

## Chloride helps collagen build its network

Extracellular chloride triggers the assembly of collagen IV networks in basement membranes.

Basement membranes are extracellular scaffolds that organize and strengthen many different tissues in the body. How the various components of basement membranes are assembled into an organized network that can regulate the behavior of overlying cells remains unclear (1), but Cummings et al. now describe how extracellular chloride ions induce the higher order assembly of collagen IV molecules outside the cell (2).

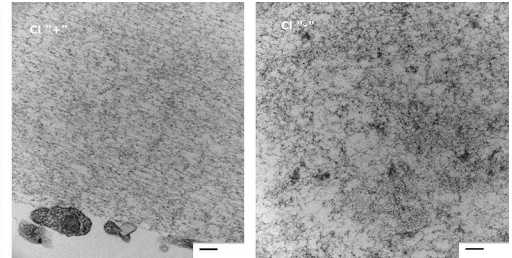
Collagen IV is the main component of basement membranes, where it forms a scaffold that binds and organizes numerous other extracellular matrix proteins and growth factors. Collagen IV assembly starts inside cells with the lateral association of three  $\alpha$ -chains to form a triple helical collagen “protomer.” After their secretion from the cell, protomers assemble with each other to form a larger network. “But what triggers this?” wonders Billy Hudson, from Vanderbilt University School of Medicine. “Why don’t collagen protomers form a network inside the cell?”

Collagen IV’s C-terminal NC1 domain plays a critical role in the assembly process, as it is involved in both the lateral association of  $\alpha$ -chains to form protomers inside the cell, and the end-on association of protomers in the extracellular space. In all, six NC1 domains come together at the interface between two protomer C-termini. Hudson and colleagues, led by Christopher Cummings, Vadim Pedchenko, and Kyle Brown, noticed that the crystal structure of NC1 hexamers contains chloride at the protomer interface, so they wondered whether this anion, which exists at a much higher concentration outside the cell than inside the cell, might regulate collagen IV assembly.

Cummings et al. purified NC1 hexamers from both cultured cells and bovine lens basement membrane. The hexamers dissociated into monomers when dialyzed into chloride-free buffer, and reassembled when



### FOCAL POINT



(Left to right) Billy Hudson, Vadim Pedchenko, Kyle Brown, Christopher Cummings, and colleagues reveal that extracellular chloride triggers the assembly of collagen IV networks during basement membrane formation. Collagen IV  $\alpha$ -chains can trimerize independently of chloride to form collagen protomers inside cells. But, once these protomers are secreted from the cell and exposed to extracellular chloride, their NC1 domains undergo a conformational change that permits protomer oligomerization and collagen network assembly. The basement membrane formed by cells *in vitro* is highly organized in the presence of chloride (center), but patchy and disorganized when the cells are cultured in low-chloride media (right).

chloride levels were restored to physiological concentrations. In isolation, however, NC1 domains don’t form a trimeric intermediate, so, to investigate which stage of hexamer assembly relies on chloride, the researchers expressed and purified recombinant  $\alpha$ -chains containing the NC1 domain and a stretch of helical repeats sufficient to mediate trimerization and protomer formation. “And we could show that chloride isn’t required for trimerization, but it is required to put two protomers together,” Hudson explains. Thus, collagen IV  $\alpha$ -chains can form protomers inside the cell, but their assembly into larger networks is only triggered when they encounter high concentrations of chloride outside the cell.

To understand how this trigger works, the researchers performed molecular dynamics simulations and found that chloride binding induces a conformational change in each NC1 domain that makes an arginine residue available to form a salt bridge with a glutamate in an opposing NC1 domain.  $\alpha$ -Chains lacking this arginine residue could still form protomers, but these protomers were incapable of forming larger assemblies. “These residues are conserved throughout the animal kingdom,” says Hudson. “So it appears

to be a fundamental mechanism controlling collagen assembly.”

To test the role of chloride ions in collagen IV and basement membrane assembly, Cummings et al. cultured mouse cells in low-chloride media, and found that they formed a disorganized, patchy extracellular matrix. Two collagen IV-binding proteins, laminin and peroxidase, were abnormally distributed within the matrix, indicating the importance of chloride and collagen IV assembly to basement membrane organization.

Consistently, *Drosophila* expressing a collagen IV mutant lacking part of its NC1 domain failed to incorporate this mutant into basement membranes, and, intriguingly, point mutations in the NC1 domain of human collagen IV  $\alpha$ -chains are associated with Alport syndrome and stroke (3, 4). Hudson says that discovering this extracellular chloride trigger is just the first step in understanding how the extracellular matrix is organized into what he calls a “smart scaffold” that can position signaling molecules and communicate with cells. “It’s just a small window into this complex extracellular machinery,” Hudson says.

1. Sherwood, D.R. 2015. *J. Cell Biol.* 210:369–372.
2. Cummings, C.F., et al. 2016. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201510065>
3. Kuo, D.S., et al. 2012. *Hum. Mol. Genet.* 21(R1):R97–R110.
4. Lemmink, H.H., et al. 1993. *Genomics.* 17:485–489.

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