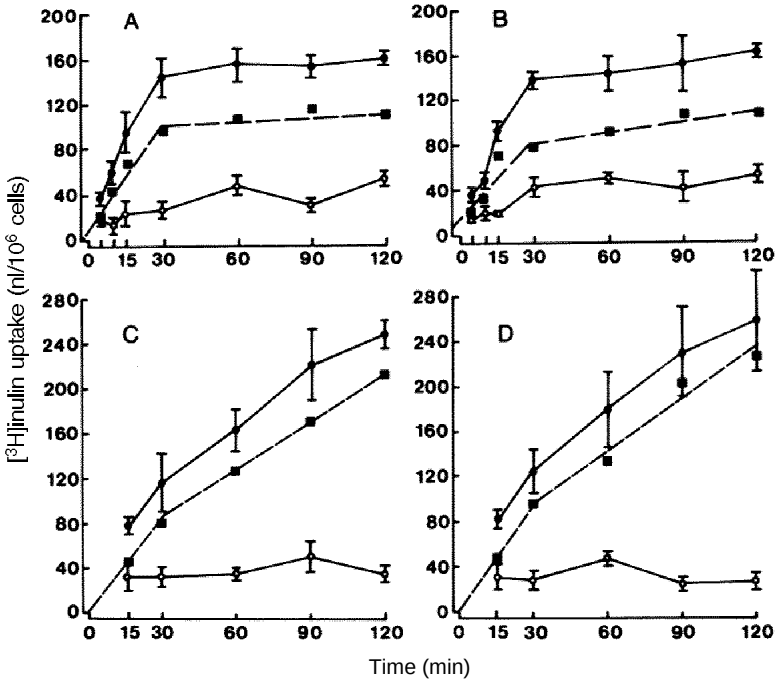

Fig. 1. Apical and basolateral uptake of the fluid-phase marker inulin by PKSV cells. Apical and basolateral [^{3}H]inulin uptake was measured on confluent PKSV-PCT (A, C) and PKSV-PR (B, D) cells grown on filters with D-glucose-supplemented medium. The uptake from the apical (A, B) and basolateral side (C, D) were measured at various incubation times at 37°C (●-●) or 4°C (○-○). Radioactivity was extracted as described in Materials and methods. Each point, expressed as nl/10^6 cells, is the mean ± s.em. from three experiments. The squares (■) represent the 4°C background-corrected mean values for the uptake from apical and basolateral sides. The broken lines represent the least squares regression line calculated from mean 4°C-corrected values before and after 30 min. In all cases uptake was biphasic and the regression line from the time points before 30 min passed through the origin.

Table 2. Morphometric analysis of PKSV cells and fluid-phase uptake of [^{3}H]inulin

	PKSV-PCT (±%)	PKSV-PR (±%)
Cell volume (μm^3)	1685 (± 18.4)	1406 (± 10.4)
Cell height (μm)	10.7 (± 6.0)	9.6 (± 6.0)
Apical membrane area (μm^2)	469.1 (± 16.6)	290.6 (± 12.8)
Lateral membrane area (μm^2)	421.9 (± 10.5)	432.7 (± 6.8)
Basal membrane area (μm^2)	150.7 (± 16.9)	150.1 (± 9.7)
	PKSV-PCT	PKSV-PR
	[^{3}H]inulin uptake (nl . min^{-1}. μm^{-2})	
Apical uptake (0-30 min)	0.657×10^{-8}	0.821×10^{-8}
Apical uptake (30-120 min)	0.029×10^{-8}	0.110×10^{-8}
Basolateral uptake (0-30 min)	0.482×10^{-8}	0.556×10^{-8}
Basolateral uptake (30-120 min)	0.256×10^{-8}	0.272×10^{-8}

Morphological parameters obtained from transverse sections of confluent cell monolayers grown on filters were derived from measurements (PKSV-PCT, n = 16; PKSV-PR, n = 17) performed on enlarged electron micrographs (× 11,200) as described in Materials and Methods. Values are expressed as the means ± the percentage of standard error of the means. The rate of [^{3}H]inulin uptake (deducted from the 4°C uptake values) from apical and basolateral sides was calculated from the slopes of the regression lines (Fig. 1) before and after 30 min incubation time at 37°C (in nl . min^{-1} . cell^{-1}) and corrected by the corresponding apical and basolateral (basal + lateral) membrane surface area.

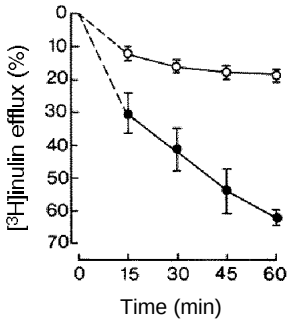

Fig. 2. [^{3}H]inulin efflux in PKSV-PCT cells. [^{3}H]inulin efflux was measured on confluent PKSV-PCT cells grown on permeable filters as described in Materials and methods. Cells were incubated with [^{3}H]inulin (2 μCi/ml) from the apical sides for 3 hours at 37°C. Circles represent the fractional loss of [^{3}H]inulin (in %) from the apical sides (○) and basolateral sides (●) of the cells measured after a 3-hour [^{3}H]inulin loading. Values are the mean ± s.e.m. from five separate filters.

radioactivity was recovered in the medium. These results strongly suggest that after its internalization by the apical membrane, the fluid-phase marker is extruded preferentially at the basolateral side of PKSV-PCT cells.

Modulation of Tag expression by D-glucose and effect on cell growth

Previous studies on the liver and on primary cultures of hepatocytes demonstrated that the L-PK gene can be activated by carbohydrates (Vaulont et al., 1986; Decaux et al., 1989). The two renal cell lines were grown and sub-cultured in medium containing 10-20 mM D-glucose. The question arises of whether the transgene could also be modulated in vitro by the culture conditions. This was tested by growing PKSV-PCT and PKSV-PR cells in modified defined medium supplemented with 20 mM D-glucose and 5 μg/ml insulin. Four days after seeding, the medium was changed to one lacking insulin and/or D-glucose. Tag gene expression, revealed by Northern blot analysis, was greatly modified under these conditions (Fig. 3A). In the presence of D-glucose plus insulin or D-glucose alone Tag transcripts were activated in both cell lines. A 72-hour incubation without these substrates blunted Tag expression. The D-glucose dependency for activation of the foreign transgene was determined by growing confluent PKSV-PCT and PKSV-PR cells in D-glucose and insulin-supplemented medium and transferring them to medium lacking these two compounds for 48 hours. The cells were then cultured in various concentrations of D-glucose (0 to 20 mM) for an additional 24 hours. Northern blot analyses revealed that the expression of Tag transcripts increased as a function of the D-glucose concentrations present in the medium (Fig. 3B).

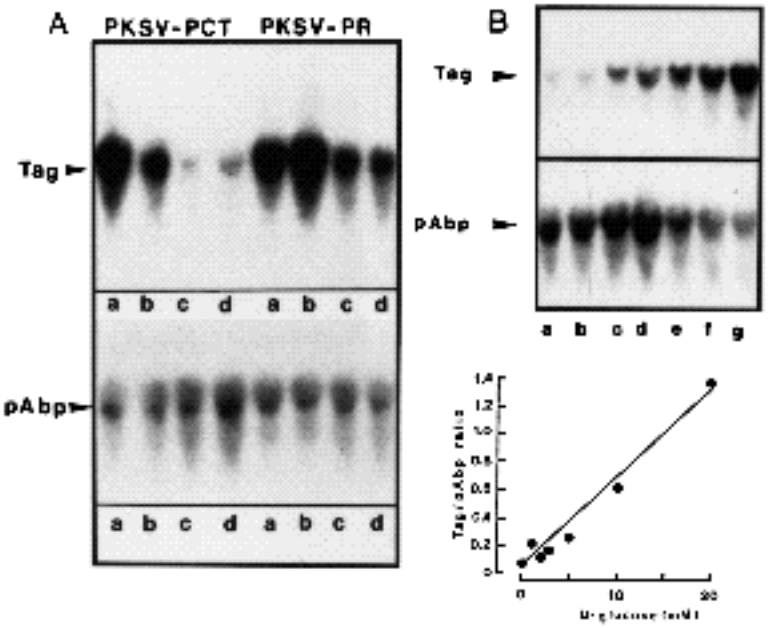

Fig. 3. SV40 Tag expression in PKSV cells. (A) PKSV-PCT and PKSV-PR cells were incubated in medium (CM) containing 20 mM D-glucose and 5 μg/ml insulin for four days (a), and then in CM plus 20 mM D-glucose (b), CM plus 5 μg/ml insulin (c) or CM alone (d) for 48 hours. Tag transcripts (Tag) were analyzed by Northern blotting as described in Materials and methods; highly labelled bands were only detected in cells cultivated in the presence of glucose plus insulin (a) or glucose (b) -supplemented medium. **(**B) PKSV-PR cells grown in D-glucose and insulin-enriched culture medium (CM) for four days were then cultured in CM alone for 48 hours. Thereafter, cells were cultivated in CM supplemented with various concentrations of D-glucose (in mM): 0 (a), 1 (b), 2 (c), 3 (d), 5 (e), 10 (f), 20 (g) for 24 hours. The Tag transcripts (Tag) increased as a function of D-glucose concentration. Identical results were obtained with PKSV-PCT cells (not shown). A and B (lower panels): autoradiograms of the same filters hybridized with the cDNA poly(A)-binding protein probe (pAbp). The lower right graph represents the Tag/pAbp mRNA ratio determined by scanning densitometry.

Consistent with these results was the observation that cell growth was also dependent on D-glucose in the culture medium. As shown in Fig. 4, both cell lines exhibited rapid rates of cell doubling when the culture medium contained D-glucose and insulin. Removal of D-glucose from the culture medium resulted in a progressive arrest of cell division. Cells could be maintained in a quiescent state for almost a week without any major change in viability (> 85-90 % viable cells). Cell growth could be restarted by incubation in medium containing D-glucose (Fig. 4). Thus, the growth kinetics and Northern blot analyses may suggest that continuous cell growth is correlated with the permanent activation of the L-PK/Tag hybrid gene induced by high concentrations of D-glucose in the culture medium.

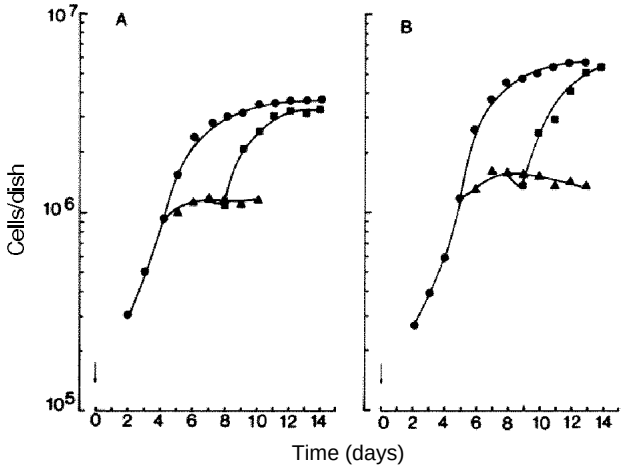

Fig. 4. Growth kinetics of PKSV cells. PKSV-PCT (A) and PKSV-PR (B) cells grown in culture medium containing 20 mM D-glucose and 5 μg/ml insulin (●) had short cell doubling times. Five days after seeding, cells were grown in medium without D-glucose (▲). The growth of both cell lines stopped 72 hours after the removal of glucose. The phenomenom was reversed when glucose was returned to the medium (■).

Influence of D-glucose on the functional expression of proximal renal cell lines

To test wether activation or inactivation of Tag and the presence of high D-glucose concentrations (20 mM) in the culture medium could affect the properties of PKSV-PCT and PKSV-PR cells, we analyzed the functions of the cells grown in the presence of D-glucose-enriched medium or shifted for 72 hours to medium where D-glucose was replaced with oxaloacetate and lactate. Immunofluorescence studies using a specific antibody directed against aminopeptidase N (APN), known to be specifically localized in apical brush-border membrane of proximal tubule and enterocytes (Kenny and Maroux, 1982), indicated that removal of D-glucose from the medium of confluent cultured cells for 72 hours does not affect the pattern of labelling (Fig. 5) corresponding to the apical membrane localization of APN as shown by laser confocal scanning microscopy on cells cultured in D-glucose-enriched medium (see companion article; Cartier et al., 1993). The transepithelial resistance from cell grown on filters was also unaffected by the absence of D-glucose from the culture medium (PKSV-PCT: CM + glucose, 37 ± 7; CM − glucose, 43 ± 3; PKSV-PR: CM + glucose, 44 ± 4; CM − glucose: 37 ± 6 Ω/cm^2, n = 5). Scanning electron microscopy from cells grown on filters showed that the number and abundance of apical microvilli was also unaffected by the culture conditions (not shown). No differences were observed in the total cellular villin content as determined by Western blot analyses or its location in the apical microvilli from cells grown in D-glucose-enriched medium or cultured in the absence of D-glucose for for 72 hours (data not shown). The specific functions of the proximal tubule were also unaffected by removal glucose from the culture medium. As shown in Fig. 6, the cells' sensitivity to PTH CT, and glucose transport capacity was not influenced by the culture conditions. However, γ-GT, APN and DPP IV activity was 1.5- to 2-fold higher in PKSV-

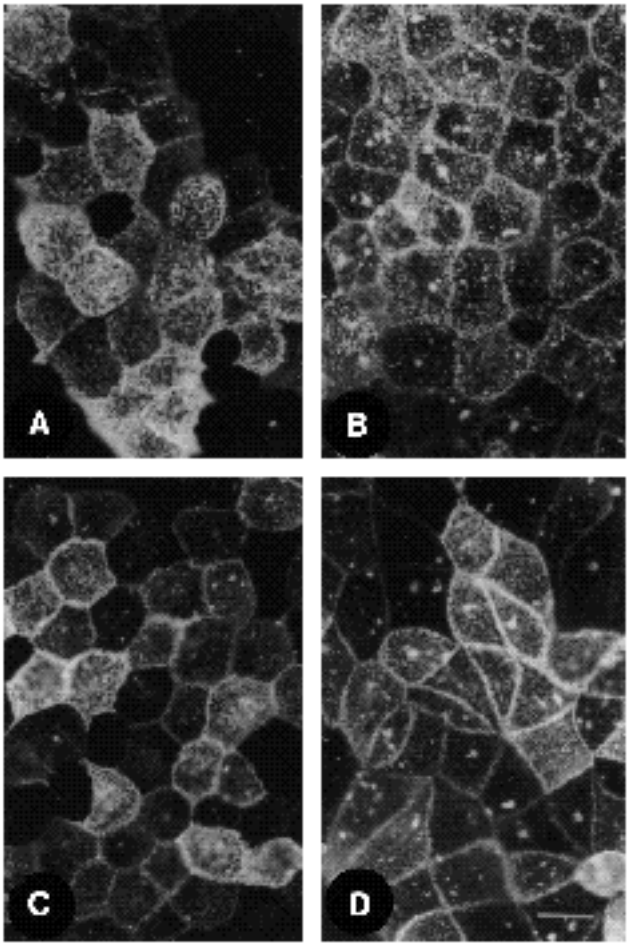

Fig. 5. Influence of D-glucose on apical localization of APN in PKSV cells. Illustrations of indirect immunofluorescence performed with the anti-APN antibody on PKSV-PCT (A, B) and PKSV-PR (C, D) were grown with D-glucose-enriched medium (A, C) or incubated in D-glucose-deprived medium (B, D) for 48 hours. Cells grown in D-glucose-enriched medium exhibit irregular labelling, whereas all cells were uniformely labelled after removal of D-glucose. Bar, 10 μm.

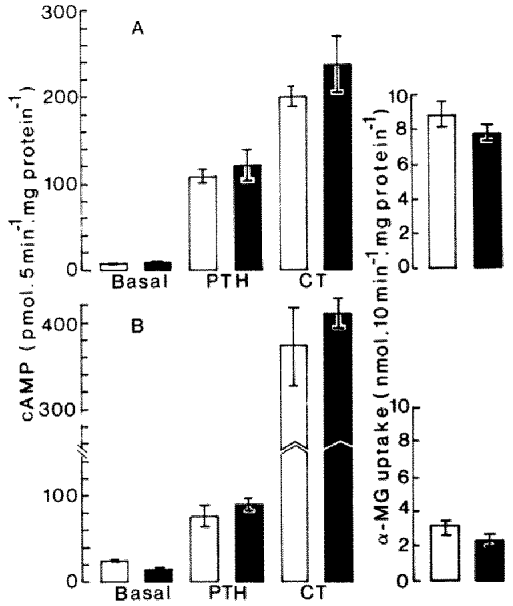

Fig. 6. Influence of D-glucose on cAMP content and sodium-dependent glucose transport. The cAMP content under PTH (10^{-7}M) and calcitonin (100 ng/ml) stimulation and α-methyl glucose (α-MG) uptake were measured on PKSV-PCT and PKSV-PR cells cells grown in CM supplemented with 10 mM D-glucose and 5 μg/ml insulin (open bars) or for 72 hours in CM without D-glucose and insulin (filled bars). The sodium-dependent glucose uptake was estimated by the difference between the α-MG uptake measured in the presence of 140 mM NaCl and that in NaCl plus 0.5 mM phlorizin. There was no significant difference between the two culture conditions tested. Values are the means ± s.e.m. from three different passages.

PCT cells grown for 48 hours in D-glucose-free medium than in D-glucose plus insulin-enriched medium (Fig. 7). PKSV-PR cells display a similar increase in hydrolase activities, but the effect was less pronounced than in PKSV-PCT cells. The activity of phosphoenolpyruvate carboxykinase, a rate-limiting enzyme for neoglucogenesis (Vandewalle et al., 1981), was also slightly enhanced within both cell lines shifted for 72 hour into D-glucose-deprived medium. Thus, these results strongly suggest that the differentiation state is not greatly influenced by the culture conditions responsible for proliferative and quiescent states.

DISCUSSION

The results from the present study indicate that both proximal convoluted and late proximal tubule cell lines derived from the L-PK/Tag1 transgenic mouse (Cartier et al., 1992) have maintained the principal functions of proximal tubule cells including: (1) parathormone and calcitonin sensitivity; (2) a sodium-dependent glucose transporter; (3) endocytic capacity; and (4) substantial amounts of brush-border hydrolase activity.

The technique of isolated single-tubule microperfusion for transport studies and the development of enzymatic microassays allowed us to determine many of the functional characteristics of early and late proximal tubule cells. The proximal convoluted and the straight proximal (Pars recta) tubule cells have the same functions, but the former exhibit higher fluid reabsorption capacities, higher net transport rates (including glucose transport) and higher cAMP sensitivity to parathormone (for reviews see, Jacobson, 1981; Morel, 1981). Both cell lines retain the properties of proximal convoluted and straight tubules. As in vivo, PKSV-PCT cells exhibited a greater sensitivity to PTH and higher rates of sodium-dependent glucose uptake than PKSV-PR cells. Moreover, the amounts of hydrolase activity, the capacity for α-methyl glucose uptake and the cAMP sensitivity to parathormone measured in PKSV-PCT cells were in the same range as that reported for primary cultures of rabbit kidney proximal convoluted tubule cells (Sakhrani et al., 1984; Vandewalle et al., 1989; Ronco et al., 1990).

Both cell lines also presented rapid rates of apical and basolateral uptake of the fluid-phase marker [^{3}H]inulin. It is well established that the kidney removes proteins, insulin, epidermal growth factor and other compounds from the cir-

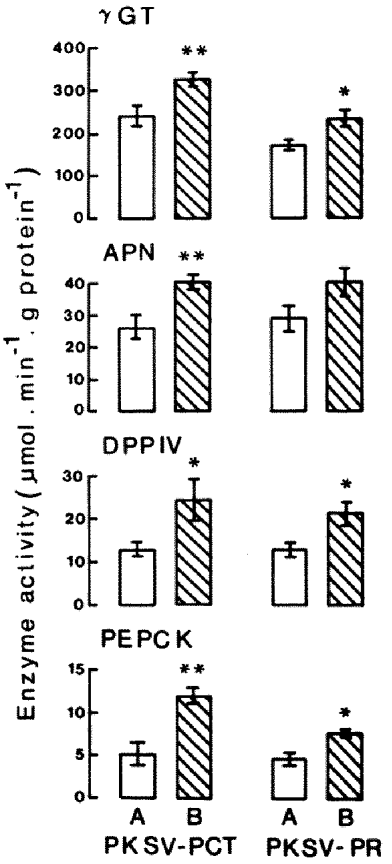

Fig. 7. Influence of D-glucose on enzyme activity. Enzyme activities were determined on PKSV-PCT (left columns) and PKSV-PR (right columns) cells grown in CM supplemented with 10 mM D-glucose and 5 μg/ml insulin (open bars) or for 72 hours in CM without D-glucose and insulin (hatched bars). γ-Glutamyl transpeptidase (γGT), aminopeptidase N (APN), dipeptidyl peptidase IV (DPPIV) and phosphoenol pyruvate carboxykinase (PEPCK) activities were measured on cell homogenates as described in Materials and methods. Values are the means ± s.e.m. from 3 to 5 different passages (* $P < 0.05$, ** $P < 0.01$; Student's *t*-test).

culation by glomerular filtration, followed by luminal endocytosis (Nielsen et al., 1987, 1989). Apical and basolateral endocytosis have been extensively studied in the distal-like MDCK renal cells (Simons and Fuller, 1985; von Bondorff et al., 1985; Bomsel et al., 1989) and differentiating intestinal HT29-18 cloned cells (Godefroy et al., 1990). Compared to MDCK cells, both PSV-PCT and PKSV-PR cells cultured in D-glucose-enriched medium exhibited greater rates of [^{3}H]inulin apical and basolateral uptake before than after 30 min of incubation. Morphometric analysis of the two proximal cell lines also showed that the basolateral to apical membrane-area ratio (Table 1) was close to that measured in intact proximal convoluted and straight PR tubule cells (reviewed by von Bonsdorf et al., 1985). Corrected for their respective surface areas, the rates of [^{3}H]inulin uptake from both the apical and basolateral sides of PKSV cells were almost equivalent. However, the rate of [^{3}H]inulin uptake by the apical sides of the cells reached a plateau after 30 min of incubation, whereas no saturation occurred for the [^{3}H]inulin uptake from the basolateral sides of the cells. The results from efflux studies show that the rate of [^{3}H]inulin efflux was markedly higher at the basal sides than at the apical sides of the cells. The existence of predominating release of [^{3}H]inulin at the basolateral sides of the cells, could be an explanation for the observed plateau of [^{3}H]inulin uptake from the apical sides after 30 min incubation. By contrast, when [^{3}H]inulin is applied to the basal sides of the cells the uptake does not reach a plateau because of the low apical-membrane extrusion capacity and the presence of labelled marker at the basal sides. Transcytosis is a common feature in polarized epithelial cells, but the magnitude and the orientation of the process may differ from one cell type to another. In keeping with previous studies on MDCK and HT29 cells, the present results obtained on proximal cultured cells show that their endocytic capacities differ from distal-like MDCK cells, which exhibit low apical uptake of [^{3}H]inulin (von Bonsdorf et al., 1985), and that, in contrast with intestinal HT29-18 cells (Godefroy et al., 1990), proximal cells exhibit a more marked basolateral than apical release of fluid-phase marker. Thus, our results suggest that PKSV cells have maintained some of the morphometric and endocytic characteristics of the parental proximal cells that have an important function in ionic reabsorption (Jacobson, 1981).

Previous studies have shown that the long-term culture of renal cell lines, derived by infection or transfection of primary cultures of isolated tubules or isolated cell suspensions with simian virus 40 (SV40) or adenovirus 12-SV40 hybrid (Scott et al., 1986; Arend et al., 1989; Vandewalle et al., 1989; Prié et al., 1991), results in the partial or complete loss of some specific functions. This dedifferentiation could be due to oncogenic viruses used to immortalize these cells, which may alter their differentiation program (Schutzbank et al., 1982; Cherington et al., 1986). In addition, cell infection studies with temperature-sensitive mutants of Rous or SV40 viruses have demonstrated that some specific functions can be restored or enhanced after inactivation of the viral genome in renal collecting-tubule epithelial cells (Prié et al., 1991) and hepatocytes (Chou and Schlegel-Haueter, 1981; Chou, 1985).

The results obtained with the two proximal cells lines derived from the transgenic mice, in which the L-PK promoter has maintained its ability to be up- or down-regulated by D-glucose (Vaulont et al., 1986; Decaux et al., 1989; Tremp et al., 1989; Cartier et al., 1992), clearly show that activation or inactivation of the Tag transcripts did not influence the specific functions of the immortalized cells except that the levels of hydrolase activity were higher in cells cultured in D-glucose-free medium than in cells cultured in D-glucose-enriched medium. Thus, our results suggest that glucose metabolism and/or cell growth may affect the regulation of apical membrane-anchored hydrolases in the PKSV proximal cell lines.

In conclusion, immortalized, differentiated cell lines established from early and late portions of the proximal renal tubule cells from LPK/Tag1 transgenic mice have maintained the proximal tubule phenotype characterized by a sensitivity to PTH and calcitonin, an active sodium-dependent glucose cotransport system, a high endocytic capacity,

a residual neoglucogenic pathway and the presence of brush-border membrane hydrolases. As a particular feature of these renal tubule cell lines, the cell growth and the expression of Tag, placed under the control of a D-glucose-sensitive promoter, can be modulated by the presence or absence of D-glucose in the medium without altering the tissue-specific functions.

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