Discovery of keratin function and role in genetic diseases: the year that 1991 was

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ABSTRACT In 1991, a set of transgenic mouse studies took the fields of cell biology and dermatology by storm in providing the first credible evidence that keratin intermediate filaments play a unique and essential role in the structural and mechanical support in keratinocytes of the epidermis. Moreover, these studies intimated that mutations altering the primary structure and function of keratin filaments underlie genetic diseases typified by cellular fragility. This Retrospective on how these studies came to be is offered as a means to highlight the 25th anniversary of these discoveries.

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Although intermediate filaments (IFs) have been characterized at some level for a longer period of time (Oshima, 2007), they were officially discovered as such as recently as 1968 by Howard Holtzer and colleagues while studying the developing skeletal muscle (Ishikawa *et al.*, 1968). The advent of gene cloning methods and monospecific antibody production in the late 1970s and throughout the 1980s led to an explosion of data and knowledge about IFs that established them as a large family of genes and proteins that are individually regulated in a tight and evolutionarily conserved tissue- and differentiation-specific manner. Researchers also uncovered some of the remarkable properties of IFs as purified elements in vitro and in living systems and recognized that they occur in the nucleus as well as in the cytoplasm. In spite of the fast pace of progress during that period, however, it was not possible to produce evidence that spoke unequivocally about the functional importance of IFs in cells and tissues, let alone their role in disease.

Beginning in the mid- to late 1980s, pioneering experimentation along two distinct lines was underway in the laboratory of Elaine Fuchs, then at the University of Chicago. The eventual merger of these approaches yielded the first formal insight into IF function in vivo, as well as into their direct involvement in human disease. In an effort to define structure–function relationships with regard to the assembly and network formation properties of IFs, one such approach was the application of systematic deletion mutagenesis to keratin 14 (K14), a type I IF that is expressed with its type II partner keratin 5 (K5) in the progenitor basal layer of the epidermis and related complex epithelia. These studies demonstrated that deleting sequences from either end of the central α-helical rod domain of the K14 protein was deleterious for filament formation in a dominant manner both in transfected cells (Albers and Fuchs, 1987, 1989; Figure 1) and the setting of IF polymerization assays involving purified proteins in vitro (e.g., Coulombe *et al.*, 1990). The second key effort in the Fuchs lab in the late 1980s resulted in the demonstration that the proximal 2.5 kb and distal 700 base pairs corresponding respectively to the 5′ upstream and 3′ downstream regions of the cloned human K14 gene were sufficient to confer tissue-specific, that is, K14-like, regulation in transgenic mice in vivo (Vassar *et al.*, 1989; Figure 1). This tour de force paved the way for the production of a human K14 gene promoter–based cassette (e.g., Saitou *et al.*, 1995) that could reliably direct the expression of any open reading frame in a K14-like manner in transgenic mice. As an aside, this tool has had a profound effect on epithelial and skin biology research.

Subsequent use of the human K14 promoter–based cassette to direct the expression of epitope-tagged and selected deletion mutants of K14 gave rise to transgenic mouse pups that exhibited extensive blistering of the skin preferentially at sites of frictional trauma (Coulombe *et al.*, 1991b; Vassar *et al.*, 1991; Figure 1). Electron microscopy showed that skin blistering occurred secondary to a loss of the integrity of keratinocytes located in the basal layer of the epidermis, that is, the precise site of mutant K14 protein accumulation. Such blistering did not occur in transgenic mice expressing a full-length version of human K14 modified to carry only an epitope tag at the C-terminus at similar or higher levels (Coulombe *et al.*, 1991b;

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Abbreviations used: EBS, epidermolysis bullosa simplex; IF, intermediate filament; K, keratin.

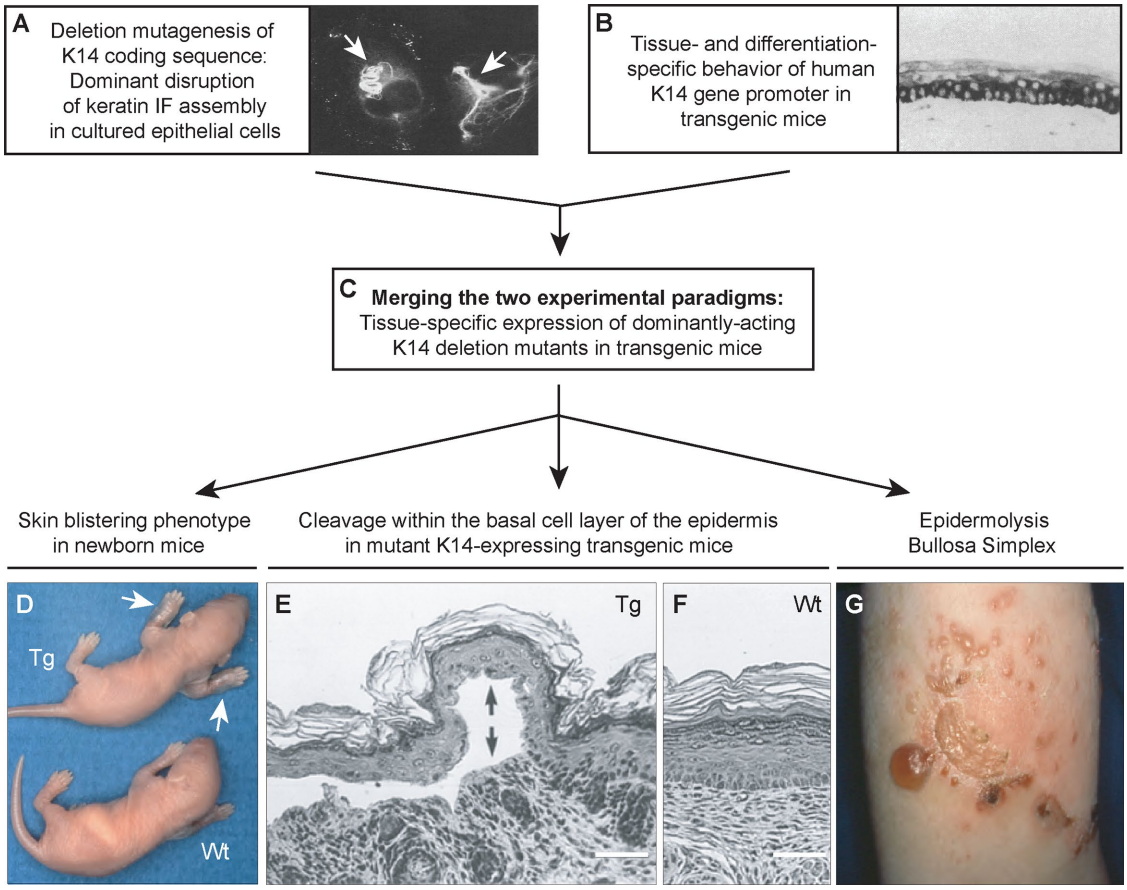

FIGURE 1: Schematic representation of the strategy and outcome of the experiments that led to the discovery of keratin function and role in genetic disease. Original figures are reproduced to give a realistic account of the data. (A) Examples of a disrupted keratin filament network in cultured epithelial cells transfected with and expressing a dominantly acting K14 deletion mutant (arrows). (Reproduced from Albers and Fuchs, 1987, with permission.) (B) Preferential expression of a substance P-epitope–tagged transgenic human K14 protein in the basal layer of tail skin epidermis in mouse, conveying the tissue- and differentiation-specific behavior of the transgene. (Reproduced from Vassar *et al.*, 1989, with permission.) (C) The two experimental approaches described in A and B were combined to assess the consequences of tissue-specific expression of dominantly acting K14 mutants in skin tissue in vivo. (D) Newborn mouse littermates. The mouse at the top is transgenic (Tg) and expresses a mutated form of K14 in the epidermis. It is showing severe skin blistering (arrows), particularly in its front paws, which are heavily used by mouse newborns to feed from their mother. The bottom mouse is a nontransgenic control showing no such blistering. (E, F) Hematoxylin-eosin–stained skin tissue sections showing the location of subepidermal cleavage within the epidermis of a K14 mutant–expressing transgenic mouse (opposing arrows in E). Cleavage occurs at the level of the basal layer, where the mutant keratin is expressed. Again, this is never seen in control wild-type (Wt) skin (F). Bar, 100 μm (E, F). (D–F are from Coulombe *et al.*, 1991b, with permission.) (G) Leg skin in a patient suffering from the Dowling–Meara form of epidermolysis bullosa simplex. Characteristic of this severe variant of this disease, several skin blisters are often grouped in a herpetiform manner (Fine *et al.*, 1991).

Vassar *et al.*, 1991). In addition, the severity of skin blistering in mutant K14–expressing transgenic pups could be directly related to the extent to which the mutant protein had been shown to disrupt filament assembly in transfected cell assays and in IF reconstitution assays in vitro. For instance, tissue-specific expression of a K14 mutant that could severely disrupt 10-nm filament assembly was associated with whole-body skin blistering and the untimely death of mouse pups and, from a pathology perspective, with "tonofilament clumping" and a paucity of visible keratin IFs in transgenic basal keratinocytes. By comparison, expression of another K14 mutant with a less deleterious effect on 10-nm IF assembly was compatible with the survival of transgenic mouse pups and resulted in skin blistering largely limited to the front paws in newborn mice together with altered organization of keratin IFs in basal keratinocytes of transgenic epidermis in situ, albeit without tonofilament clumping. This initial set of mouse strains thus revealed the existence of a direct link between the so-called "genotype" (i.e., mutant K14 characteristics) and the skin phenotype (Coulombe *et al.*, 1991b; Vassar *et al.*, 1991; Fuchs and Coulombe, 1992). Electrophoretic analyses of protein samples confirmed that the K14 mutant proteins acted dominantly to produce such spectacular phenotypes in transgenic mouse skin. Finally, blistering also occurred in the mutant K14–expressing transgenic mice in other stratified epithelia known both to express K14 and experience trauma, notably in the oral mucosa (Coulombe *et al.*, 1991b; Vassar *et al.*, 1991).

It is worth celebrating the 25th anniversary of these pioneering experiments for the following two reasons. First, the study of these mice provided the first formal demonstration that keratin IFs play a

fundamentally important role in structural support in surface epithelia such as the epidermis and oral mucosa. Without proper IF support, epidermal keratinocytes are rendered fragile and cannot sustain trivial frictional stress (Coulombe *et al.*, 1991b; Fuchs and Coulombe, 1992). The second reason is the observation that the phenotype of these K14 mutant–expressing mice proved eerily similar to those of individuals afflicted with the disease epidermolysis bullosa simplex (EBS), a rare, dominantly inherited and debilitating skin condition in which the epidermis and oral mucosa undergo blistering after exposure to trivial mechanical trauma. As observed in the mouse model, tissue cleavage had been shown to result from the loss of integrity of keratinocytes located in the basal layer (Fine *et al.*, 1991). Further, other researchers had previously reported on anomalies in the organization of keratin IFs in the basal epidermal keratinocytes of EBS patients (Anton-Lamprecht, 1983; Ito *et al.*, 1991) or in cultures of epidermal keratinocytes established from EBS patients (Kitajima *et al.*, 1989). The Fuchs laboratory thus teamed up with Amy Paller, a physician-scientist and pediatric dermatologist with deep expertise in genodermatoses, and mutations were soon discovered in the K14 gene of two independent and sporadic cases of a severe variant of the disease known as Dowling–Meara EBS (Coulombe *et al.*, 1991a; Figure 1). The two mutations were heterozygous missense alleles that affected the very same codon in K14 (Arg-125) and were correctly predicted at the time to correspond to a mutational hot spot in type I keratin genes. The mutations were shown to dominantly disrupt 10-nm IF assembly in vitro and/or in transfected keratinocytes in culture (Coulombe *et al.*, 1991a). Soon thereafter, a team led by Ervin Epstein at University of California, San Francisco (San Francisco, CA), reported on the use of classical linkage analysis to uncover a missense mutation in the K14 gene of a small pedigree with Koebner-type EBS, a less severe variant of the disease (Bonifas *et al.*, 1991). The next year, Birgit Lane and colleagues (Lane *et al.*, 1992) reported on the occurrence of mutations in keratin 5 (K5), the formal type II keratin assembly partner for K14 in vivo, in another instance of Dowling–Meara EBS.

In the years since 1991, a role in structural support has been formally demonstrated for all classes of IFs (Coulombe *et al.*, 2009), including the nuclear-localized lamins (e.g., Lammerding *et al.*, 2004). Moreover, we now know of several hundred independent instances of mutations in either K5 or K14 in the setting of the EBS disease, with the vast majority of those consisting of dominantly acting missense alleles (Szeverenyi *et al.*, 2008; Human Intermediate Filament Database, www.interfil.org, maintained at the Centre for Molecular Medicine and Bioinformatics Institute, Singapore). We also learned that, as anticipated, EBS largely represents a loss-of-function phenotype, since K14-null mice (Lloyd *et al.*, 1995), K14-null individuals (Chan *et al.*, 1994; Rugg *et al.*, 1994), and K5-null mice (Peters *et al.*, 2001) all exhibit an EBS-like skin-blistering phenotype (Coulombe *et al.*, 2009). Mutations such as Arg125Cys in K14 markedly compromise the remarkable mechanical properties of keratin filaments (Ma *et al.*, 2001), as well as the steady-state dynamics of keratin filaments in transfected keratinocytes in culture (Werner *et al.*, 2004). Finally, mutations affecting the coding sequence of IF genes have been shown to underlie >100 diseases affecting the human population (Omary *et al.*, 2004; Szeverenyi *et al.*, 2008; www.interfil.org). Consistent with the exquisite tissue- and cell type–specific regulation of IF genes, these diseases collectively affect a myriad of tissues and organs and are relevant to nearly all branches of medicine. These observations attest to the importance and profound effect that the generation and characterization of mutant K14–expressing transgenic mice has had for cell biology, epithelial physiology, dermatology, and medicine.

Many thoughts spring to mind when reminiscing about my involvement with this body of work. First, this effort was prescient of the power of team science and, in particular, of the potential effect of close collaborations involving biologists and physician-scientists. I learned a great deal and benefited immensely from working closely with many colleagues on this project, including Bob Vassar, Kathryn Albers, Linda Degenstein, Liz Hutton, Anthony Letai, Amy Paller, and, last but not least, my postdoctoral mentor and the laboratory head, Elaine Fuchs. Second, there is no substitute for elements such as innovation, hard work, perseverance, boldness, accountability, and great leadership. Elaine had the vision and created the exceptional circumstances necessary to make this set of discoveries possible, and, of equal importance, she was an integral part of the day-to-day progress and maturation of the entire project. Finally, as we all know, there is an intangible element of luck involved in discovery research. In this instance, a strong argument can be made that the studies highlighted here may not have had such a deep and defining effect had the effort been devoted to any IF other than the K5–K14 keratin pairing.

What are some of the lingering issues regarding this specific topic that preoccupy us still, 25 years later? Two challenges loom particularly large. First, we have yet to achieve a satisfactory understanding of how mutations in keratin proteins can cause disease. This is due in part to the lack of an atomic-level understanding of the core structure of IFs (which has been a tough nut to crack; Lee *et al.*, 2012), along with the reality that, for any relevant IF gene, there is a broad variety of disease-associated (mostly missense) mutations that pepper their primary structure (www.interfil.org). Second, we have yet to achieve success toward the treatment of EBS or any IF-based disorder. Disease characteristics such as low incidence, a dominantly inherited character, genetic heterogeneity (e.g., broad mutational landscape), and, in the case of EBS and related conditions, an intrinsically high rate of cell turnover within the main target tissue significantly add to the challenge of devising safe and effective therapeutic strategies (Coulombe *et al.*, 2009). Although efforts are still underway to foster progress on these two challenging issues, the field as a whole has made significant progress in uncovering a plethora of noncanonical functions of keratin IFs (Hobbs *et al.*, 2016) in addition to understanding their regulation, dynamics, and many remarkable properties.

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REFERENCES

Albers K, Fuchs E (1987). The expression of mutant epidermal keratin cDNAs transfected in simple epithelial and squamous cell carcinoma lines. J Cell Biol 105, 791–806.

Albers K, Fuchs E (1989). Expression of mutant keratin cDNAs in epithelial cells reveals possible mechanisms for initiation and assembly of intermediate filaments. J Cell Biol 108, 1477–1493.

Anton-Lamprecht I (1983). Genetically induced abnormalities of epidermal differentiation and ultrastructure in ichthyoses and epidermolyses: pathogenesis, heterogeneity, fetal manifestation, and prenatal diagnosis. J Invest Dermatol 81, 149s–153s.

Bonifas JM, Rothman AL, Epstein E (1991). Epidermolysis bullosa simplex: evidence in two families for keratin gene abnormalities. Science 254, 1202–1205.

Chan Y, Anton-Lamprecht I, Yu QC, Jackel A, Zabel B, Ernst JP, Fuchs E (1994). A human keratin 14 "knockout": the absence of K14 leads to severe epidermolysis bullosa simplex and a function for an intermediate filament protein. Genes Dev 8, 2574–2587.

Coulombe PA, Chan YM, Albers K, Fuchs E (1990). Deletions in epidermal keratins leading to alterations in filament organization in vivo and in intermediate filament assembly in vitro. J Cell Biol 111, 3049–3064.
Coulombe PA, Hutton ME, Letai A, Hebert A, Paller AS, Fuchs E (1991a). Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analyses. Cell 66, 1301–1311.
Coulombe PA, Hutton ME, Vassar R, Fuchs E (1991b). A function for keratins and a common thread among different types of epidermolysis bullosa simplex diseases. J Cell Biol 115, 1661–1674.
Coulombe PA, Kerns ML, Fuchs E (2009). Epidermolysis bullosa simplex: a paradigm for disorders of tissue fragility. J Clin Invest 119, 1784–1793.
Fine JD, Bauer EA, Briggaman RA, Carter DM, Eady RA, Esterly NB, Holbrook KA, Hurwitz S, Johnson L, Lin A, *et al*. (1991). Revised clinical and laboratory criteria for subtypes of inherited epidermolysis bullosa. A consensus report by the Subcommittee on Diagnosis and Classification of the National Epidermolysis Bullosa Registry. J Am Acad Dermatol 24, 119–135.
Fuchs E, Coulombe PA (1992). Of mice and men: genetic skin diseases of keratin. Cell 69, 899–902.
Hobbs RP, Jacob JT, Coulombe PA (2016). Keratins are going nuclear. Dev Cell 38, 227–233.
Ishikawa H, Bischoff R, Holtzer H (1968). Mitosis and intermediate-sized filaments in developing skeletal muscle. J Cell Biol 38, 538–555.
Ito M, Okuda C, Shimizy N, Tazawa T, Sato Y (1991). Epidermolysis bullosa simplex (koebner) is a keratin disorder. Ultrastructural and immunohoistochemical study. Arch Dermatol 127, 367–72.
Kitajima Y, Inoue S, Yaoita H (1989). Abnormal organization of keratin intermediate filaments in cultured keratinocytes of epidermolysis bullosa simplex. Arch Dermatol Res 281, 5–10.
Lammerding J, Schulze PC, Takahashi T, Kozlov S, Sullivan T, Kamm RD, Stewart CL, Lee RT (2004). Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. J Clin Invest 113, 370–378.
Lane EB, Rugg EL, Navsaria H, Leigh IM, Heagerty AH, Ishida YA, Eady RA (1992). A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. Nature 356, 244–246.
Lee CH, Kim MS, Chung BM, Leahy DJ, Coulombe PA (2012). Structural basis for heteromeric assembly and perinuclear organization of keratin filaments. Nat Struct Mol Biol 19, 707–715.
Lloyd C, Yu QC, Cheng J, Turksen K, Degenstein L, Hutton E, Fuchs E (1995). The basal keratin network of stratified squamous epithelia: defining K15 function in the absence of K14. J Cell Biol 129, 1329–1344.
Ma L, Yamada S, Wirtz D, Coulombe PA (2001). A "hot-spot" mutation alters the mechanical properties of keratin filament networks. Nat Cell Biol 3, 503–506.
Omary MB, Coulombe PA, McLean WHI (2004). Intermediate filament proteins and their associated diseases. N Engl J Med 351, 2087–2100.
Oshima RG (2007). Intermediate filaments: a historical perspective. Exp Cell Res 313, 1981–1994.
Peters B, Kirfel J, Bussow H, Vidal M, Magin TM (2001). Complete cytolysis and neonatal lethality in keratin 5 knockout mice reveal its fundamental role in skin integrity and in epidermolysis bullosa simplex. Mol Biol Cell 12, 1775–1789.
Rugg EL, McLean WH, Lane EB, Pitera R, McMillan JR, Dopping-Hepenstal PJ, Navsaria HA, Leigh IM, Eady RA (1994). A functional "knockout" of human keratin 14. Genes Dev 8, 2563–2573.
Saitou M, Sugai S, Tanaka T, Shimouchi K, Fuchs E, Narumiya S, Kakizuka A (1995). Inhibition of skin development by targeted expression of a dominant-negative retinoic acid receptor. Nature 374, 159–162.
Szeverenyi I, Cassidy AJ, Chung CW, Lee BT, Common JE, Ogg SC, Chen H, Sim SY, Goh WL, Ng KW, *et al*. (2008). The Human Intermediate Filament Database: comprehensive information on a gene family involved in many human diseases. Hum Mutat 29, 351–360.
Vassar R, Coulombe PA, Degenstein L, Albers K, Fuchs E (1991). Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin disease. Cell 64, 365–380.
Vassar R, Rosenberg M, Ross S, Tyner A, Fuchs E (1989). Tissue-specific and differentiation-specific expression of a human K14 keratin gene in transgenic mice. Proc Natl Acad Sci USA 86, 1563–1567.
Werner NS, Windoffer R, Strnad P, Grund C, Leube RE, Magin TM (2004). Epidermolysis bullosa simplex-type mutations alter the dynamics of the keratin cytoskeleton and reveal a contribution of actin to the transport of keratin subunits. Mol Biol Cell 15, 990–1002.