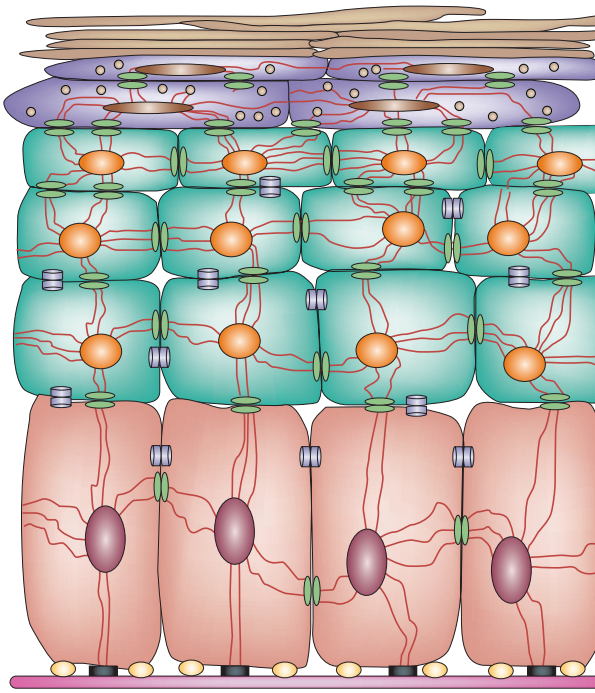
MILESTONE 19

Deciphering keratin function



> These papers were a wake-up call, showing that intermediate filaments are likely to have critically important roles within the context of a whole animal. It was also a beautiful demonstration of the strength of transgenic approaches for asking basic cell biology questions in an intact vertebrate.
> *William Bement*

Some of the early insights that linked mutations in cytoskeletal proteins to disease came from experiments that recapitulated the disease phenotype in transgenic mice that expressed mutant genes encoding cytoskeletal proteins — and from the functional analysis of mutant proteins isolated from patients.

Intermediate filament (IF) networks can be found in most vertebrate cells (see Milestone 7); however, their function remained elusive until a 1991 study from Fuchs and colleagues identified a function for keratin filaments, which constitute a class of IFs that form networks in epithelial cells. The authors generated transgenic mice expressing a truncated mutant keratin-14 (*K14*) gene in the basal layer of the epidermis and the related stratified squamous epithelia. Mice carrying the mutant gene had a high level of neonatal mortality correlating with extensive skin blistering following mild trauma. Skin keratinocytes from the transgenic mice were found to have disrupted keratin networks, and aggregates containing the mutant keratin and endogenous native keratin proteins were found in basal keratinocytes. In addition to providing compelling evidence for the structural support function of keratins, the phenotype exhibited by these mice was similar to epidermolysis bullosa simplex (EBS), which is a rare condition characterized by epidermal fragility that correlates with defects in keratin-filament organization.

In a subsequent study, Fuchs and colleagues carried out a genetic and functional analysis of the *K14* gene in patients with EBS. Focusing on the Dowling–Meara subtype of EBS, the authors showed that keratinocytes from these patients had an aberrant keratin-filament network: keratins purified from these cells formed markedly shorter 10-nm filaments *in vitro*. Sequencing reverse-transcribed mRNA and genomic DNA from two EBS patients revealed a point mutation in the *K14* gene that translated to a mutation in a highly conserved Arg residue at position 125 within the rod domain of K14. To complete the analysis, the authors showed that K14 carrying a mutation in Arg125 perturbs the keratin network when expressed in cells, and that the mutant K14 is only able to form short filaments *in vitro*.

These studies unequivocally demonstrated the significance of IFs — previously thought to be dispensable for life — in development and disease.

Sowmya Swaminathan, Senior Editor,
Nature Cell Biology

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