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Chloride channels ClC-2 and ICln mRNA expression differs in renal epithelial ontogeny

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Chloride channels ClC-2 and ICln mRNA expression differs in renal epithelial ontogeny. Development-dependent mRNA expression of the chloride channels ClC-2 and ICln was studied by quantitative reverse transcriptase-polymerase chain reaction in rat ureteric bud and cortical collecting duct primary monolayer cultures. Abundance of ClC-2 mRNA increased in ureteric bud cells between embryonic day 15 (E15) and E17, peaked at postnatal day 3 (P3), and was down-regulated at P7 when morphogenesis is complete, suggesting a specific embryonic function. Expression of ICln mRNA, in contrast, up-regulated continuously with development.

The permanent, metanephric kidney is derived from two distinct embryonic cell populations: the mesenchymal and the epithelial cells of the ureteric bud (UB). The mesenchymal blastema generates the nephron up to the connecting tubule, whereas the UB branches to give rise to the collecting duct (CD) system [1]. The volume-regulatory chloride channel ClC-2 [2] is highly expressed in fetal lung and is down-regulated after birth [3]. Because lung and CD epithelia have branching morphogenesis in common, the temporal pattern of ClC-2 mRNA expression was analyzed in primary UB and cortical CD (CCD) monolayer cultures and compared with that of ICln [4], a further volume-regulatory Cl channel (or channel regulator).

METHODS

Primary monolayer cultures were obtained as described previously [5, 6] from the most distal dichotomous tubular branches of the outer renal cortex of Wistar rats at different embryonic and postnatal stages [embryonic day 15 (E15) to postnatal day 14 (P14; Fig. 1)]. cDNA was prepared as follows. Total RNA from six corresponding monolayers (approximately 5-mm diameter) was isolated according to [7] using Tri Reagent (Biozol, Eching, Germany), pooled, and reversely transcribed. cDNA was precipitated, washed, diluted in 40 μl 10 mM Tris/1 mM EDTA, pH 8.0, and stored at −80°C. Within an experimental series, cDNA preparations from different developmental stages were adjusted to equal concentrations of a housekeeper [3], the β-actin template, such that amounts of β-actin polymerase chain reaction (PCR) products did not deviate by more than a factor of two. Fragments of cDNA encoding the Cl channel ClC-2 (gene bank accession #X64139), ICln (gene bank accession #L26450), and β-actin (gene bank accession #M12481) were amplified using sequence-specific oligonucleotide primers with products of 375, 326, and 351 bp lengths, respectively [ClC-2 sense, 5′-CAA GCC TCC AGG AAG GTA C-3′ (bp 2306 to bp 2324); ClC-2 antisense, 5′-TCC CAA TGA GTC TGC CAA TG-3′ (bp 2661 to bp 2680); ICln sense, 5′-ATG TCA TGG TGA ATG CCA GA-3′ (bp 276 to bp 295); ICln antisense, 5′-TAA TCT CTC CAG CGT GGC TT-3′ (bp 582 to bp 601); β-actin sense, 5′-AAC CGC GAG AAG ATG ACC CAG ATC ATG TTT-3′ (bp 265 to bp 294); β-actin antisense, 5′-AGC AGC CGT GGC CAT CTC TTG CTC GAA GTC-3′ (bp 586 to bp 615); primers were purchased from Gibco BRL, Eggenstein, Germany]. The reaction mixture contained 2-μl serially (in H_2O) diluted cDNA (dilution factors, 1:5 to 1:25 for ClC-2 and ICln or 1:100 to 1:10,000 for β-actin), 20.6 μl H_2O, 0.2 μl dNTPs (25 mM; Promega, Ingelheim, Germany), 0.25 μl sense primer, 0.25 μl antisense primer (both 10 μM), 0.125 μl AmpliTaq Gold polymerase (5 U/μl), and 2.5 μl 10-fold buffer (15 mM $MgCl_2$; both Perkin Elmer, Weiterstadt, Germany). Samples were incubated in a MJ-Research thermal cycler (DNA Engine PTC 200; Biozym, Oldendorf, Germany) initially for 10 minutes at 95°C followed by 30 cycles of 45 seconds at 94°C, 1 minute at 56°C, and 1 minute at 72°C and a final extension at 72°C for 7 minutes. A 12 μl aliquot of each PCR reaction was separated by electrophoresis in a nondenaturating 5% acrylamide gel. Specificity of the PCR products was confirmed by restriction analysis and sequencing. The PCR product was evaluated quantitatively as

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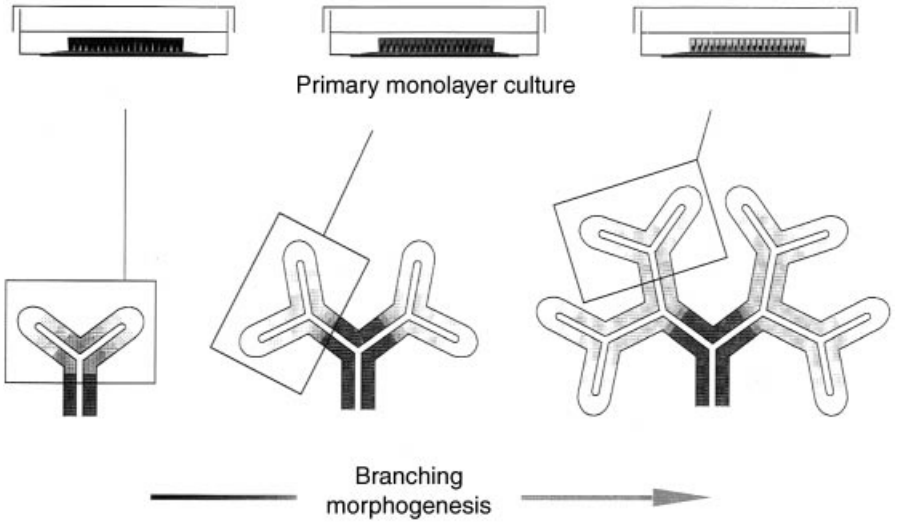

Fig. 1. Scheme of growing ureteric bud and cortical collecting duct epithelium *in vitro*. Monolayer cultures were obtained by microdissecting and explanting the outermost dichotomous branches of outgrowing ureteric trees at various embryonic and postnatal developmental stages.

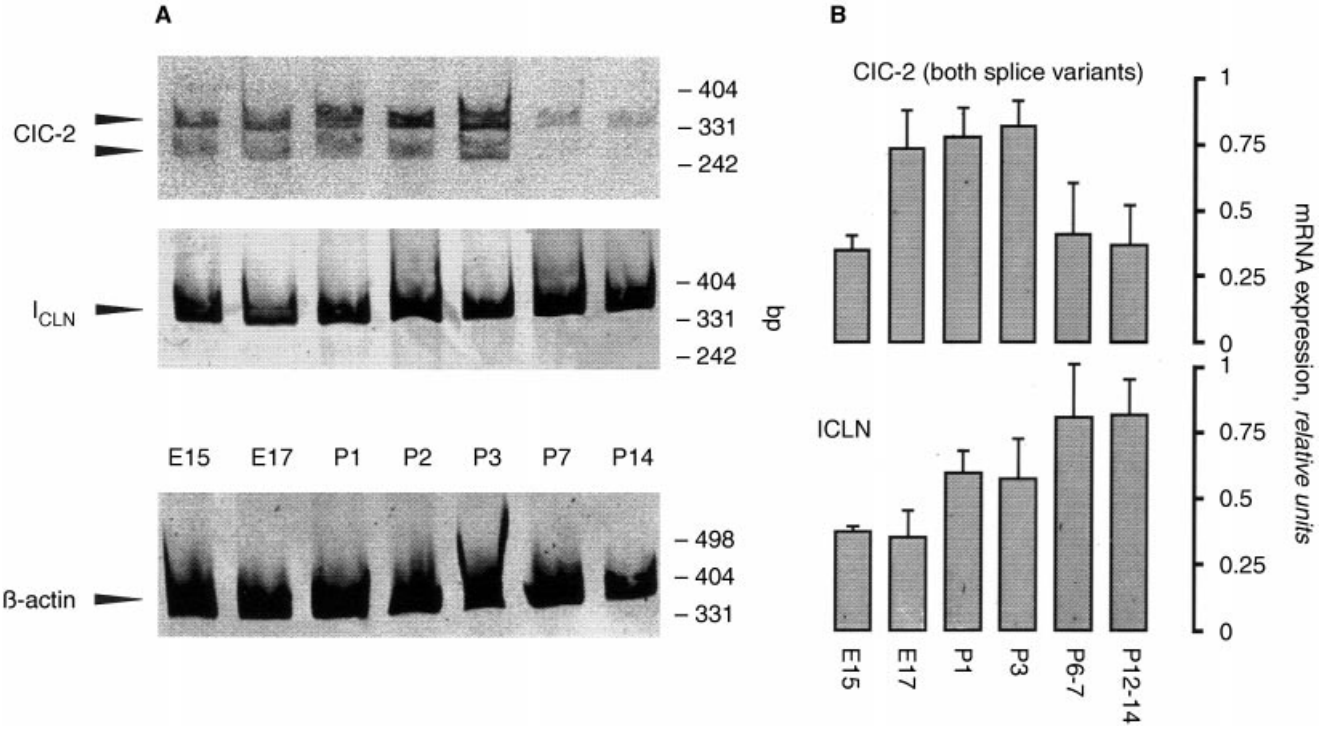

Fig. 2. Semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). (*A*) ClC-2— (upper gel), ICln- (middle gel), and β-actin— (lower gel) specific polymerase chain reaction (PCR) products amplified from ureteric bud and cortical collecting duct epithelium at various embryonic (E15 to E17) and postnatal (P1 to P14) developmental stages. PCR products were separated by acrylamide gelelectrophoresis, stained by VistraGreen fluorescence dye, and visualized on a Fluorophosphorimager. (*B*) Amount of DNA in gel bands (A), normalized by the β-actin bands, and plotted against the developmental stages (means ± SE, $N = 3$ to 4).

follows. Gel bands were stained with fluorescence DNA-dye (Vistragreen ™, Amersham), visualized, and quantified by ImageQuant Software on a Storm Fluorophosphorimager (both Molecular Dynamics, Krefeld, Germany). Within the serial cDNA dilutions applied (1:100 to 1:10,000), the amount of β-actin PCR product was directly proportional to the template concentration indicating the nonsaturating behavior of the PCR reaction. The amount of ClC-2 or ICln PCR product was normalized for the β-actin value. Within each series of cDNA preparation, β-actin—normalized values X_i of the different developmental stages were divided by the largest value X_{max} to normalize between the different experimental series further.

RESULTS AND DISCUSSION

Ureteric bud and CCD epithelia in primary monolayer culture conserve their embryonic and mature phenotype, respectively, as characterized by patch-clamp techniques [6]. In this study, UB and CCD monolayers expressed mRNAs encoding the Cl channel ClC-2 and the Cl channel or regulator ICln, respectively. Besides the expected 375-bp CLC-2 band, a 312-bp fragment was amplified from all cDNAs investigated. In this shorter ClC-2 PCR product, 63 bps (bp 2398 to bp 2460) were deleted, and the following 5′-CTG GCG-3′ (bp 2461 to bp 2466) was exchanged to 5′-TCA GAA-3′ by alternative splicing, resulting in a loss of 21 amino acids and in an exchange of two AA at the cytoplasmic C terminus of the channel between the putative transmembrane domain D12 and region D13 [2]. Messenger RNA expression of both ClC-2 splice variants increased on days E15 to E17 peaked between E17 to P3 and decreased postnatally between days P3 to P6 (Fig. 2). A similar time course of ClC-2 mRNA expression is seen in the developing lung, where the postnatal down-regulation of ClC-2 coincides with the switch from fluid secretion to reabsorption of alveolar cells [3]. In contrast to ClC-2, mRNA expression of ICln increased during the entire embryonic and postnatal period of development (Fig. 2). This pattern is consistent with the appearance of a VSOAC-like, swelling-activated Cl conductance [8] in perinatal UB monolayers (S. Huber, M. Horster, unpublished observations). In conclusion, the temporal pattern of ClC-2 expression suggests a specific embryonic function of this channel in epithelia undergoing branching morphogenesis, whereas the increasing expression of ICln mRNA might reflect the development of vectorial transport with its enhanced requirement for volume regulation (after days P6 to P7) when morphogenesis has been completed.

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