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Differentiation of renal β -intercalated cells to α -intercalated and principal cells in culture

(kidney/collecting duct/ion transport/antigens)

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ABSTRACT The renal collecting duct is a heterogeneous epithelium consisting of intercalated cells (ICC) and principal cells (PC). The origin of this cellular heterogeneity is not clear. To test the hypothesis that the two cell types might originate from one another, pure populations of ICC (β subtype) and PC were isolated by fluorescence-activated cell sorting and grown on permeable supports. After the monolayers reached confluence, the expression of ICC- and PC-specific functions and antigens was monitored. Cultures of sorted β -ICC, in addition to expressing ICC-specific functions (such as an electrogenic H^+ secretion) and antigens, progressively acquired PC functions (amiloride-sensitive Na^+ transport and K^+ secretion). On day 6, cultures of sorted β -ICC exhibited a lumen-negative transepithelial potential difference of 83 ± 4 mV and a short circuit current of $107 \pm 15 \mu A/cm^2$ and created a lumen-to-bath K^+ concentration ratio of ≈ 10 . The percentage of cells staining with two PC-specific antibodies was 53% and 65%. On the other hand, cultures of sorted PC failed to acquire ICC-specific functions while maintaining PC characteristics. To rule out preferential proliferation of a few contaminating PC as an explanation of these results, we have generated a continuous collecting duct cell line (M-1) originating from mice transgenic for the early region of simian virus 40. Cell lines cloned from M-1 cells exhibit both PC and ICC functions and show mutually exclusive heterogeneous expression of PC and ICC antigens, demonstrating a common origin of the two cell types. These data indicate that while β -ICC in culture can give rise to both α -ICC and PC, PC cannot convert to ICC, which raises the possibility that β -ICC is the stem cell in the renal collecting duct. Differentiation of ICC to PC may explain the cellular heterogeneity in the cortical collecting duct.

The two cell types of the cortical collecting duct (CCD) in the mammalian kidney, principal cells (PC) and intercalated cells (ICC), exhibit profound morphological differences (1, 2) and serve markedly different functions. PC are believed to reabsorb Na^+ and secrete K^+ , whereas ICC are thought to be involved in H^+ and bicarbonate transport. According to the current PC model, Na^+ reabsorption and K^+ secretion involve a basolateral Na^+/K^+ -ATPase and amiloride-sensitive Na^+ channels and Ba^{2+} -sensitive K^+ channels in the apical membrane (3). HCO_3^- transport in the CCD is bidirectional, and the two opposite functions (i.e., HCO_3^- reabsorption and secretion) seem to be segregated into two ICC subtypes: α - and β -ICC, respectively (2, 4). α -ICC are generally modeled with an apical H^+ -ATPase, a basolateral Cl^-/HCO_3^- exchanger, and an intracellular carbonic anhydrase. β -ICC are thought to be mirror images of α -ICC, with a basolateral H^+ -ATPase and an apical Cl^-/HCO_3^- exchanger (4, 5).

In addition to these functional differences, PC and ICC differ in hormonal responsiveness. PC are the main targets

for vasopressin (6, 7) and aldosterone (8), whereas ICC respond mainly to β -adrenergic agents (7).

The physiological importance and the origin of such a cellular heterogeneity are not clear, and very little is known about the relationship between the various cell types. To test the hypothesis that the two cell types might originate from one another, we isolated homogeneous populations of β -ICC and PC from rabbit CCD by using fluorescence-activated cell sorting (7, 9) and studied their characteristics in primary culture. Here we report that β -ICC in primary culture are able to convert into both α -ICC and PC, as judged by the expression of functions and antigens specific for these two cell types.

METHODS

Separation of PC and ICC. PC and β -ICC were isolated as described (7, 9). In brief, small CCD fragments, containing both PC and ICC, were isolated by immunoselection using monoclonal antibody (mAb) ST.12, which reacts with an ectoantigen on CCD cells (10, 11). CCD suspensions were then stained with a fluorescein isothiocyanate (FITC)-conjugated PC-specific antibody (DT.17; ref. 7) and the β -ICC marker peanut lectin agglutinin (PNA; cf. ref. 4) conjugated with phycoerythrin, and the two cell types were separated by fluorescence-activated cell sorting (7, 9). Since the PNA-positive population includes a few α/β -ICC hybrids (7, 12), in the present study such hybrid cells were excluded by taking advantage of the fact that such cells exhibit weak PNA binding (12). To maximize cell yields, gating parameters for 90° and forward scatter were chosen more liberally than described (7).

The purity of sorted β -ICC, as assessed by flow cytometric reanalysis and staining with other cell-specific mAbs, was $>98\%$, whereas that of sorted PC was $\approx 95\%$. Viability of β -ICC was $85\% \pm 1\%$ and that of PC was $68\% \pm 3\%$.

Culture of Sorted PC and ICC. Sorted cells were seeded on porous-bottom dishes with a surface area of 0.6 cm^2 (Millicell HA, Millipore) at a near saturating density of $4\text{--}6 \times 10^5$ viable cells per cm^2 . In each experiment, seeding density for PC and β -ICC was the same. The filter cups were placed into the central wells of organ culture dishes and incubated with 0.4 ml of medium at the apical (inner) and 0.8 ml at the basolateral (outer) side. The cultures were grown in PC-1 medium (Ventrex) supplemented with penicillin (50 units/ml), streptomycin (50 $\mu\text{g}/\text{ml}$), tylosin (12 $\mu\text{g}/\text{ml}$), and 5% decomplexed fetal bovine serum (Hyclone) and equilibrated with 5% $\text{CO}_2/95\%$ air. After the cultures reached confluence, medium was changed every 24 h.

To assess plating efficiency, in three experiments the number of nonadhered cells was determined 24 h after seeding. Plating efficiency was $49\% \pm 2\%$ in PC and $60\% \pm 2\%$ in ICC cultures.

Measurement of Transepithelial Potential Differences (PD) and Short-Circuit Currents (SCC) and Determination of Ion Concentrations. PD and SCC were measured in monolayers, using a voltage-current clamp apparatus, as described (11, 13). Electrical resistance was calculated from the ratio of PD to SCC. Pilot experiments revealed that transepithelial electrical parameters reached steady-state values at day 5–7; therefore, experiments were carried out in this time window. In experiments testing the effects of amiloride, acetazolamide, or changes in apical K^+ concentrations or pH, SCC was measured every 30 sec for 2-sec intervals, thus maintaining the cultures in a predominantly open-circuit condition. Na^+ and K^+ concentrations were determined by flame photometry in media collected, after a 24-h incubation, from the two sides of the monolayers.

Immunocytochemistry. Cells from confluent monolayers, grown on permeable supports, were detached and incubated in suspension with cell-specific mAbs followed by staining with FITC-conjugated anti-mouse immunoglobulin as in ref. 10. Cells were then fixed and mounted in 1% paraformaldehyde and inspected by fluorescence microscopy. On each sample, at least 400 cells were counted. Staining for band-3 antigen was performed by the indirect immunoperoxidase technique on acetone-fixed cytopins of cells removed from the monolayers (10) using a mAb (IVF12; generously provided by V. L. Schuster) that, in addition to basolateral band-3 antigen, also recognizes a renal mitochondrial protein that is preferentially present in α -ICC (14).

In a few cases, intact monolayers were stained at 0°C with a combination of FITC- and Texas Red-labeled cell-specific antibodies for 30 min, fixed with 1% paraformaldehyde, and mounted on coverslips for fluorescence microscopy.

RESULTS

Characteristics of Cultures Initiated with β -ICC. Sorted β -ICC in primary culture reached confluence in 3–4 days, as detected by the development of a transepithelial resistance of $885 \pm 320 \Omega \cdot cm^2$. Surprisingly, the cultures secreted acid into the apical medium, resulting in a marked transepithelial pH difference (apical pH, 5.90 ± 0.2 ; basolateral pH, 7.41 ± 0.03 ; $P < 0.001$, $n = 11$). The cultures continued to acidify the apical medium throughout the duration of the experiment. At day 6, after a 24-h incubation, the cultures generated a significant apical-to-basolateral pH difference of 1.91 ± 0.11 ($P < 0.001$) (Fig. 1 *Left*). Thus, already 3 days after seeding,

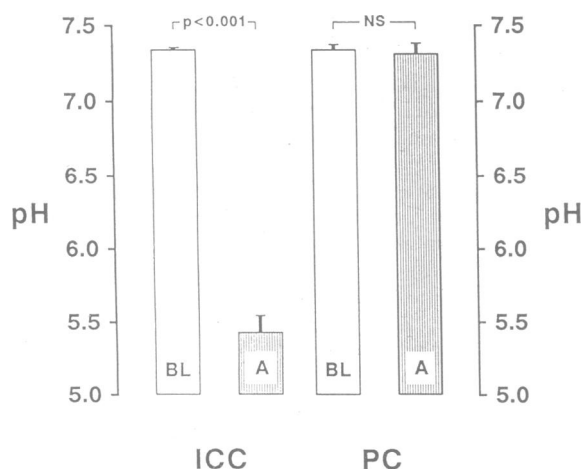


FIG. 1. Basolateral (BL) and apical (A) pH in cultures initiated with sorted β -ICC (*Left*; $n = 18$) and PC (*Right*; $n = 6$) after a 24-h period (from day 5 to day 6). Data are the means \pm SE. NS, not significant.

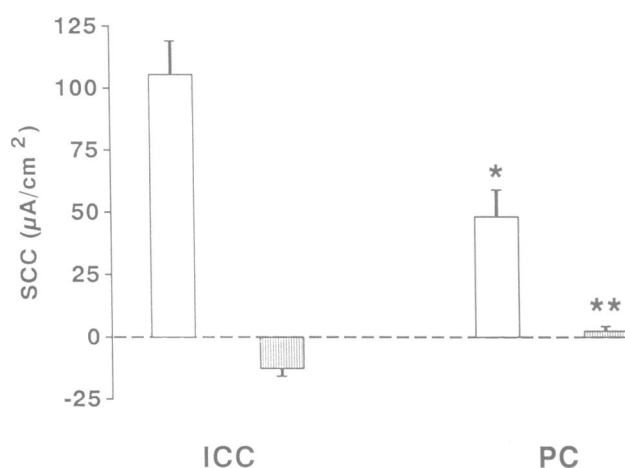


FIG. 2. Effect of amiloride ($20 \mu M$; apical side) on the SCC in cultures initiated with β -ICC (left side; $n = 8$) or PC (right side; $n = 5$). Open bars, control; hatched bars, amiloride. The effect of amiloride was statistically significant in both groups ($P < 0.0001$ for ICC and $P < 0.01$ for PC). *, $P < 0.05$ (ICC vs. PC, control period); **, $P < 0.01$ (ICC vs. PC after amiloride addition).

the initially pure β -ICC cultures exhibited a function that is believed to be specific for α -ICC *in vivo*.

Even more surprising, the same monolayers progressively acquired PC-specific functions. On day 6, transepithelial PD averaged -84.0 ± 3.5 mV while SCC was $116.7 \pm 12.3 \mu A/cm^2$ ($n = 17$). To test whether the lumen-negative PD and associated SCC are consequences of a Na^+ current through apical Na^+ channels, we examined the effect of amiloride. In cultures initiated with β -ICC, luminal addition of $20 \mu M$ amiloride not only decreased SCC dramatically (by $118.6 \pm 9.1 \mu A/cm^2$; $P < 0.01$) but also reversed its direction (Fig. 2 left side). The lumen-negative PD was also reversed (from -65.9 ± 8.9 to $+12.8 \pm 2.7$; $P < 0.001$), while transepithelial resistance increased from 623 ± 52 to $1355 \pm 289 \Omega \cdot cm^2$ following amiloride addition ($P < 0.001$).

The reversal of SCC and PD suggested that inhibition of the Na^+ current unmasked an electrogenic H^+ current. This assumption was confirmed by examining the effects of apical acidification and the carbonic anhydrase inhibitor, acetazolamide. Both interventions inhibited the reversed current. Representative experiments are shown in Fig. 3, and the mean response to acetazolamide is summarized in Fig. 4. In aggregate, these data indicate the presence of an electrogenic H^+ -secreting mechanism in cultures initiated with β -ICC.

If the expression of an amiloride-sensitive Na^+ current is due to the acquisition of PC phenotype, one would expect to see other PC-related functions as well. Since another characteristic of PC *in situ* is electrogenic K^+ secretion, we tested for the presence of this function in cultures of sorted β -ICC. Determination of K^+ and Na^+ concentrations in the basolat-

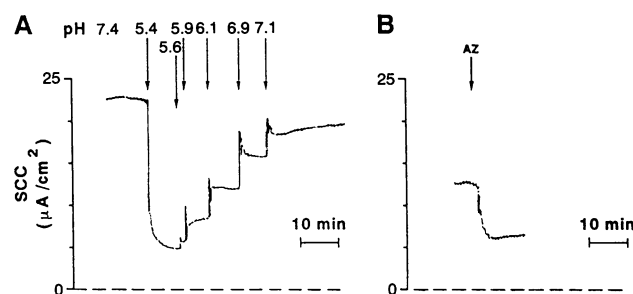


FIG. 3. Representative tracings of the effects of apical pH (A) and acetazolamide (B) on the reverse SCC in cultures of sorted β -ICC. AZ, acetazolamide (0.1 mM) on the basolateral side.

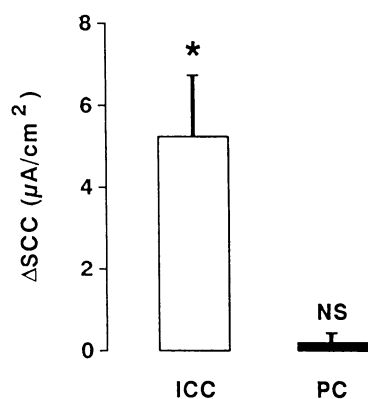


FIG. 4. Acetazolamide-induced changes in the reverse SCC in cultures of sorted β -ICC ($n = 7$) and PC ($n = 5$). *, $P < 0.01$. NS, not significant.

eral and apical medium after 24 h of incubation revealed that monolayers of sorted β -ICC generated impressive gradients for K^+ concentration (apical-to-basolateral ratio, 9.81 ± 1.13), accompanied by marked Na^+ concentration gradients in the other direction (basolateral-to-apical ratio, 1.53 ± 0.07), indicating significant K^+ secretion and Na^+ reabsorption (Fig. 5 Left). To test for the presence of apical K^+ conductances more directly, we examined the effect of increasing apical K^+ concentrations on transepithelial PD. Stepwise increments in apical K^+ concentration were associated with a significant increase in transepithelial PD (slope, 42.1 ± 10 mV per decade). This increase in PD was significantly reduced by 2 mM Ba^{2+} , a known K^+ channel inhibitor, confirming the presence of an apical K^+ conductance.

The acquisition of PC-related functions by sorted β -ICC upon culture was accompanied by the expression of PC-specific antigens. Table 1 summarizes staining of such cultures with different ICC- and PC-specific mAbs. On day 6, the percentage of cells expressing PC antigens was in the range of 45–81%, as assessed by staining with two antibodies against different antigens. At the same time, 53–80% of the cells expressed β -ICC antigens, suggesting the presence of PC/ICC hybrid cells. Double staining of β -ICC monolayers with a PC- and a β -ICC-specific antibody (DT.17 and B63, respectively) confirmed that $39.2\% \pm 4.1\%$ of the cells stained with both antibodies and thus had a dual phenotype. A representative staining of a monolayer initiated with β -ICC is shown in Fig. 6. Neither cells expressing PC antigens nor those with ICC markers formed colonies; rather, similar to the *in situ* epithelium, the two cell types were intermingled with one another. In addition to the presence of PC and β -ICC antigens, and consonant with the presence of an electrogenic H^+ secretion, $\approx 25\%$ of the cultured β -ICC also expressed band-3 antigen, a putative α -ICC marker (Table 1).

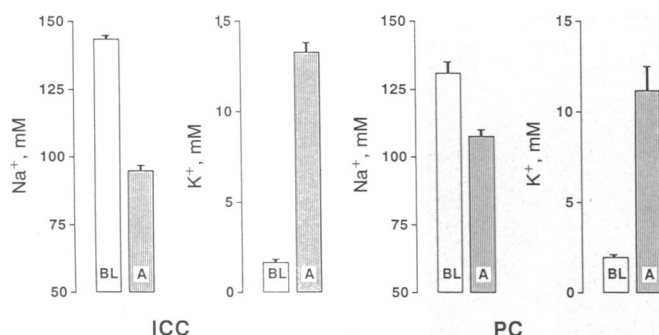


FIG. 5. Distribution of Na^+ and K^+ on the basolateral (BL) and apical (A) side of cultured β -ICC ($n = 22$) and PC ($n = 7$) after 24 h of incubation.

Table 1. Antigenic characterization of cultures initiated with sorted β -ICC and PC

| Marker specificity | mAb | % cells stained | |
|--------------------|-------------|-----------------------|----------------|
| | | β -ICC cultures | PC cultures |
| PC | ST.9 | 64.8 ± 6.1 | 69.9 ± 8.3 |
| | DT.17 | 53.4 ± 3.2 | 54.2 ± 7.2 |
| β -ICC | B63 | 66.1 ± 4.0 | 10.7 ± 1.8 |
| | B601 | 59.3 ± 2.8 | 9.6 ± 2.2 |
| α -ICC | Anti-band 3 | 25.3 ± 3.1 | 0 |

Data are the means \pm SE; $n = 5$ for β -ICC cultures and $n = 3$ for PC cultures. At day 6, cultured cells were detached from the filters and stained with mAb as described in *Methods*. For each marker, at least 400 cells were inspected.

Characteristics of Cultures Initiated with Sorted PC. On day 6, primary cultures of sorted PC, grown under the same conditions as β -ICC, had a lumen-negative PD of -63.4 ± 8.7 mV and SCC of 39.5 ± 6.5 $\mu A/cm^2$; the transepithelial resistance averaged 1675 ± 486 $\Omega \cdot cm^2$ ($n = 6$). It should be noted that, paradoxically, both the lumen-negative PD and the inward current were consistently lower in PC cultures than in those initiated with β -ICC ($P < 0.01$), whereas resistance was significantly higher in PC than in ICC cultures ($P < 0.002$). In PC cultures, SCC was also inhibited by amiloride (by 45.7 ± 9.1 $\mu A/cm^2$; $P < 0.01$; Fig. 2 right side), but, in contrast to β -ICC cultures, there was no evidence for a reversed current, and acetazolamide had no effect on SCC (Fig. 4). PC cultures did not acidify the apical medium (Fig. 1 Right), a result that is compatible with the lack of electro-

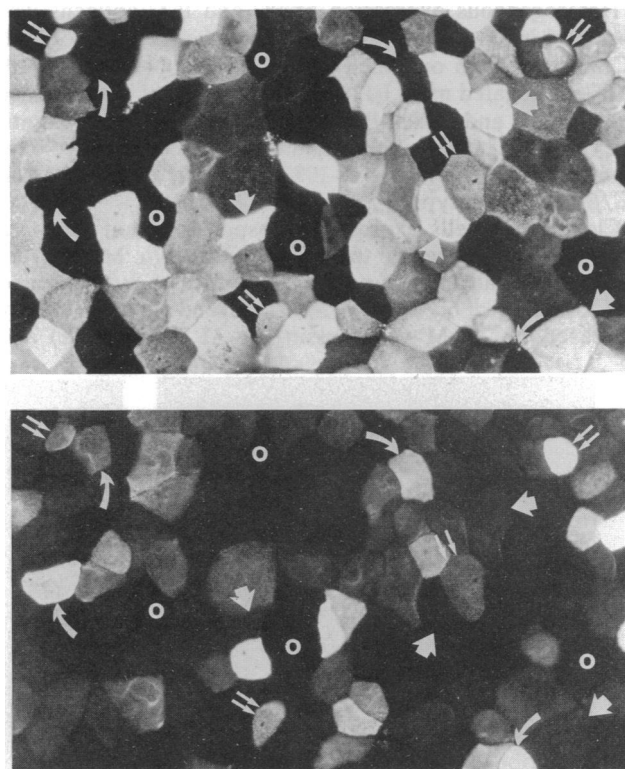


FIG. 6. Fluorescence micrographs of the distribution of the Texas Red-labeled PC marker DT.17 (Upper) and the FITC-labeled β -ICC marker B63 (Lower) in a filter-grown primary culture of sorted β -ICC at day 6. Typical examples of the four cell types present are labeled as follows. Curved arrows indicate cells staining for the β -ICC marker only, thick arrows point to cells staining for the PC marker only, double arrows indicate PC/ β -ICC hybrid cells, and O labels cells that are negative for both markers. ($\times 320$.)

genic H^+ secretion. Thus, there was no indication that sorted PC acquired α -ICC-related functions upon culture.

PC cultures also reabsorbed Na^+ and secreted K^+ , as indicated by the formation of transepithelial Na^+ and K^+ concentration gradients (Fig. 5 *Right*), albeit at somewhat slower rate than those initiated with β -ICC. Diffusion potentials evoked by increasing apical K^+ concentration were also considerably lower in PC than in β -ICC cultures (27.9 ± 3.3 vs. 42.1 ± 10 mV per decade).

Staining with PC- and ICC-specific markers revealed that the majority of sorted PC retained PC-related antigens; no staining was observed for band-3 antigen, whereas about 10% of the cells expressed β -ICC markers (Table 1). Whether the presence of β -ICC is related to lower purity of the initial isolates or represents back conversion could not be determined.

Evidence for Cellular Interconversion in a Cloned CCD Cell Line. Unequivocal evidence for cellular interconversion would be the demonstration that cloned ICC are able to convert into PC. Attempts to clone sorted rabbit CCD cells were unsuccessful, as the cells failed to grow at clonal density. To circumvent this difficulty, we have generated a continuous CCD cell line (M-1) from a mouse transgenic for the early region of simian virus 40. The characteristics of this cell line are described elsewhere (15). M-1 cells were dispersed by prolonged trypsinization and cloned by either limiting dilution to an average plating density of 0.5 cell per well or by single-cell deposition with a cell sorter. The presence of a maximum of one cell per well was verified by microscopic inspection. Expression of PC- and ICC-specific functions and antigens were then studied in four sublines obtained by limiting dilution and in five others obtained by single-cell deposition.

Heterogeneous expression of PC and ICC antigens was observed in all cell lines. Fig. 7 shows the presence of both PC and β -ICC in one such colony after dual staining with FITC-conjugated mAb F13/0121, a PC marker in the mouse kidney (15), and Texas Red-conjugated B63, a β -ICC antibody (15, 16). Although the majority of the cells stained only for one of these markers, ≈ 5 –10% of the cells exhibited a dual PC/ β -ICC phenotype.

Cloned M-1 cells expressed transport functions that are thought to be segregated to PC and ICC *in situ*. Monolayers of such cells had a lumen-negative PD of -43.5 ± 4.3 mV and a SCC of $52.7 \pm 6.7 \mu A/cm^2$. This current was significantly increased by 1 nM vasopressin (by $31.5\% \pm 5.3\%$) and

reduced by $91\% \pm 1.8\%$ after apical administration of amiloride, indicating the presence of PC-related functions. The same monolayers also developed an apical-to-basolateral pH difference of 0.25 ± 0.04 units (lower at the basolateral side) after 24 h of incubation, suggesting the presence of HCO_3^- -secreting cells (i.e., β -ICC) in the monolayer.

In Situ Hybrids. If interconversion between the different cell types of the CCD also occurs *in vivo*, one would expect to see hybrid cells expressing both ICC- and PC-specific antigens. Indeed, immunohistochemical staining of rabbit kidney sections with combinations of cell-specific antibodies revealed the presence of a minority cell population that was double-stained with PC and ICC markers. The percentage of cells double stained with an antibody against H^+ -ATPase (ICC marker; see ref. 17) and DT.17 (PC specific) or with PNA and DT.17 was $\approx 1\%$. Congruent observations were made on mouse kidney sections double stained with mAb F13/0121 (PC specific) and B63 (β -ICC marker). A more common type of hybrid cell was seen on sections dual stained with B63 and an antibody against band 3. These β/α -ICC hybrids occurred with a frequency of ≈ 3 –5%.

To exclude the possibility that double staining might be an artifact due to projection of one cell over the other and to quantitate the frequency of hybrid cells, suspensions of freshly isolated CCD cells were costained with mAb DT.17 (PC specific) and PNA (β -ICC marker). Flow cytometric analysis revealed that $0.8\% \pm 0.1\%$ of the CCD population was PC/ β -ICC hybrid, reacting with both markers. Hybrid cells were then sorted out and examined by fluorescence microscopy to verify that the dual fluorescence indeed originated from single cells and not from doublets.

DISCUSSION

One of the unique features of distal urinary epithelia is cellular heterogeneity. Just like the urinary bladder of the turtle and the toad (18), the mammalian collecting duct consists of two functionally and morphologically different cell types intermingled with one another: PC and ICC.

The mechanism by which the cellular heterogeneity of distal urinary epithelia arises and is maintained is not well understood. Since even in the mature kidney, cells of the CCD do divide, albeit at a slow rate (19), there must be a mechanism that maintains the observed constant ratio of the two cell types. In theory, this can be achieved in two ways:

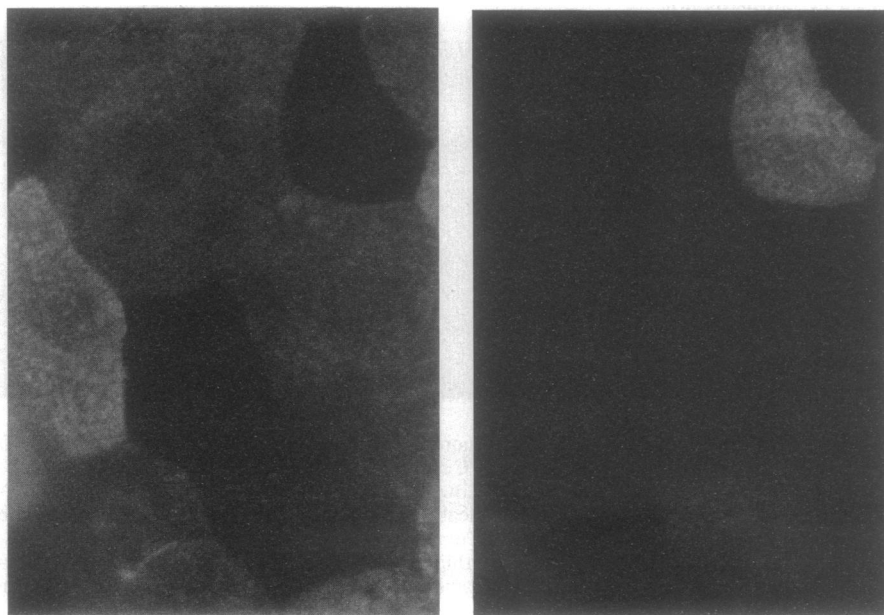


FIG. 7. Distribution of the fluorescence associated with the FITC-labeled PC antibody F13/0121 (*Left*) and the Texas Red-labeled β -ICC antibody B63 (*Right*) in clonal M-1 cells grown on permeable support. ($\times 1300$.)

PC and ICC might represent independent lineages whose division rates are well coordinated or, alternatively, the two cell types might originate from one another in a coordinated way. Although PC and ICC are generally assumed to represent separate cell lineages, experimental evidence for this assumption is lacking. The possibility that PC and ICC might originate from one another has not been raised thus far, although a study by Schwartz *et al.* (20) suggested that β -ICC might convert to α -ICC.

The present data demonstrate that sorted β -ICC are able to give rise to H^+ -secreting cultures, clearly indicating the presence of functional α -ICC. Such cells were identified by immunocytochemistry by the presence of band-3 antigen. The relatively low frequency of cells staining with the anti-band-3 antibody might be due to penetration problems, as this antibody recognizes an intracellular antigen (14), and due to a low expression of band-3 antigen in cultured cells.

Our results indicate an even greater plasticity of β -ICC than previously thought, because cultures initiated with sorted β -ICC exhibited, in addition to α -ICC-related features, functions and antigens characteristic for PC *in vivo*. The exact sequence of events leading to the appearance of PC in β -ICC cultures cannot be determined from the present data. It is possible that β -ICC first convert to α -ICC and then differentiate further into PC, which would be compatible with the observation that in cultures of sorted β -ICC the appearance of α -ICC-related functions precedes that of PC functions (G.F.-T., unpublished results). Nevertheless, the possibility that α -ICC and PC might both originate from β -ICC directly cannot be excluded, especially since we observed hybrid cells expressing both β -ICC and PC antigens. It is also possible that sorted β -ICC might first dedifferentiate into a precursor cell that, in turn, could later differentiate into α -ICC and/or PC. This possibility, however, seems unlikely because β -ICC markers remained expressed during the entire observation period (up to 7 days in culture), and, as mentioned above, many hybrid cells could be observed coexpressing β -ICC and PC markers. Nevertheless, we cannot exclude the possibility that sorted β -ICC might contain a subpopulation of precursors that might give rise to α -ICC and PC. In any event, it seems that β -ICC have the potential to recreate the characteristic cellular heterogeneity of the CCD.

That the cellular heterogeneity of cultured β -ICC is due to bona fide cellular interconversion and not to preferential proliferation of a few contaminating PC is supported by the following evidence. First, cultures were initiated with highly purified β -ICC populations at near saturating densities. Furthermore, even if re-sorted cells, with a purity of 99.9%, were used, the characteristics of the cultures arising were identical (data not shown). Thus the few contaminating PC (<0.1% in re-sorted cells) would need to have markedly higher division rates to overgrow ICC during the short period (≈ 3 days) of monolayer formation. This, however, seems unlikely, since sorted PC had a lower plating efficiency and grew slower than sorted ICC. In fact, in every respect (electrophysiological parameters, Na^+ reabsorption, and K^+ secretion), monolayers arising from β -ICC exhibited more pronounced PC-related functions than those originating from sorted PC, which raises the possibility that PC might be terminally differentiated. One could argue that for optimal proliferation PC need some growth factors produced by ICC, and thus, a few contaminating PC in the ICC cultures might proliferate at a much higher rate than those in the PC cultures. If this were the case, one would expect to see PC growing in colonies in the ICC cultures. However, we have never observed such PC "islands".

Another line of evidence for cellular interconversion comes from the experiments performed on cloned cells of the M-1 CCD cell line (15). Colonies arising from single cells exhibited both PC and β -ICC specific antigens, segregated into different

cells. In addition, monolayers of cloned M-1 cells expressed both β -ICC- and PC-related functions. Apparently, such morphological and functional heterogeneity is not unique for clonal M-1 cells: both A-6 cells, which originate from *Xenopus* kidney, and COMMA-D, a mammary cell line, have been shown to express antigens and functions heterogeneously even after cloning (21, 22).

The finding that cultured β -ICC can give rise to α -ICC and PC, while PC do not show significant conversion to ICC, suggests that, among the CCD populations, β -ICC behave as stem cells. Still, one cannot extrapolate from the present data that the same sequence of events (i.e., β -ICC to α -ICC and PC) occurs during embryonic development. It is also possible that the culture conditions used in this study accelerate or alter the natural progression. Nevertheless, the presence of PC/ β -ICC hybrids *in situ*, observed in this study, or the presence of α / β -ICC hybrids, described earlier (7, 12), are consonant with the idea of cellular conversion occurring *in vivo*. In addition, the observation that *in vivo* β -ICC seem to be able to convert into α -ICC while the reverse process apparently does not occur (20, 23) suggests that conversion of the two ICC subtypes might also be unidirectional.

On the basis of the results presented here, we conclude that the characteristic cellular heterogeneity of the CCD might be due to conversion of one cell type to the other. Our results point to a remarkably plasticity of the CCD, because conversion of a β -ICC to α -ICC or PC requires major remodeling of the cell. The spontaneous cell differentiation observed in primary cultures of sorted cells makes this system a particularly attractive model to unravel important events in epithelial cell differentiation.

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- Kaissling, B. & Kriz, W. (1979) *Adv. Anat. Embryol. Cell Biol.* **56**.
- Madsen, K. M. & Tischer, C. C. (1986) *Am. J. Physiol.* **250**, F1-F15.
- Wright, F. S. & Giebisch, G. (1985) In *The Kidney*, eds. Seldin, D. W. & Giebisch, G. (Raven, New York), pp. 1224-1249.
- Schuster, V. L. & Stokes, J. B. (1987) *Am. J. Physiol.* **253**, F203-F212.
- Brown, D., Hirsch, S. & Gluck, S. (1988) *Nature (London)* **331**, 622-624.
- Kirk, K. L., Buku, A. & Eggena, P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6000-6004.
- Fejes-Tóth, G. & Náray-Fejes-Tóth, A. (1989) *Am. J. Physiol.* **256**, F742-F750.
- Muto, S., Giebisch, G. & Sansom, S. (1987) *Am. J. Physiol.* **253**, F742-F752.
- Fejes-Tóth, G. & Náray-Fejes-Tóth, A. (1991) *J. Tissue Cult. Methods* **13**, 173-178.
- Fejes-Tóth, G. & Náray-Fejes-Tóth, A. (1987) *Am. J. Physiol.* **253**, F1302-F1307.
- Náray-Fejes-Tóth, A. & Fejes-Tóth, G. (1991) *J. Tissue Cult. Methods* **13**, 179-184.
- Schuster, V. L., Fejes-Tóth, G., Náray-Fejes-Tóth, A. & Gluck, S. (1991) *Am. J. Physiol.* **260**, F506-F517.
- Náray-Fejes-Tóth, A. & Fejes-Tóth, G. (1990) *Am. J. Physiol.* **259**, F672-F678.
- Ostergaard, L. S., Jennings, M. J., Karniski, L. P. & Schuster, V. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 981-985.
- Stoos, B., Náray-Fejes-Tóth, A., Carretero, O. A., Ito, S. & Fejes-Tóth, G. (1991) *Kidney Int.* **39**, 1168-1175.
- Fejes-Tóth, G., Mehrgut, F. M., Náray-Fejes-Tóth, A. & Schwartz, G. J. (1990) *Kidney Int.* **37**, 53.
- Bastani, B., Purcell, H., Hemken, P., Trigg, D. & Gluck, S. (1991) *J. Clin. Invest.* **88**, 126-136.
- Steinmetz, P. R. (1986) *Am. J. Physiol.* **251**, F173-F187.
- Jakobsson, B., Bohman, S.-O., Sundelin, B. & Aperia, A. (1988) *Kidney Int.* **33**, 662-666.
- Schwartz, G. J., Barasch, J. & Al-Awqati, Q. (1985) *Nature (London)* **318**, 368-371.
- Moberly, J. B. & Fanestil, D. D. (1987) *Am. J. Physiol.* **253**, C37-C44.
- Campbell, M., Taha, M. M., Medina, D. & Rosen, J. M. (1988) *Exp. Cell Res.* **177**, 109-121.
- Hayashi, M., Schuster, V. L. & Stokes, J. B. (1988) *Am. J. Physiol.* **255**, F220-F228.