Role of SGK in mineralocorticoid-regulated sodium transport

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Role of SGK in mineralocorticoid-regulated sodium transport. Mineralocorticoids stimulate electrogenic Na^+ transport in tight epithelia by altering the transcription of specific genes. Although the earliest mineralocorticoid effect is to increase the activity of the epithelial sodium channel (ENaC), ENaC mRNA and protein levels do not change. Instead, physiologic observations suggest that a mineralocorticoid target gene(s) encodes an ENaC regulator(s). To begin to identify and characterize mineralocorticoid-regulated target genes, we used suppression-subtractive hybridization to generate a cDNA library from A6 cells, a stable cell line of *Xenopus laevis* of distal nephron origin. A serine-threonine kinase, SGK, was identified from this screen. Sequence comparison revealed that frog, rat, and human SGK are 92% identical and 96% similar at the amino acid level. SGK mRNA was confirmed by Northern blot to be strongly and rapidly corticosteroid stimulated in A6 cells. In situ hybridization revealed that SGK was strongly stimulated by aldosterone in rat collecting duct but not proximal tubule cells. Low levels of SGK were present in rat glomeruli, but SGK was unregulated in this structure. Finally, SGK stimulated ENaC activity approximately sevenfold when coexpressed in *Xenopus laevis* oocytes. These data suggest that SGK is an important mediator of aldosterone effects on Na^+ transport in tight epithelia. In view of the existence of SGK homologues in invertebrates, it is interesting to speculate that SGK is an ancient kinase that was adapted to the control of epithelial Na^+ transport by early vertebrates as they made the transition from a marine to a freshwater environment.

Environmental pressures began to shape vertebrate epithelial sodium transport when early vertebrates or possibly protovertebrates made their initial forays into estuaries, lagoons, and rivers in search of food and to escape from predators [1]. As these primitive chordates left the sodium-rich environment of the sea, they entered a milieu with minute amounts of sodium and unlimited free water. Although there is little certainty about the characteristics of the earliest vertebrates, it is likely that they were anadromous (that is, they lived part of their lives in fresh and part in salt water), and thus, the ability to respond to dramatic changes in environmental sodium was a prerequisite to subsequent vertebrate evolution [2]. Comparative physiologic observations suggest that glucocorticoids, prolactin, and osmolarity itself may have been important regulators of epithelial Na^+ transport in these ancient organisms [2, 3]. Later, with the development of the renin-angiotensin-aldosterone system (RAAS) in conjunction with the nicotinamide adenine dinucleotide (NAD)-dependent form of 11-β-hydroxysteroid dehydrogenase (11-HSD2) [4, 5], aldosterone became the principal regulator of epithelial Na^+ transport. By responding to changes in blood pressure and renal tubular sodium—rather than the extracellular sodium concentration—the RAAS provided a mechanism for land vertebrates to mount a homeostatic response to changes in extracellular fluid volume independently from changes in osmolarity [6]. 11-HSD2, on the other hand, allowed mineralocorticoids such as aldosterone to stimulate Na^+ transport selectively by converting glucocorticoids to inactive metabolites [7].

Thus, although there has been considerable phylogenetic change in the hormonal and nonhormonal regulators, it seems likely that the ability to regulate distal nephron Na^+ transport is ancient and that the intracellular signaling mechanisms might be conserved. Recently, we demonstrated that a member of the serine-threonine kinase pathway, SGK (also referred to in the lower case, sgk), is a mineralocorticoid-induced regulator of epithelial sodium channel (ENaC) activity [8]. SGK mRNA is rapidly stimulated by mineralocorticoids in both *Xenopus laevis* A6 kidney cells and in rat cortical duct (CD). SGK, in turn, stimulates ENaC activity when coexpressed with ENaC subunits in *Xenopus* oocytes. In this review, we discuss these data, as well as evidence that SGK is an ancient kinase regulated by a variety of factors, including osmolarity. The potential relevance of these observations to vertebrate evolution is also addressed.

CLONING OF SGK AND REGULATION BY CORTICOSTEROIDS

Suppression subtractive hybridization [9] was used to generate a library of partial-length cDNAs representing

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Key words: mineralocorticoid, sodium channel, ENaC, A6 cells, serine-threonine kinase.

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Rat    MTVKTEAA-- RSTLTYSRMR GMVAILIAFM KQRRMGLNDF IQKLANNS-Y    47
Xen    MTVKTETAAG ASTLTYSKMR GMVALLIAFM KQRRMGLNDF IQKIATNSSY    50

Rat    ACKHPEVQSY LKISQPQEPE LMNANPSPPP SPSQQINLGP SSNPHAKPSD    97
Xen    ACKPSEVQSI LNISPPQEPE LLNENSSPPP SPSQQINLGP SSNPHAKPSD   100

Rat    FHFLKVIGKG SFGKVLLARH KAEEAFYAVK VLQKKAILKK KEEKHIMSER   147
Xen    FQFLKIIGKG SFGKVLLARH QSDEKFYAVK VLQKKAILKK KEEKHIMSER   150
                                 ATP

Rat    NVLLKNVKHP FLVGLHFSFQ TADKLYFVLD YINGGELFYH LQRERCFLEP   197
Xen    NVLLKNVKHP FLVGLHFSFQ TTSRLYFILD YINGGELFYH LQRERCFLEP   200

Rat    RARFYAAEIA SALGYLHSLN IVYRDLKPEN ILLDSQGHIV LTDFGLCKEN   247
Xen    RARFYAAEIA SALGYLHSLN IVYRDLKPEN ILLDSQGHIV LTDFGLCKEN   250

Rat    IEHNGTTSTF CGTPEYLAPE VLHKQPYDRT VDWWCLGAVL YEMLYGLPPF   297
Xen    IEPNGTTSTF CGTPEYLAPE VLHKQPYDRT VDWWCLGAVL YEMLYGLPPF   300
                PDK1

Rat    YSRNTAEMYD NILNKPLQLK PNITNSARHL LEGLLQKDRT KRLGAKDDFM   347
Xen    YSRNTAEMYD NILNKPLQLK PNITNSARNL LEGLLQKDRT KRIGAKNDFM   350

Rat    EIKSHIFFSL INWDDLINKK ITPPFNPNVS GPSDLRHFDP EFTEEPVPSS   397
Xen    EIKNHIFFSP INWDDLINKK ITPPFNPNVS GPSDLQHFDP EFTEEPVPNS   400

Rat    IGRSPDSILV TASVKEAAEA FLGFSYAPPM DSFL 431
Xen    IGQSPDSILI TASIKEAAEA FMGFSYAPPM ESYL 434
                PDK2
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Fig. 1. Sequence comparison between rat [10] and *Xenopus* SGK [8]. Full-length *Xenopus* SGK was cloned and sequenced as described by Chen et al [8]. ATP-binding, PDK1, and PDK2 regulatory domains are underlined. Lys130 (*Xenopus* numbering), which contacts ATP, is bold, as is Thr259 (phosphorylated by PDK1) and Ser425 (phosphorylated by PDK2). The overall homology between rat and *Xenopus* is extremely high: 92% identity and 96% similarity at the amino acid level. Human [18], rabbit [17], and dogfish [30] SGK share similar levels of homology (data not shown). *C. elegans* SGK shares high homology (nearly 50%) with vertebrate SGKs [32], while yeast YPK1 and YKR2 show less homology but are functionally related to SGK [33].

rapidly induced mRNAs in A6 cells. Clone A83 had 92% amino acid identity (96% similarity) with rat SGK, which had been cloned previously as a glucocorticoid-regulated member of the serine-threonine protein kinase family [10]. A83 was subsequently referred to as *Xenopus* SGK (xSGK; Fig. 1). In view of previous evidence suggesting that ENaCs were regulated through phosphorylation by an unknown serine-threonine kinase [11, 12], SGK was selected for further characterization.

SGK mRNA was rapidly and strongly induced by dexamethasone, threefold within 15 minutes and more than 15-fold by 45 minutes (Fig. 2A). [Note that corticosteroid stimulation of SGK gene transcription is mediated by the mineralocorticoid and glucocorticoid receptors (MR and GR, respectively), two members of the nuclear receptor superfamily. A6 cells express predominantly GR [13, 14], and since dexamethasone activates GR more potently than aldosterone, it was used in the experiments shown in Figure 2. In other experiments not shown, SGK mRNA was stimulated by aldosterone as well.]

When followed over 24 hours, SGK mRNA reached a peak of 20-fold at 1 hour and then declined to 1.6-fold above basal at 24 hours (Fig. 2B). The response of SGK mRNA to dexamethasone was not inhibited by the protein synthesis inhibitor cycloheximide at levels that blocked more than 90% of Na^+ transport, indicating direct regulation (Fig. 2C). Importantly, SGK immunoreactive protein was also rapidly and strongly increased by dexamethasone [8].

We next sought to determine whether SGK is regulated by the physiologic mineralocorticoid, aldosterone, in the mammalian kidney. Thus, aldosterone was administered to adrenalectomized (adx) rats. Kidneys were harvested, and the SGK response was examined by in situ hybridization (Fig. 3). In adx animals (Fig. 3A), SGK was expressed focally within the cortex, at low levels in outer medulla, and moderate levels in inner medulla and papilla. Interestingly, examination of emulsion-dipped sections counterstained with hematoxylin and eosin (Fig. 3 B–E) revealed that in adx animals, SGK expression in cortex was primarily within glomeruli (Fig. 3B), while very little was seen in tubules (Fig. 3 B–D). Basal expression in outer medulla was quite low, while expression levels in inner medulla and papilla were moderate and appeared to be in tubule cells (Fig. 3 E, F). As seen in the autoradiogram of whole kidney (Fig. 3A), SGK expression was strongly induced by aldosterone, focally in cortex, and more widely in medulla, in a pattern suggestive of distal nephron (note the signal in the juxtamedullary portions of the cortex that resembles medullary rays). Inspection of emulsion-dipped sections revealed that glomerular expression was not stimulated by aldosterone (Fig. 3B); instead, SGK expression was strongly induced in cells of only the distal nephron. Proximal tubule cell levels of SGK were below background in both the presence and absence of aldosterone (Fig. 3 C, D). Importantly, corticosterone did not stimulate SGK expression in distal nephron, glomerulus, or proximal tubule (data not shown). We conclude that the principal mineralocorticoid aldosterone, but not the principal glucocorticoid corticosterone, selectively induces SGK expression in rat distal nephron.

SGK STIMULATION OF EPITHELIAL SODIUM CHANNEL ACTIVITY

Since the principal early effect of aldosterone is to stimulate ENaC activity [11, 15], we next directly tested the effects of SGK on ENaC activity by coexpressing
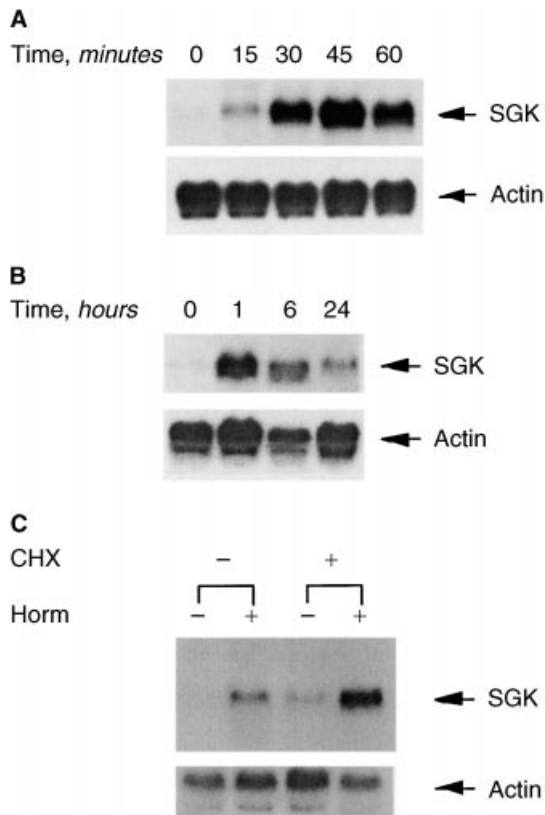
Fig. 2. SGK mRNA is rapidly and directly stimulated by dexamethasone in A6 cells. A6 cells were grown on permeable supports and parallel cultures were treated with vehicle or 10^{-7} mol/L dexamethasone for the times shown. Blots were hybridized with a probe for *Xenopus* SGK, stripped, and rehybridized with a probe for *Xenopus* type 8 actin. (*A* and *B*) Northern blots representing 1- and 24-hour time courses of hormone treatment, respectively. SGK message constituted a single major band on Northern blot, with a size of 2.6 kb pairs. (*C*) Hormone stimulation of SGK does not require new protein synthesis. A6 cells were incubated with cycloheximide (chx) for two hours, and then cells were treated with vehicle or 10^{-7} mol/L dexamethasone for an additional two hours, as shown. Cycloheximide blocked hormone induction of PD/R by 90% (not shown), as previously described [7].

them in *Xenopus* oocytes, as described previously [8]. As shown in Figure 4A, SGK stimulated ENaC-mediated Na$^+$ current approximately sevenfold (6.9 ± 1.5). In the absence of coexpressed ENaC subunits, amiloride-inhibitable Na$^+$ current was very low (<0.1 μA) and was unaffected by SGK as was oocyte morphology (data not shown). Finally, as shown in Figure 4B, SGK did not stimulate ROMK2-mediated K$^+$ current when the two were coexpressed in oocytes. It is of interest that the ROMK2 potassium channel was not activated by SGK, since it likely represents the major apical K$^+$-secreting channel in ENaC-expressing cortical distal nephron cells [16]. This observation is consistent with the idea that the major stimulus for increased K$^+$ secretion during the early phase of the mineralocorticoid response is an increase in driving force because of the depolarizing effect of Na$^+$ influx.

ROLE OF SGK IN MINERALOCORTICOID-STIMULATED SODIUM TRANSPORT

The data presented here suggest that SGK is an important mediator of aldosterone-induced epithelial sodium transport. The induction of SGK mRNA and protein by mineralocorticoids is robust and precedes the induction of Na$^+$ transport. Moreover, SGK stimulates ENaC-mediated Na$^+$ current when they are coexpressed in *Xenopus* oocytes. Further support for SGK as a mediator of mineralocorticoid action comes from a recent report demonstrating that aldosterone regulates SGK expression in primary cultures of rabbit CD cells [17].

SGK was originally identified as a glucocorticoid-regulated mRNA in mammary epithelial cells [10] and subsequently has been found in most vertebrate tissues, as well as in cultured cells, albeit at widely variable levels [10, 18]. Although SGK had all of the earmarks of a serine-threonine kinase, initial attempts at demonstrating kinase activity were unsuccessful. Indeed, SGK does not phosphorylate a number of nonspecific kinase substrates such as myelin basic protein or histone H1. Recently, a synthetic peptide phosphorylated by SGK was identified [19], although cellular targets remain unidentified. In view of functional [11] and biochemical [12] evidence implicating a serine-threonine kinase in ENaC regulation, it seems probable that SGK regulates Na$^+$ transport through protein phosphorylation. However, it is uncertain whether ENaC is the direct target of SGK. Other possible SGK targets include a methyl transferase [20], a channel-associated G protein [21], another kinase [22], or a component of the plasma membrane trafficking machinery [23].

MINERALOCORTICOID REGULATION OF SGK GENE TRANSCRIPTION

Mineralocorticoids directly modulate SGK gene transcription, and furthermore, a simple hormone response element (HRE) was found in the rat SGK 5′-flanking region [10]. It is important to note that MR and GR both bind to and activate transcription from simple HREs and have distinct activities at only composite or compound HREs [24, 25]. Their similar transcriptional activities together with the similar effects they mediate on Na$^+$ transport in cultured CD cells [13, 14, 26] make it highly likely that MR and GR regulate SGK gene transcription by similar mechanisms. Notably, in all cases examined thus far, the pattern of SGK gene regulation by MR and GR parallels the regulation of Na$^+$ transport by these same receptors. These observations further support the idea that MR and GR mediate similar effects on cortical collecting duct (CCD) and that hormone specificity is due to the action of glucocorticoid-limiting factors such as 11-HSD2 and possibly hormone transporters [27, 28].
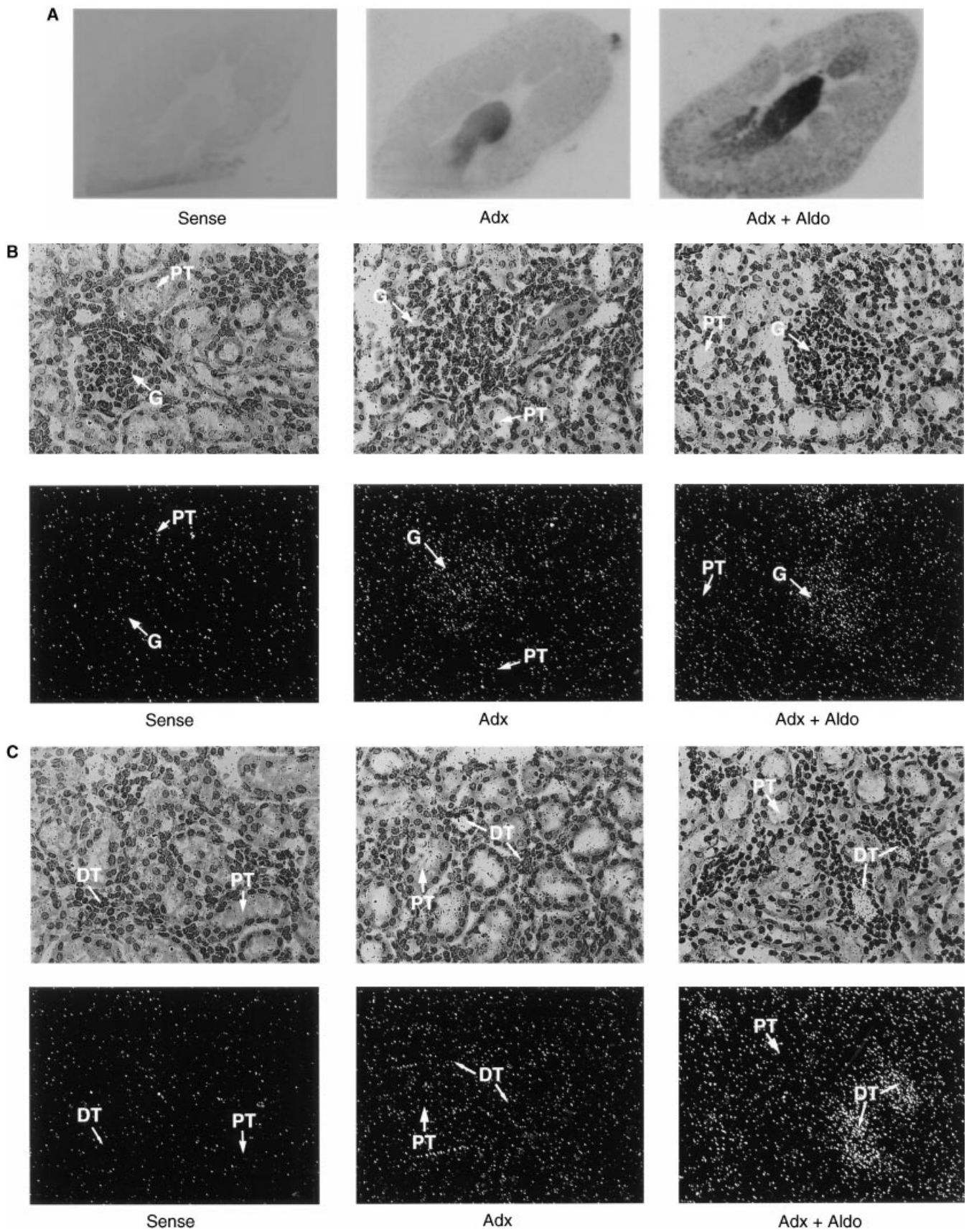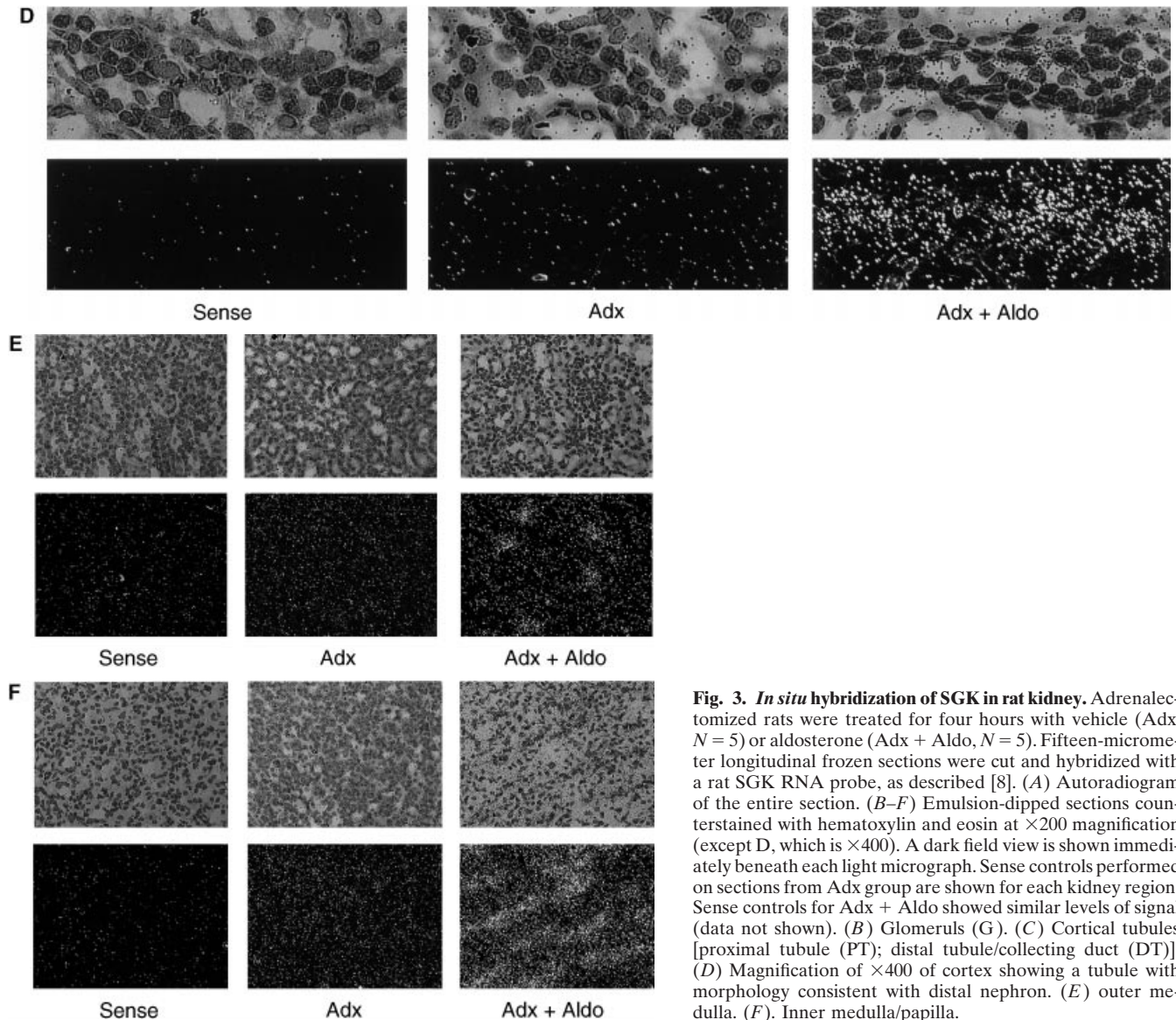
Fig. 3. (See p. 1287 for legend).

Fig. 3. *In situ* **hybridization of SGK in rat kidney.** Adrenalectomized rats were treated for four hours with vehicle (Adx, $N = 5$) or aldosterone (Adx + Aldo, $N = 5$). Fifteen-micrometer longitudinal frozen sections were cut and hybridized with a rat SGK RNA probe, as described [8]. (*A*) Autoradiogram of the entire section. (*B–F*) Emulsion-dipped sections counterstained with hematoxylin and eosin at ×200 magnification (except D, which is ×400). A dark field view is shown immediately beneath each light micrograph. Sense controls performed on sections from Adx group are shown for each kidney region. Sense controls for Adx + Aldo showed similar levels of signal (data not shown). (*B*) Glomeruls (G). (*C*) Cortical tubules [proximal tubule (PT); distal tubule/collecting duct (DT)]. (*D*) Magnification of ×400 of cortex showing a tubule with morphology consistent with distal nephron. (*E*) outer medulla. (*F*). Inner medulla/papilla.

PHOSPHATIDYLINOSITOL-3-KINASE AND SGK ACTIVATION OF THE EPITHELIAL SODIUM CHANNEL

SGK's closest homologue is PKB/Akt, a serine-threonine kinase that lies in the phosphatidylinositol-3-kinase (PI3K) signaling pathway and mediates many of the effects of insulin [23]. Recently, SGK phosphorylation of a synthetic oligopeptide ("SGK-tide") was also shown to be PI3K dependent. It is well established that PI3K and the kinases it regulates, particularly PKB/Akt, are important for insulin's effects on proliferation and glucose metabolism [23], and recent evidence supports a role for PI3K in insulin-stimulated Na^+ transport [29]. In view of the sequence homology between PKB/Akt and SGK, as well as recent evidence establishing that PI3K activates SGK, it is interesting to speculate that PI3K activity is required for mineralocorticoid-stimulated Na^+ transport. Indeed, SGK may be an integrator of peptide and steroid hormone signals. According to this view, mineralocorticoids control the abundance of SGK protein, while the PI3K pathway controls SGK activity (through a phosphorylation cascade), and thus, both stimuli must be present for high-level vectoral Na^+ transport. Blockade of either pathway should disrupt the Na^+ current. This form of dual regulation may provide an effective check on the system so that Na^+ transport is activated under only the appropriate conditions.

SIGNIFICANCE OF SGK TO VERTEBRATE EVOLUTION

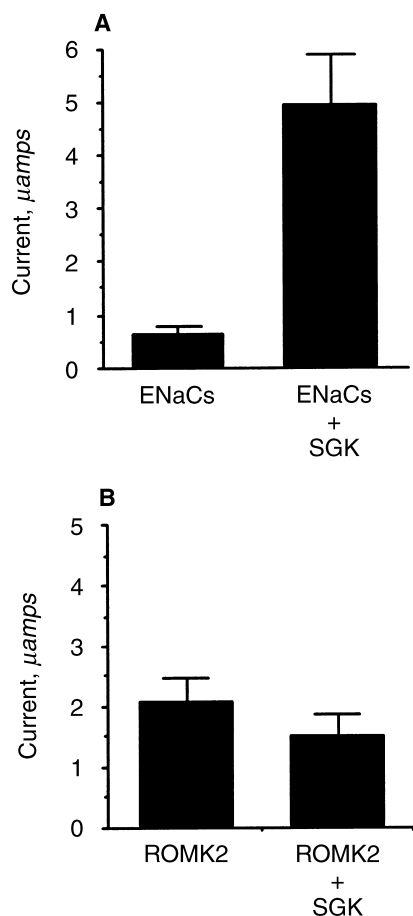The data presented here, as well as another recent report, strongly suggest that SGK plays an important

Fig. 4. (A) Epithelial sodium channel (ENaC) activity is stimulated by SGK when they are coexpressed in *Xenopus laevis* oocytes. In vitro transcribed RNA for each of the three *Xenopus* ENaC subunits was injected into oocytes with or without SGK. Shown is amiloride-sensitive current (Iami) from a representative experiment with six oocytes per condition (mean ± SE). The mean increase in amiloride-inhibitable Na$^+$ current was 6.9-fold ± 1.5-fold for 12 independent experiments (6 oocytes per condition in each experiment). (*B*) Potassium channel ROMK2 is not stimulated by SGK. Experiments were performed in *Xenopus* oocytes as in (A). Shown is a representative experiment with six oocytes per condition. Similar results were obtained in two independent experiments performed on different days.

role in controlling epithelial Na$^+$ transport in kidney cells [8, 17]. The role of SGK in other cell types is less clear. However, all cells must respond to changes in volume, and it is interesting to note that in liver cells and shark rectal gland cells, SGK is stimulated by hyperosmolarity [18, 30], a stimulus known to shrink cells and stimulate Na$^+$ transport [31]. Indeed, hyperosmotic shock has been shown to activate an amiloride-sensitive Na$^+$ channel in hepatocytes [31]. Furthermore, SGK appears to be an ancient kinase, with homologues expressed in organisms as distantly related to vertebrates as *Caenorhabditis elegans* and Saccharomyces *cerevesiae* [32, 33]. Taken together, these observations suggest the interesting possibility that in early marine organisms, SGK was

involved in cellular osmoregulation and that with the invasion of fresh water by protovertebrates, it was adopted for the control of systemic Na$^+$ balance. The principal regulators of SGK expression (and activity) in the tight epithelia of the early vertebrates are uncertain, but probably included osmolarity itself and perhaps glucocorticoids and prolactin or a prolactin precursor [34, 35]. Later, with the development of the RAAS in advanced fishes and amphibia, SGK expression in tight epithelia came under the control of aldosterone. The RAAS increased in importance until, in mammals, it became the predominant regulator of Na$^+$ metabolism in tight epithelia, perhaps in part because aldosterone became the principal regulator of SGK gene transcription. A persistent role of SGK in osmoregulation is suggested by its expression in a variety of cell types and its regulation by osmotic shock. SGK's other roles remain to be discovered.

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