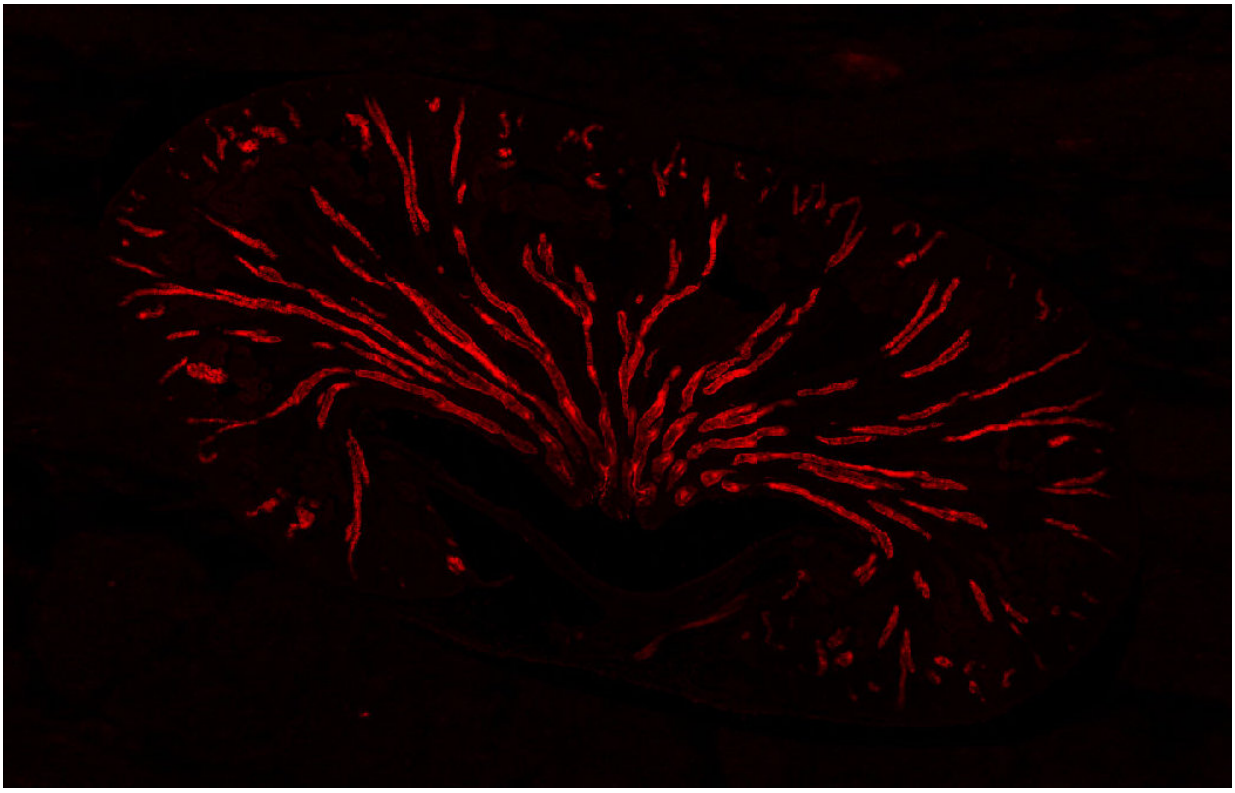
How the kidneys produce concentrated urine

December 13 2017



The collecting duct system of the mouse kidney is colored red with a fluorescent dye. Credit: Janett Ruffert, Kai Schmidt-Ott, MDC

When we drink little, we produce less urine. But how is this process regulated? An international team of scientists led by Prof. Kai Schmidt-Ott of the Max Delbrück Center for Molecular Medicine (MDC) has now shed light on how the kidneys concentrate urine.

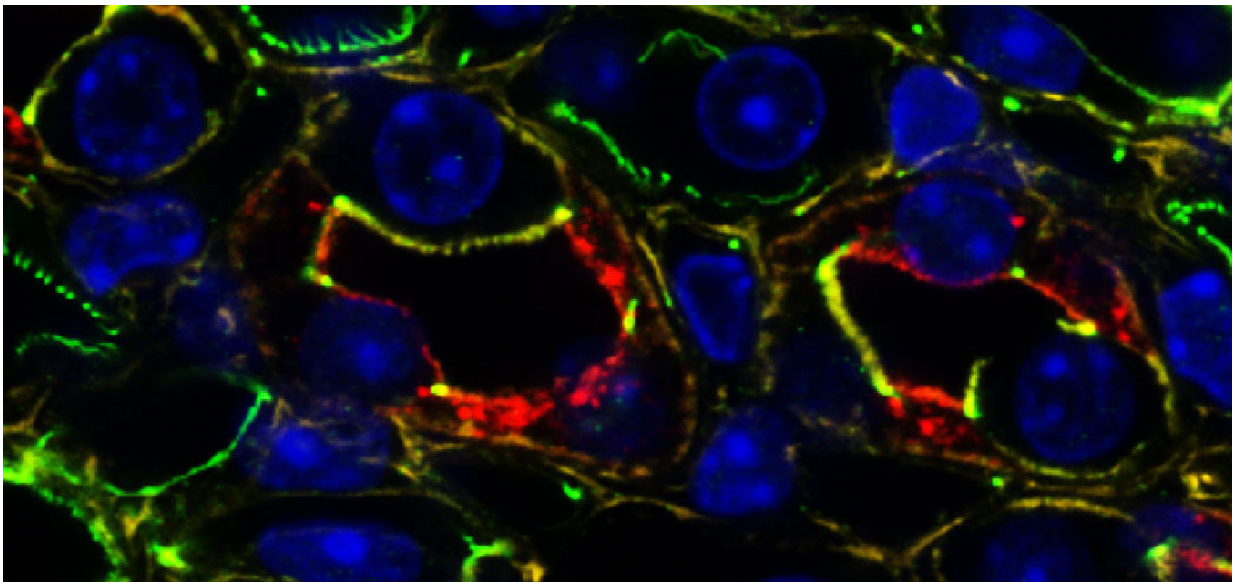
When water intake is low, humans and other higher organisms produce very small quantities of urine. "To help the body retain as much fluid as possible, water is reabsorbed from urine within the kidneys' collecting duct system. This process is vital," explains Prof. Kai Schmidt-Ott of the MDC and Charité's Department of Nephrology and Medical Intensive Care.

For this reabsorption to work, the renal medulla that surrounds the collecting ducts must accumulate large quantities of salt and urea. Only then can water follow the osmotic gradient and be absorbed from the collecting duct into the renal medulla, where it eventually re-enters the circulation.

GRHL2 makes collecting duct cells impermeable

"For the first time, we have identified an important molecular switch that is able to maintain a high salt concentration in the renal medulla," says the first author of the study, Dr. Christian Hinze of the MDC. This "switch" is the protein grainyhead-like 2, or GRHL2 - a transcription factor that can control the activity of genes.

The molecule is produced in the collecting duct cells and enables these cells to form a tight barrier between the urine and the medulla. Together with colleagues from Charité in Berlin and researchers from Kiel, Norway and the United States, the MDC scientists have now published their findings in the *Journal of the American Society of Nephrology*.



Cross-sections of two collecting ducts are shown. Tissue proteins are colored with fluorescent dyes: aquaporin 2 (red), F-actin (yellow), tight junction protein 1 (green) and cell nuclei (DAPI, blue). Credit: Janett Ruffert, Kai Schmidt-Ott, MDC

Genetically modified mice produce more urine

Experiments conducted in collecting duct cell cultures showed that GRHL2 has a significant impact on the permeability of the connection between cells. "Normally, the collecting duct cells form a tight barrier between the urine and surrounding tissue," says Dr. Hinze. "But cells lacking GRHL2 become permeable to certain substances." Experiments showed that collecting duct cells lacking the molecule GRHL2 become leaky and allow salts and urea to pass across cell-to-cell contacts.

Next, the scientists used an animal model to test whether these findings could be extrapolated to a living organism. They generated mice that lacked the gene encoding GRHL2 in the collecting duct system of the

kidneys.

"These genetically engineered mice appeared normal and healthy at first sight," says Schmidt-Ott. He adds that even their kidneys looked almost completely normal; only under the microscope could they see that the collecting duct cells were slightly smaller than usual. "However, the genetically altered mice produced more urine than usual, and this urine was also more dilute," explains Hinze. Furthermore, the scientists found that the medullary region of their kidneys contained a reduced concentration of sodium.

Kidneys failed when the mice lacked water

The increased urine production became a problem as soon as the mice had limited access to water. Their creatinine and urea levels - two important laboratory indicators of kidney function - shot up drastically. "It appeared that the kidneys of these mice were failing," says Hinze.

"This way, we were able to demonstrate for the first time how important the collecting duct cell barriers are for maintaining high concentrations of solutes in the kidney's interstitium- and thus for regulating the concentration of urine," adds principle investigator Schmidt-Ott. Given that the human kidney also produces GRHL2, the researchers anticipate that these results will be relevant for humans.

GRHL2 a potential target for new treatments

"What we found is fundamentally new information, which we can now use to further investigate conditions like diabetes insipidus, a severe and potentially devastating disease in humans," says Schmidt-Ott. This disorder involves the kidneys excreting abnormally large amounts of urine, resulting in a frequent need to urinate and to drink excessive

amounts of fluids. Researchers at the MDC are now interested in finding out whether GRHL2 can be controlled in order to one day offer better treatment options for patients with disorders of their water balance.

More information: Christian Hinze et al, GRHL2 Is Required for Collecting Duct Epithelial Barrier Function and Renal Osmoregulation, *Journal of the American Society of Nephrology* (2017). DOI: 10.1681/ASN.2017030353

Provided by Max Delbrück Center for Molecular Medicine