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Mechanics of soft epithelial keratin networks depend on modular filament assembly kinetics

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ABSTRACT

Structural adaptability is a pivotal requirement of cytoskeletal structures, enabling their reorganization to meet the cellular needs. Shear stress, for instance, results in large morphological network changes of the human soft epithelial keratin pair K8:K18, and is accompanied by an increase in keratin phosphorylation levels. Yet the mechanisms responsible for the disruption of the network structure *in vivo* remain poorly understood. To understand the effect of the stress-related site-specific phosphorylation of the K8:K18 pair, we created phosphomimicry mutants – K8(S431E), K8(S73E), K18(S52E) – *in vitro*, and investigated the various steps of keratin assembly from monomer to network structure using fluorescence and electron microscopy, and using rheology characterized their network mechanical properties. We find that the addition of a charged group produces networks with depleted intra-connectivity, which translates to a mechanically weaker and more deformable network. This large variation in network structure is achieved by the formation of shorter mutant filaments, which exhibit differing assembly kinetics and a manifestly reduced capacity to form the extended structures characteristic of the wild-type system. The similarity in outcome for all the phosphomimicry mutants explored points to a more general mechanism of structural modulation of intermediate filaments via phosphorylation. Understanding the role of kinetic effects in the construction of these cytoskeletal biopolymer networks is critical to elucidating their structure-function properties, providing new insight for the design of keratin-inspired biomaterials.

Statement of Significance

Structural remodeling of cytoskeletal networks accompanies many cellular processes. Interestingly, levels of phosphorylation of the human soft epithelial keratin pair K8:K18 increase during their stress-related structural remodeling. Our multi-scale study sheds light on the poorly understood mechanism with which site-specific phosphorylation induces disruption of the keratin network structure *in vivo*. We show how phosphorylation reduces keratin filament length, an effect that propagates through to the mesoscopic structure, resulting in the formation of connectivity-depleted and mechanically weaker networks. We determine that the intrinsically-set filament-to-filament attractions that drive bundle assembly give rise to the structural variability by enabling the formation of kinetically-arrested structures. Overall, our results shed light on how self-assembled intermediate filament structures can be tailored to exhibit different structural functionalities.

1. Introduction

The keratin 8 (K8), keratin 18 (K18) pair is the cytoskeletal intermediate filament (IF) system found in soft epithelia [1–3]. Recent work has shown the K8:K18 system to be adaptable, with a time-dependent high turnover in the cell [1,3,4], and subject to multiple and site-specific post-translational modifications [5]. Of particular interest is site-specific kinase-dependent keratin phosphorylation, whose levels increase in periods of cell stress and mitosis, and is coupled to stages of the cell life-cycle that see large structural reorganization to its internal network components [6–9]. *In vivo* studies of phosphorylation-dependent IF structural change show a significant break in network homogeneity

Abbreviations: IF, intermediate filament; K8, keratin 8; K18, keratin 18; WT, wild-type; Ser/S, serine; Glu/E, glutamic acid; Ala/A, alanine; ULF, unit-length filament; TEM, transmission electron microscopy; STED, stimulated emission depletion.

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throughout the cell, with regions of high protein density (with no observable mesh) and other IF-depleted regions [6–10].

The self-assembly of the K8:K18 pair [11–15], schematically depicted in Fig. 1a, begins with the necessary heterodimerization of the Type I K18 monomer with its pair-corresponding Type II K8 monomer. The heterodimers further assemble into tetramers, and the lateral association of four tetramers gives rise to the repeat denomination referred to as the Unit-Length Filament (ULF), Fig. 1a [11–15]. The longitudinal assembly of the ULFs creates the mature filament (diameter ≈ 10 nm), and the lateral association of mature filaments, driven by the presence of salt, creates the bundles of filaments [16–19], Fig 1a. The bundles further associate with one another, also by salt-mediated non-specific interactions and henceforth referred to as the crosslinks, forming the base physiological network structure observed within cells [4,20–22].

Previous work has shown that the non-helical domains of IF monomers play a role in subunit lateral alignment within filaments, filament elongation, and filament stabilization [18,23–25]. The localization of a phosphate group, generally within the head or tail domain of the monomers, has been found to affect the solubility, polymerization and network-forming propensities of the IF systems, with cases of hyperphosphorylation correlating to complete network collapse [4,9,10,26]. Serine (Ser, S) 431 of K8, for instance, is a basally phosphorylated site whose levels of phosphorylation increase during mitosis and with the increased expression of EGF-regulated-kinase [8]. Phosphorylation at Ser 73 of K8 (a substrate of p38 α-kinase) however, proceeds in an on/off manner [6]. In addition, K18 has also been found to be a substrate for kinases, with the most notable site corresponding to Ser 52 [7]. *In vivo* experiments on IF-depleted cells transfected with keratin

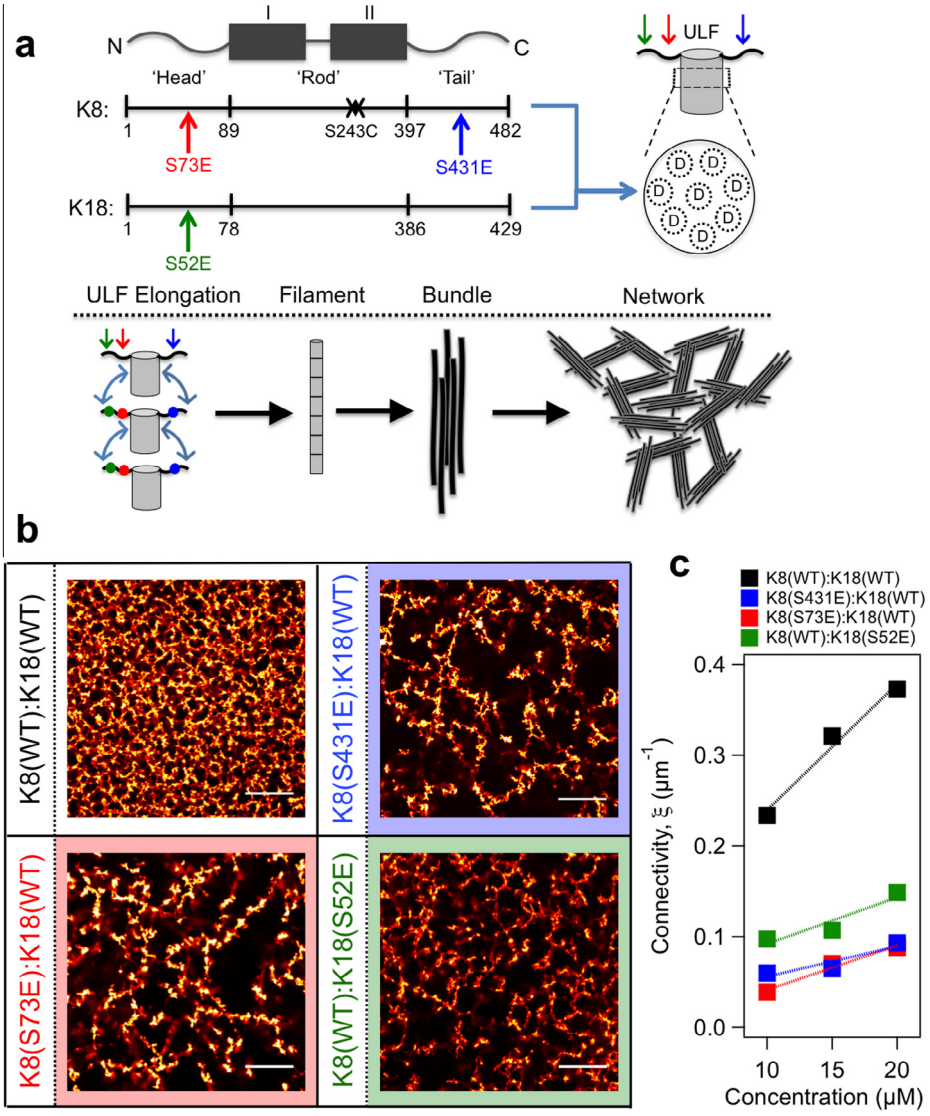

Fig. 1. Schematics of keratin monomers and network assembly, and structural variations between K8(WT):K18(WT) and phosphomimicry mutant networks. (a) Schematic representation of the keratin monomeric structure, with arrows marking the phosphomimicry mutation sites (numbering excludes the first methionine). Dimers (denoted as "D") are assembled from each of K8 and K18, and further associate to form the repeat unit – the unit length filament (ULF) – composed of 8 dimers in the lateral dimension. ULFs stack longitudinally, forming the mature filament; and the lateral association of the filaments drives bundle formation, which is the structural base of network formation (only 2 tails are schematically represented in the ULF, and omitted from the other structures for simplicity). (b) Confocal microscopy images of labeled keratin networks at 15 μM protein concentration highlight the significant structural impact of the introduced mutations, specifically in reducing network connectivity. Scale bar is 25 μm. (c) Graphical representation of network connectivity in μm^{-1} determined from confocal microscopy images of the various networks at the corresponding protein concentrations (for confocal images of the keratin networks at different protein concentrations, refer to Supplementary Figs. S1–S3). The network connectivity increases proportionally to the protein concentration, with that of the K8(WT):K18(WT) network being consistently larger (by ≈2-fold) than those observed for the phosphomimicry mutant systems. Error bars representing the fit result error for network connectivity, <0.005 μm^{-1}, are plotted in (c) but are smaller than their corresponding markers.