Mini-Review on the Cellular Mechanisms of Disease

Intermediate Filaments and Disease: Mutations that Cripple Cell Strength

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There are nearly 50 different intermediate filament (IF) proteins of five major types (for reviews, see references 16, 23, and 59). Four of the IF types are cytoplasmic, and they are expressed differentially in all tissues at various stages of embryonic and adult development. Type V IF proteins are the nuclear lamins, which form a fibrous meshwork on the inner surface of the nuclear membrane, and they are universally expressed in higher eukaryotes. Although the functions of cytoplasmic IFs remain largely unknown, defects in the genes encoding these proteins have been shown to give rise to autosomal dominant, tissue-specific degenerative disorders. This article reviews how a knowledge of IF mutations in disease has advanced our knowledge of IF structure, and how the clinical features of IF diseases have, in turn, enhanced our understanding of the roles of IFs in higher eukaryotic organisms.

IF Assembly: A Lesson in Protein Design

IF proteins share common structural features enabling them to self-assemble into complex 10-nm filaments in vitro in the absence of auxiliary proteins or factors (Fig. 1). IFs are rope-like structures made of ~4.5-nm protofilaments, which in turn are made from smaller fibers called protofilaments (2). Protofilaments contain linear arrays of IF dimer subunits, arranged in head to tail fashion (1, 31). Although the molecular details of protofilaments have not yet been precisely defined, each protofilament appears to be constructed from two antiparallel chains of these dimers.

The IF dimer is a coiled coil of two in-register molecules aligned in a parallel fashion. Cytoskeletal IF proteins have a central 310-amino acid residue α-helical domain, the rod, which is flanked by nonhelical head (amino end), and tail (carboxy end) domains. The formation of the coiled coil is guided by heptad repeats of hydrophobic amino acids within the rod. This feature has been widely used in nature to facilitate interactions between two or more proteins. Most IF types are able to form homodimers. Keratins are unusual in that they form obligatory heterodimers of a type I and a type II subunit.

In solution, stable tetramers form from two dimers arranged in an unparallel fashion, either in an unstaggered alignment or in a near half-staggered alignment with an amino terminal overlap (for review see reference 23). Chemical cross-linking data support the existence of both forms in IFs (25, 56, 57). It has been postulated that the staggered alignments occur at the level of the protofilament; i.e., in alignment of the two antiparallel linear arrays of dimers (which could also be viewed as end-to-end linkages of staggered tetramers). The unstaggered alignments may be used during higher ordered lateral interactions, perhaps between protofilaments (30).

Further insights into the molecular associations in IFs have been gained by secondary structure analyses, sequence comparisons, and molecular mutagenesis of IF subunits. The IF α-helical rod is subdivided by three short nonhelical linker segments, L1, L1-2, and L2 (Fig. 1, thin lines). These segments often contain proline or multiple glycine residues, and they are predicted to break the α-helical rod at locations that are conserved among all IF proteins (16, 27). The four resulting rod segments are referred to as helices 1A, 2A, 1B, and 2B. The sequence identity among all IF proteins is particularly high at the start of helix 1A and near the end of helix 2B, making these domains the most diagnostic for an IF protein. Deletion mutagenesis studies indicate that the ends of the rod play a special role in IF assembly (3, 19, 26, 51, 68). Even subtle point mutations in these highly conserved sequences can have deleterious effects (28, 29, 41) that are more severe than proline mutations more centrally in the rod (41). It has recently been postulated that in the linear, head-to-tail arrays of dimers, the rod ends of one dimer may overlap slightly with the rod ends of an adjacent dimer, thereby accounting for their special importance in IF structure (30, 56; Fig. 1).

Despite extensive mutagenesis studies, the functions of the nonhelical head and tail segments of IF proteins remain unresolved. These domains vary greatly in length (~10-1,500 residues) and amino acid composition, making it doubtful that the heads and tails of IFs produce a common structure. Indeed, deletion analyses of tail segments are suggestive that much of this portion of IF proteins is often dispensable to...
IF structure, although some studies suggest that tails may play a role in governing lateral associations at the protofilament and protofibril level (30, 36, 39, 43). In contrast, IF head domains have more prominent and diverse roles in filament structure, and roles in both lateral and end-to-end linkages have been proposed (26, 30, 36, 57, 68). It is also likely that at least a portion of the head and/or tail segments protrude along the IF surface, wrapping the filament in a cloak of tissue-specific sequences.

Finally, sequences in the head and tail are sites for phosphorylation, which can orchestrate dynamic IF assembly and disassembly events in vivo. Thus, mitosis-mediated reorganization of vimentin filaments occurs concomitantly with a transient increase in IF protein phosphorylation, and a cell cycle-dependent increase in phosphorylation of the lamins coincides with nuclear lamina disassembly (for review see reference 47). Both of these in vivo processes are governed by cdc2 kinase–mediated phosphorylation of tail and/or head sequences that immediately flank the IF rod domain. The changes to IF assembly/function that accompany tail or head phosphorylations may extend beyond cell cycle–mediated changes in IF networks, and may range from modulation of surface properties of filaments (32) to influencing axonal caliber of neurofilaments (NFs), and possibly slowing axonal transport (for review, see reference 65).

**Genetic Diseases of Keratin IFs and Refinements on IF Structure**

In contrast to microfilaments, microtubules, or the nuclear lamina, cytoplasmic IFs seem to be dispensable for cells growing on a plastic surface in tissue culture. Thus, if nature had a purpose in mind for IFs, it must be to perform functions tailored to suit specialized needs of eukaryotic cells within their natural environment. It seems reasonable to surmise that compromising such functions could lead to human diseases whose phenotypes are confined to specific tissues. Since genetically engineered mutations in IF genes typically act in a dominant negative fashion to perturb cytoskeletal architectures, candidate diseases should be primarily autosomal dominant.

Epidermolysis bullosa simplex (EBS) is the first group of diseases discovered to be IF disorders. Affecting ~1:50,000 in the population and with clinical features present at birth, EBS is characterized by mechanical stress-induced skin blistering caused by cytolysis within the basal layer of the epidermis (see Fig. 2). The disease is typically transmitted in an autosomal dominant fashion, although recessive inheritance has been reported. There are three major types of EBS: (a) Dowling-Meara (D-M) EBS is characterized by generalized, severe intraepidermal blistering and clumping of often afilamentous keratin within the basal cell cytoplasm (4); (b) Koebner (K) EBS is intermediate in severity; and (c) Weber-Cockayne (W-C) EBS patients have mild basal cell cytolysis and blistering predominantly on the hands and feet, and the basal keratin filaments often appear nearly normal, with occasionally only mild aggregation of filaments. In all EBS types, suprabasal layers are unperturbed, reflective of a normal differentiation process.

From electron microscopy studies, it was noted that in...
D-M EBS, tonofilament clumping exists even in cells that have not undergone lysis, hinting that anomalies in keratin networks may be early events in the blistering process (4). Additionally, cultured D-M EBS keratinocytes display perturbed keratin networks, resembling those of normal keratinocytes transfected with a gene encoding a mutant basal type I keratin, K14 (37, 64). Most notably, transgenic mice engineered to express a truncated human K14, missing the tail and about one third of the α-helical rod segment, expressed 10-nm filaments. This leads to cell degeneration upon mechanical trauma in the basal cells, presumably because the mechanical strength of these cells has been compromised. In EH, cells express mutant K1 or K10 proteins, generating aberrant IFs and cell fragility in the suprabasal cells.

Within the past 2 yr, a second autosomal dominant disease has been found to be an IF disorder. Like EBS, epidermolytic hyperkeratosis (EH) is typified by epidermal cytolysis, but in this case, the basal epidermal layer has normal morphology, and the cytolysis occurs in suprabasal layers (Fig. 2; for review, see reference 4). In severe cases, tonofilament clumping and perinuclear shells of tonofilament aggregates are found in suprabasal cells, and there is a markedly thickened granular layer and stratum corneum in EH skin. Given the striking similarities between EBS and EH and the knowledge that epidermal cells switch to the expression of K1 (type II) and K10 (type I) keratins as they commit to terminally differentiate, it was predicted that EH might be a disorder of K1 and K10 (64). This was later confirmed by transgenic mouse studies (24), by genetic mapping of an EH family (15) and by sequence analyses of the K1 and K10 genes of EH patients (12, 13, 54; see also 14, 44, 60, 69). Recently, yet another disease, epidermolytic palmoplantar keratoderma (EPPK) has been found to be a keratin disorder (52, 62). EPPK is similar to EH, but with blistering localized to suprabasal layers of palmoplantar skin. The gene often mutated in EPPK encodes K9, a type I keratin whose pattern of expression mirrors the blistering.

As shown in Fig. 1, many of the same principles that operate on the loci of K5 and K14 mutations in the various grades of EBS also apply to K1 and K10 mutations in EH and K9 mutations in EPPK. Remarkably, the arginine mutated in the K14 gene of D-M EBS (R125) is often mutated in the K10 gene of patients with severe EH and the K9 gene of patients with EPPK (12, 52, 54, 60). Thus, the same mutation in a highly conserved residue of three genes can give rise to three distinct genetic diseases by virtue of the differential expression of the genes. The high frequency with which this residue is mutated to a cysteine or a histidine in EBS, EH, and EPPK is partly caused by the fact that this residue is a hot spot for C to T mutagenesis by CpG methylation and deamination (17). However, for both EBS and EH, mutations tend to be clustered and are frequently at the same or equivalent residues, even when they are not obvious hot spots for mutagenesis.

In cases where genetic arguments cannot account for a high frequency of mutation of a particular residue, a compromise of function is likely to be the underlying explanation. Indeed, the overriding feature of the EBS, EH, and EPPK mutations is that they are critical for IF structure (10, 12, 13, 20, 29, 42, 60). In this regard, structural studies on EBS mutations have revealed a correlation between the degree to which a mutation perturbs IF assembly, the specific location of a mutation, and the severity of the EBS or EH disease (42). Such studies have chiseled new details into a
previously faceless IF sculpture. Thus, to explain the clustering of severe mutations in the rod ends and to account for why these mutations primarily affect filament elongation in vitro (20), it has been proposed that the rod ends of end-to-end linked dimers may overlap slightly (56, 57), a feature that also explains repeat lengths in paracrystalline IF structure (30).

In contrast to the filament-shortening keratin mutations of severe EBS or EH, the L1-2 linker mutants of W-C EBS produce filaments in vitro that are unrvaled, suggesting that lateral interactions may be affected (10). How might the L1-2 linker segment play a role in lateral interactions? Intriguingly, chemical cross-linking data predict that two staggered dimers are aligned so that the carboxy half of the L1-2 domain in one dimer is in close proximity to, and possibly even directly opposite, the helix 1A segment in an adjacent dimer (25, 56, 57). While these values are only accurate to 1 to ~10 residues (25), when considered in conjunction with the putative overlap of the ends of two linearly aligned dimers (57), it is possible that the rod ends and the L1-2 sequences all reside at a common junction. In such a model, the L1-2 segment would play a clear role in the lateral alignment of staggered dimers. While more refined analyses will be necessary to unequivocally establish this alignment, perturbations at this level could readily influence lateral associations at higher levels of IF structure (10).

The fourth region where mutations are clustered is the H1 domain of the head of the type II keratins (9, 13, 69). The concentration of mutations in this region underscores its importance to IF structure. These studies also substantiate previous conclusions from in vitro assembly (67) and computer studies (16; see also 57) that this region of the head domain of type II keratins plays a role in IF assembly. Precisely how the head domain is involved is presently unknown. However, in other IF types, this general region serves as a regulatory domain for phosphorylation-dependent control of IF assembly. In this regard, it is intriguing that in two unrelated W-C EBS families, the mutation is L161S, creating a potential protein kinase C site (9). Whether this site is actually phosphorylated in W-C EBS remains to be determined.

**IF Disorders in General**

The knowledge that EBS, EH, and EPPK are disorders of keratins has enabled predictions of other human genetic diseases that may be disorders of IF genes. In general, IF diseases are expected to be autosomal dominant, given the multimeric nature of IFs and the dominant negative action of most IF mutants. In addition, IF diseases are expected to display perturbations in the IF networks, as well as subsequent degeneration and cytolysis in cells in which the IF gene is expressed.

Several possible candidate hair diseases share these features (for review see reference 4). The autosomal dominant mouse mutants, Re, Bsk, and Re*, map in close proximity to the type I epidermal keratin genes (45 and references therein): Rex mice have curly whiskers and bent hair shafts, and denuded mutant mice and bareskin mice undergo hair loss after completion of the first hair cycle. Another class of candidate IF disorders are those that might involve NF genes. Although not present in all neurons, NFs are preferentially located in the axon, where they are thought to provide mechanical strength. Recently, Ohara et al. (48) discovered that the Quv mutant of Japanese quail is a recessive, nonsense mutation in the NF-L gene resulting in the absence of NF-L, a dramatic deficiency of NFs, a reduction in the caliber of resulting axons, and a mild, generalized quivering. NF defects have also been suspected in the pathogenesis of several types of neurodegenerative diseases, such as amyotrophic lateral sclerosis or ALS and infantile spinal muscular atrophy (66 and references therein). Interestingly, transgenic mice that overexpress wild-type NFs or produce mutant NFs exhibit the hallmarks of motor neuron disease, including axonal degeneration and skeletal muscle atrophy (18, 70). Thus, recent studies on the relation between NF aberrations and motor neuron diseases are promising, and future studies should reveal if such human diseases involve defects in NF genes.

Other potential IF diseases include some forms of familial cardiomyopathies. Many of these disorders are myosin defects (61). However, some are not, and in several of these cases, abnormalities in desmin filament networks have been observed electron microscopically (49). Hence, although human studies have not yet been conducted, it seems possible that some of these cases may turn out to result from desmin mutations.

**How Do IF Mutations Cause Cell Lysis?**

A number of findings point to the hypothesis that without a proper IF network, cells become fragile and prone to breakage upon mechanical stress. Thus, in mice and humans, EBS cells often rupture in a defined zone beneath the nucleus and above the hemidesmosomes. This is the longest portion of the columnar basal cell, and the zone expected to be most fragile when mutations arise that compromise filament elongation, as do many of the EBS, EH, and EPPK mutations. Hence, an important function of keratin filaments seems to be to impart a mechanical framework to an epidermal cell.

Do other IFs also play a role as mechanical integrators of space as suggested many years ago? Several lines of evidence suggest that they do. Treatment of female rats with anabolic steroids increases the number of IFs in heart muscle, perhaps providing the heart strengthening sought after by athletes (7). In addition, rheologic studies show that type III IFs harden and resist breakage under stresses where other cytoskeletal networks will rupture (35). Conversely, a Xenopus oocyte extract depleted of its single lamin (Ldm) is able to encapsulate chromatin by fused membrane vesicles and assemble nuclear pores, but the resulting nuclei are fragile (46). Similarly, transgenic mice overexpressing a wild-type hair keratin gene have cortical cells that are fragile, leading to brittleness and hair breakage (50).

If most IFs function in part to provide mechanical integrity to cells, then it seems likely that genetic defects in other IF genes might lead to cell fragility and degeneration in other tissues, as suggested above. However, it is also expected that the mechanical strength requirements of a cell will vary dramatically, depending on its shape and environment. In fact, when D-M EBS keratinocytes are flattened either during wound healing in vivo or on a petri dish in vitro, lysis is reduced (21). Moreover, K8 and K18 seem to be essential in Xenopus gastrulation (38, 63) and in liver development (6), but not in early mouse development (5, 6), a finding that is difficult to explain without invoking either (a) tissue and developmental-specific differences in mechanical strength...
requirements; or (b) additional functions for IFs, which at present remain obscure. In the future, elucidating the specialized requirements of cells and tissues for IFs will be paramount in determining the extent to which defects in the present remain obscure. In the future, elucidating the ~50 human IF genes might give rise to a broad family of specialized requirements of cells and tissues for IFs will be important in understanding the roles of IFs in various cellular functions.

References