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Cells in focus

# The renal connecting tubule: Resolved and unresolved issues in $Ca^{2+}$ transport

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ABSTRACT

The renal connecting tubule (CNT) localizes to the distal part of the nephron between the distal convoluted tubule and the collecting duct, and consists of two different cell types: segment-specific and intercalated cells. The former reabsorb water ($H_2O$), sodium ($Na^+$) and calcium ($Ca^{2+}$) ions to the blood compartment, while secreting potassium ions ($K^+$) into the pro-urine. The latter cells contribute to the renal control of the acid-base balance. Several factors and hormones tightly regulate these transport processes. Although the CNT reabsorbs only ~15% of filtered $Ca^{2+}$ load, this segment is finally decisive for the amount of $Ca^{2+}$ that appears in the urine. Impaired $Ca^{2+}$ transport across CNT can provoke severe urinary $Ca^{2+}$ excretion, called hypercalciuria. This review mainly focuses on the activity, abundance and expression of the epithelial $Ca^{2+}$ channel named Transient Receptor Potential Vanilloid 5 (TRPV5) that is the gatekeeper of active $Ca^{2+}$ reabsorption in the CNT.



**Cell facts**

- The renal connecting tubule is located in the distal part of the nephron and consists of segment-specific and intercalated cells.
- The segment-specific cells finely regulate the reabsorption of $H_2O$, $Na^+$, $K^+$ and $Ca^{2+}$.
- The segment-specific cells express TRPV5 which facilitates the rate-limiting step in active transcellular $Ca^{2+}$ transport.

## 1. Introduction

The kidneys control the maintenance of body ion balance and arterial blood pressure. Blood is filtrated by the glomeruli and the electrolytes are reabsorbed from the pro-urine along the various segments constituting the nephron. The distal part of the nephron that comprises the distal convoluted tubule (DCT), the connecting duct (CNT) and the collecting duct (CD) achieves the final adjustment of renal ion excretion which is regulated by various hormones.

The CNT displays different morphologic characteristics. While the CNT is short and directly linked to the CD in the superficial cortex, it is longer and merges with other CNT segments in the juxtamedullar region to form the "arcades" before flowing into the cortical collecting duct (Fig. 1A). The epithelium of CNT is composed of two different cell types: intercalated and non-intercalated cells that are intermingled with one another at a ratio of approximately 1:2. The nomenclature of these cells is not yet unambiguously defined. The non-intercalated cells have been named CNT cells (Seldin and Giebisch, 1992). However, the term "CNT cells" is confusing since intercalated cells are also CNT cells, i.e. cells originating from the CNT. Alternatively, authors (Verrey, 2001; Ronzaud et al., 2007; Wang et al., 2010) proposed the term "principal cells" to characterize the non-intercalated cells of the aldosterone-sensitive distal part of the nephron, composed of DCT, CNT and CD. However, in a strict sense, the term "principal cells" refers only to the non-intercalated cells of the CD (Seldin and Giebisch, 1992). Thus, in this review, we prefer to use the term "segment-specific cells" to describe the non-intercalated cells of the CNT (Loffing and Korbmacher, 2009).

Segment-specific cells of the CNT, which have a polygonal shape, express many transporters involved in transcellular ion transport. They actively transport $H_2O$, $Na^+$, $K^+$ and $Ca^{2+}$ (Fig. 1B). Intercalated cells that have a rounded shape regulate the urinary acidification. Both cell types contain a high density of mitochondria (Fig. 1C and D).

## 2. CNT origin

The embryological origin of CNT is not entirely clear. It has been proposed that it originates from the nephrogenic blastema (Neiss and Klehn, 1981) or from the branching ureteric bud (Howie et al., 1993). However, others suggest that the CNT develops by mutual induction processes initiated from adjoining segments. This last

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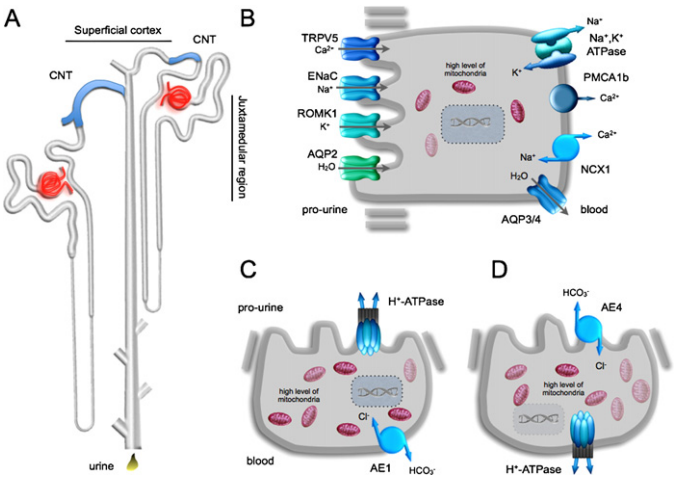


**Fig. 1.** The morphologic characteristics and cell types of the CNT. (A) The CNT (*in blue*) is located to the distal part of the nephron and consists of segment-specific and intercalated cells. While the CNT is short in the superficial cortex, it is longer in the juxtamedullar region. (B) Segment-specific cells of CNT are characterized by different transporters: (1) in the apical side: TRPV5 mediates the influx of $Ca^{2+}$, ENaC mediates the influx of $Na^+$, ROMK1 mediates the efflux of $K^+$, AQP2 permits $H_2O$ transport inside cell; (2) in the basolateral side; $Ca^{2+}$ is extruded by the concerted action of the PMCA1b pump and the NCX1 exchanger, $Na^+$ is extruded by the $Na^+/K^+$-ATPase which also permits the entry of $K^+$, $H_2O$ is extruded in the blood compartment by the AQP3 or AQP4. (C) Intercalated type A cells apically secrete $H^+$ in the pro-urine compartment by a $H^+$-ATPase and basolaterally extrudes $HCO_3^-$ in the blood compartment by an $Cl^-/HCO_3^-$ exchanger (AE1). (D) Intercalated type B cells apically secretes $HCO_3^-$ in the pro-urine compartment by an $Cl^-/HCO_3^-$ exchanger (AE4) and basolaterally extrudes $H^+$ in the blood compartment by a $H^+$-ATPase.

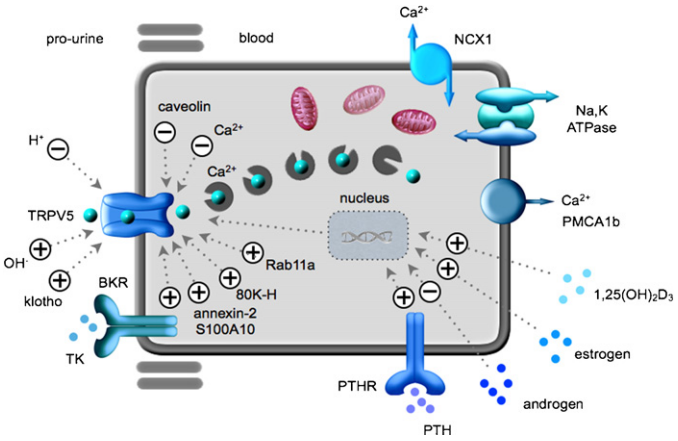


**Fig. 2.** $Ca^{2+}$ transport across segment-specific cells of CNT. TRPV5 mediates the apical entry of $Ca^{2+}$ inside the cell. In the urinary compartment, acidic conditions ($H^+$) decrease the activity of TRPV5. Klotho directly interacts with TRPV5 and tissue kallikrein binds to the bradykinin receptor (BKR) located to the apical membrane. Both klotho and tissue kallikrein (TK) increase the apical abundance of TRPV5. Intracellularly, $Ca^{2+}$ is buffered by calbindin-$D_{28K}$ and is shuttled to the basolateral membrane that contains the NCX1 exchanger and the PMCA1b pump. Free cytosolic $Ca^{2+}$ decreases the open probability of TRPV5 while 80K-H increases its activity. Rab11a and the annexin-2-S100A complex increase the apical insertion of TRPV5 while caveolin decrease its plasma membrane abundance. In the blood compartment, PTH, $1,25(OH)_2D_3$ and estrogen increase the transcription and protein expression of TRPV5 while androgen inhibits its level of expression.

hypothesis is consistent with the fact that the CNT shares characteristics of both the DCT and CD. Like the DCT, the CNT expresses TRPV5 and like the CD, the CNT has numerous intercalated cells (Schmitt et al., 1999).

## 3. Function

### 3.1. Segment-specific cells of CNT

#### 3.1.1. $H_2O$ transport

Vasopressin increases the $H_2O$ reabsorption in the CNT by binding to the basolateral $V_2$ receptor that stimulates a cyclic adenosine monophosphate and protein kinase A (cAMP-PKA)-dependent signaling pathway. This ultimately leads to the apical translocation and activation of the water channel aquaporin 2 (AQP2). $H_2O$ is finally transported at the basolateral side by aquaporin 3 (AQP3) and 4 (AQP4) channels (Coleman et al., 2000).

#### 3.1.2. $Na^+$ transport

Active $Na^+$ transport in the CNT is a two-step process that involves $Na^+$ uptake by the apical ENaC and $Na^+$ extrusion by the basolateral $Na^+$ pump $Na^+/K^+$-ATPase. It is stimulated by several hormones including vasopressin (Bugaj et al., 2009), insulin (Markadieu et al., 2009) and aldosterone (Verrey et al., 2008). Aldosterone binds to the mineralocorticoid receptor and increases activity and expression of $Na^+$ transporters and thus stimulates the transepithelial $Na^+$ reabsorption. The existence of genetic disorders associated with a loss-of-function or hyperactivity of ENaC, leading to renal salt wasting syndromes and arterial hypertension, respectively, emphasizes that ENaC is the rate-limiting step of active $Na^+$ transport (Loffing and Korbmacher, 2009).

#### 3.1.3. $K^+$ transport

Segment-specific cells are also involved in the regulation of $K^+$ secretion. $K^+$ entry into the CNT cell is facilitated by the $Na^+$–$K^+$/ATPase located in the basolateral membrane. Subsequently, accumulated $K^+$ ions are released in the pro-urine via the renal outer medulla potassium channel ROMK1. The main factors regulating $K^+$ secretion are the dietary $K^+$ intake and aldosterone (Frindt et al., 2009).

#### 3.1.4. $Ca^{2+}$ transport

$Ca^{2+}$ plays a fundamental role in many cellular processes and its extracellular concentration is controlled by the kidney, intestine and bone in a concerted fashion. In kidney, the late part of DCT and the CNT are the site of active $Ca^{2+}$ transport and precisely regulate $Ca^{2+}$ reabsorption. Although these distal segment reabsorb only ~15% of the $Ca^{2+}$ filtered, they are responsible for the final adjustment of urinary $Ca^{2+}$ excretion and are tightly regulated by several hormones. $Ca^{2+}$ enters the CNT cell through TRPV5 (Hoenderop et al., 1999) and is extruded into the blood compartment by the concerted action of the plasma membrane $Ca^{2+}$ pump (PMCA1b) and the $Na^+/Ca^{2+}$ exchanger (NCX1). TRPV5 knockout ($TRPV5^{-/-}$) mice exhibit severe urinary $Ca^{2+}$ wasting, providing evidence for a gatekeeper role of TRPV5 in active renal $Ca^{2+}$ transport (Hoenderop et al., 2003). *In vivo* micropuncture experiments confirmed that $Ca^{2+}$ reabsorption is indeed impaired in the CNT. Moreover, $TRPV5^{-/-}$ mice displayed phosphaturia, compensatory intestinal $Ca^{2+}$ hyperabsorption, reduced bone thickness, polyuria and urinary acidification (Hoenderop et al., 2003). Intracellular $Ca^{2+}$ concentration is kept low with respect to the extracellular fluid. Calbindin-$D_{28K}$, a $Ca^{2+}$-binding protein, appears to shuttle $Ca^{2+}$ from the TRPV5 channel to the basolateral $Ca^{2+}$ transporters. Simultaneously, calbindin-$D_{28K}$ buffers $Ca^{2+}$ during high $Ca^{2+}$ influx providing protection against toxic high cytosolic $Ca^{2+}$ concentrations (Lambers et al., 2006).

The mechanisms by which TRPV5 is regulated can be classified in the following categories: (i) increase in TRPV5 protein expression; (ii) plasma membrane insertion of TRPV5; (iii) direct TRPV5 channel activation; and (iv) plasma membrane TRPV5 retrieval (Fig. 2 and Table 1). Calciotropic hormones, such as vitamin $D_3$ and parathyroid hormone (PTH), increase the protein expression of TRPV5 (Boros et al., 2009). The active form of vitamin $D_3$, 1,25-