

The proteasome helps epithelial cells set up KAMPs

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Study describes how epithelial cells process keratin 6a to generate antimicrobial peptides that prevent bacteria from colonizing the cornea.

As barriers to the outside world, skin and mucosal epithelia are the body's first line of defense against many different pathogens present in the environment. One defense mechanism commonly used by these epithelia is the production of antimicrobial peptides that can kill invading pathogens and activate the host immune response (1). In this issue, Chan et al. describe how, in response to bacterial ligands, corneal epithelial cells generate antimicrobial peptides from their keratin 6a intermediate filaments (2).

Intermediate filaments provide mechanical support to epithelial cells but soluble keratin subunits can have other important functions as well (3). While she was a research scientist at the University of California, Berkeley, Connie Tam discovered that human corneal epithelial cells constitutively produce short peptide fragments from the C-terminal region of keratin 6a that have potent bactericidal activity against a variety of gram-positive and gram-negative bacteria (4). "When I started my own lab at Cleveland Clinic, I wanted to study how cells produce these keratin-derived antimicrobial peptides, or KAMPs," Tam explains.

Tam and colleagues, led by research scientist Jonathan Chan, first examined how the filamentous keratin network of corneal epithelial cells responded to the presence of bacterial surface molecules, such as LPS, flagellin, or lipoteichoic acid, that can be detected by host cell Toll-like receptors (2). "When cells were exposed to these bacterial ligands, the filamentous network seemed to disassemble because it appeared more diffuse," Tam says. With more, soluble keratin 6a present in the cytosol, the cells were able to produce increased amounts of KAMPs, without up-regulating keratin 6a gene expression.

Keratin filament disassembly is often regulated by phosphorylation, and Chan et al. found that bacterial ligands enhanced the phosphorylation of several serine residues in keratin 6a's N-terminal domain. Mutating



Focal Point. Jonathan Chan (left), Connie Tam (center), and colleagues describe how corneal epithelial cells produce keratin-derived antimicrobial peptides (KAMPs) in response to a variety of gram-positive and gram-negative bacteria. In a control cell (top right), keratin 6a (green) forms a filamentous network but, in the presence of the bacterial surface ligand flagellin (bottom right), keratin 6a is phosphorylated and the network is disassembled. Soluble, cytosolic keratin 6a subunits can then be ubiquitinated by cullin-RING E3 ligases and targeted to the proteasome, which produces short peptide fragments from keratin 6a's C-terminal domain that have potent bactericidal activity. PHOTOS COURTESY OF THE AUTHORS.

these residues to nonphosphorylatable alanine residues reduced keratin 6a solubility.

But how are soluble keratin 6a subunits subsequently processed into KAMPs? Other classes of antimicrobial peptide can be generated by specific cytosolic proteases that cleave longer, precursor proteins, or by targeting precursors to lysosomes via the autophagy pathway. In other contexts, however, soluble keratin subunits can be ubiquitinated and targeted to the proteasome for degradation (5).

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Chan et al. found that keratin 6a can be ubiquitinated by cullin-RING E3 ligases. Treating organotypic corneal cell cultures with the proteasome inhibitor epoxomicin caused the cells to accumulate ubiquitinated keratin 6a and reduced the production of KAMPs in response to bacterial ligands. Extracts from epoxomicin-treated corneal cultures showed reduced bactericidal activity compared with control extracts. "So,

the proteasome is involved in cleaving full-length, cytosolic keratin 6a to generate these antimicrobial fragments," Tam says.

Accordingly, the researchers found that administering epoxomicin to the eyes of live mice impaired the clearance of *Pseudomonas aeruginosa*, an important cause of corneal infection and blindness. The proteasome is well known for its role in generating antigenic fragments during adaptive immunity, but Chan et al.'s results suggest that it also produces antimicrobial peptides as part of the innate immune response.

Tam and colleagues are now examining ways to manipulate KAMP production and to test whether these antimicrobial peptides can be used to treat corneal infections in the clinic. In addition, they are investigating whether full-length keratin 6a plays other roles during inflammation.

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