that GSK-3β regulates the MPT (9). The MPT is poorly understood, and the precise molecular composition of the MPT needs to be defined (16). Advancement in cardioprotection and apoptosis will require a better understanding of the composition and regulation of the MPT and its role in apoptosis. Perhaps the most important issue is whether inhibition of GSK-3β is protective if it is initiated upon reperfusion. Inhibition of several kinases upon reperfusion has been reported to block protection afforded by insulin or other protective drugs administered upon reperfusion. Since these kinases phosphorylate GSK-3β, it is plausible that GSK-3β is a target during reperfusion. Consistent with this, a recent study by Gross et al. (11) showed that addition of GSK-3β inhibitors 5 minutes before the start of reperfusion resulted in a significant reduction in infarct size.

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A fork in the road of cell differentiation in the kidney tubule

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The collecting ducts of the kidney are composed of intercalated cells (responsible for acid/base transport), principal cells (mediating salt and water absorption), and inner medullary cells, which mediate all three types of transport. Forkhead box (*Fox*) genes are a large family of transcription factors that are important in cell-type specification during organogenesis. In this issue, Blomqvist et al. find that mice lacking *Foxi1* have no intercalated cells in the kidney (see the related article beginning on page 1560). The collecting ducts of the null mice contained primitive cells that expressed both intercalated cell and principal cell proteins, yet the acid/base transport function of the kidney was disrupted and the mice exhibited distal renal tubular acidosis. These findings suggest that *Foxi1* plays a critical role in determining cell identity during collecting duct development.

The mechanism of generation of the 400 or more cell types of mammals from a single cell remains the principal problem of developmental cell biology. Classical embryologists discovered that progenitor cells first undergo *specification* before they become *determined* to form a differentiated cell type. Under certain conditions, specified cells can differentiate in a reversible manner, whereas determined cells autonomously differentiate even if placed in a different location in the embryo. The molecular mechanism of these changes in cell fate depends on signals received from the niche or "ecosystem" in which the cell is located, but ultimately it must involve the activation of a network of transcription factors that is specific for each tissue and cell type.

Renal collecting duct

Each nephron of the adult mammalian kidney is composed of 12 epithelial segments. The collecting duct has three segments, some of which have more than one cell type. These tubular segments begin to develop when the ureteric bud (an outgrowth of the wolffian duct) invades the metanephric mesenchyme. The tip of each ureteric bud induces the conversion of this mesenchyme into an epithelial structure that undergoes morphogenetic transformation to form the nephron from the glomerulus to the end of the distal tubule, while the ureteric bud

forms the collecting duct. The adult collecting duct has several cell types, one of which — the intercalated cell — is specialized for the transport of H+ and HCO₃⁻. The other type — the principal cell — mediates salt and water transport. A third cell type, present only in the inner medullary collecting duct, performs salt, water, and acid transport. All of these cells derive from the ureteric bud, but the pathway of their differentiation remains obscure. The paper by Blomqvist et al. in this issue of the *JCI* is the first to provide insight into the molecular mechanism of cell-identity determination of at least the intercalated cell (1).

Intercalated cells

The intercalated cell exists in two functionally distinct subtypes in the cortical collecting duct: one secretes HCO₃⁻, while the other absorbs it. Reabsorption of HCO₃⁻ (i.e., secretion of protons) is accomplished in α-intercalated cells by an apical H+-ATPase and a basolateral Cl⁻/HCO₃⁻ exchanger. Secretion of HCO₃⁻ by β-intercalated cells is mediated by the same kinds of transporters but located on the opposite cell membranes. We found that when animals were fed an acid diet, the β form converted to the α phenotype (2). Others have shown, using a variety of methods, that there are more than two subtypes of intercalated cells, but we interpret these findings as showing a continuity of changes taking place between the two extremes of α and β. Recent studies based on single identified β-intercalated cells showed that these cells converted to an α phenotype in response to acidification of the basolateral media (3). Recently, we found in an intercalated cell line that this conversion represents an instance of terminal differentiation, with the β-intercalated cell representing a less differentiated (protoepithelial) type and the α phenotype being more differentiated (4).

Foxi1

Blomqvist et al. (1) find that mice lacking *Foxi1*, a forkhead transcription factor, have no intercalated cells as judged by electron microscopy, or by the absence of specific proteins of these cells. *Foxi1* is expressed in the intercalated cell and in the inner ear. Could the transcription factor be a critical determinant of cell identity of these cells? The authors show a remarkable result: each cell of the collecting ducts of the mutant mice expresses both intercalated proteins (carbonic anhydrase II) and principal cell proteins (aquaporin 2). It would have been useful to

find out whether the embryonic collecting duct also resembled the mutant ducts in their pattern of expression. Although studies using these excellent markers have not been performed, the entire collecting duct seems to be composed of a single cell type, staining weakly for aquaporin 2 (K. Madsen, personal communication).

Development of the collecting duct

Nephrogenesis proceeds in mice, rats, and rabbits for one week postnatally. Electron microscopic studies have shown that the cortical collecting duct of the newborn shows a single cell type, resembling a principal cell, with a single cilium, few organelles, smooth apical and basolateral surfaces, and large glycogen deposits (5). No intercalated cells can be demonstrated in the developing neonatal collecting duct, using physiological criteria (6). Minuth and colleagues found that the early collecting tubule contains a "scrambled" expression of principal and intercalated lectin-binding sites (7). But it remains to be shown definitively that development of the collecting tubule lineage progresses from a generic ureteric bud cell to a specified collecting tubule cell that finally terminally differentiates into principal, intercalated, and inner medullary collecting duct types. Incidentally, the latter cell contains both principal and intercalated cell proteins, which raises the question of whether these cells have been arrested in their developmental program at the specification stage.

A kidney–ear axis?

There is an interesting connection between renal anomalies and deafness. Deletion of ATP6B, of pendrin, and of Kcc4, the K-Cl cotransporter present in intercalated cells, can cause deafness. One of the supporting cells of the sensory epithelium in the vestibular epithelium has many similarities to the intercalated cell, and it serves to control the acidic pH of the endolymph. Remarkably, deletion of *Foxi1* depletes the epithelium of these cells, causing deafness (8).

Forkhead genes and intercalated cell identity

Forkhead genes (termed *Fox*, for forkhead box) encode a subset of the helix-turn-helix class of transcription factors (9). They are divided into 17 classes (A to Q) and are involved in a wide range of cell-type specification during organogenesis in animals and humans. In *Caenorhabditis elegans*, a forkhead protein, pha-4 (an orthologue of

FoxA), specifies the identity of the pharynx and causes the expression of all pharyngeal genes (10). Remarkably, the order of appearance of the pharyngeal genes depends on the affinity of the promoters to the forkhead protein, providing an elegant mechanism that controls the timing of appearance of these genes.

Foxi1 controls the expression of pendrin and the band 3 anion exchangers (AE1), and probably also the ATP6B subunit of the vacuolar H+-ATPase, all characteristic proteins of the intercalated cells. But the absence of this factor resulted not only in the absence of these genes but also in the lack of this cell type. The intercalated cells contain a very large number of mitochondria, and their shape is quite characteristic because of the presence of vigorous apical endocytosis and exuberant microvilli. It seems unlikely that all of these characteristics are secondary events produced by expression of a few transport proteins. One possibility is that absence of the transport proteins leads to cell death during development. But since the expression of carbonic anhydrase and aquaporin 2 becomes scrambled in the mutant mice, it is likely that *Foxi1* activates a whole program of expression of genes that control cell shape, organellar biogenesis, apical structure (microvilli and microplicae), and apical functions (endocytosis and exocytosis). Using microarray analysis it should be possible to identify the developmental program that specifies this cell type. But what is the developmental pathway by which *Foxi1* determines the identity of the intercalated cell? Blomqvist et al. favor the idea that it has a simple and direct effect of cell determination similar to the proposed mechanism of pha-4 (1). However, it appears that *Foxi1* is expressed in the kidneys at embryonic day 16 in a uniform pattern in the ureteric bud and collecting duct (ref. 11; note that this is our interpretation of Figure 2A of ref. 11, which differs from the opinion of the authors). This raises the question of whether *Foxi1* plays a more complex role in cell specification in the collecting duct. For instance, its disappearance could also mediate principal cell differentiation. Forkhead genes often collaborate with other transcription factors to control the timing of differentiation, exerting negative and positive influences on these processes. For instance, *Foxe1* collaborates with *pax8* and *TTF1* to control the differentiation of the thyroid cell. *Foxe1* mutations result in thyroid agenesis (12), yet the gene is expressed in the thyroid

before the differentiation of thyrocytes and is suppressed when thyroglobulin begins to be expressed (13). What seems clear is that the identification of *Foxi1*'s role in intercalated cell differentiation has opened the door to an exciting new chapter in the development of the kidney.

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The case of visceral fat: argument for the defense

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Increased plasma fatty acid concentrations may be responsible for many of the metabolic abnormalities associated with abdominal obesity. Excessive visceral fat is associated with insulin resistance and other metabolic risk factors for coronary heart disease. A study reported in this issue of the *JCI* evaluates the relative contribution of fatty acids released during lipolysis of visceral adipose tissue triglycerides to portal and systemic fatty acid flux in human subjects (see the related article beginning on page 1582).

The relationship between excess abdominal fat mass and insulin resistance was recognized a half century ago, when Jean Vague, a French physician, reported an association between a "masculine" or "android" obesity phenotype and diabetes (1). Subsequently, many large epidemiological and smaller physiological studies have confirmed the relationship between abdominal obesity and insulin resistance, diabetes, and other metabolic risk factors for coronary heart disease (2–5). In fact, excess abdominal fat is even associated with impaired insulin-mediated glucose uptake in lean adults (6).

Abdominal fat is composed of several distinct anatomic depots: subcutaneous fat, which can be divided into anterior and posterior or superficial and deep layers, and intraabdominal fat, which can be divided into intraperitoneal and retroperitoneal sites. Intraperitoneal fat, also known as visceral fat, is composed of mesenteric and omental fat masses. Although the absolute amount of each of these depots is much larger in upper-body obese than in lean persons, the relative amount of abdominal fat with respect to total body fat mass is often similar in both groups. For example, visceral fat constitutes about 10% of total body fat mass in lean and obese men (7).

The close relationship between abdominal fat (i.e., total, subcutaneous, and/or visceral fat) and metabolic disease has stimulated a clinical interest in identifying high-risk patients. Waist circumference is often used as a surrogate marker of abdominal fat because it correlates closely with total abdominal fat mass measured by computed tomography (8) and it is not practical to directly measure abdominal fat mass in a clinical setting. Based on data from epidemiological studies, the Expert Panel on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults, convened by the NIH, proposed that men with a waist circumference greater than 102 cm (40 in.) and women with a waist circumference greater than 88 cm (35 in.) are at increased risk for metabolic diseases (9).

Fatty acid metabolism and insulin resistance

The association between abdominal fat and insulin resistance does not prove causality, and it is possible that environmental, biological, or inherited factors that induce insulin resistance also cause abdominal fat accumulation (10). Nonetheless, it has been proposed that alterations in fatty acid metabolism associated with abdominal obesity are responsible for impaired insulin action because excessive circulating FFAs inhibit the ability of insulin to stimulate muscle glucose uptake and to suppress hepatic glucose production (11). The notion of a link between abdominal fat, FFA metabolism, and insulin resistance is supported by the observation that basal whole-body FFA flux rates are greater in upper-body obese than in lower-body obese and lean subjects (12, 13) and that diet-induced weight loss decreases whole-body FFA flux and improves insulin sensitivity (14). It has been hypothesized that excess visceral fat is more harmful than excess subcutaneous fat, because lipolysis of visceral adipose tis-