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Review

Perspectives on the Molecular and Biological Implications of Tropoelastin in Human Tissue Elasticity*

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The elasticity of a range of vertebrate and particularly human tissues depends on the dynamic and persistent protein elastin. This elasticity is diverse, and comprises skin, blood vessels, and lung, and is essential for tissue viability. Elastin is predominantly made by assembling tropoelastin, which is an asymmetric 20-nm-long protein molecule. This overview considers tropoelastin's molecular features and biological interactions in the context of its value in tissue repair.

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Introduction

Mammalian tissue comprises an ensemble of molecular and cellular components that reflect its blended physical and biological makeup.[1] This interplay means that their biopolymer components need to not only deliver durable tensile and compression performance, they also need to participate in cross-talk with other resident molecules and with cells.[2,3] That harmony encompasses the flow of blood through the vasculature to exchange gases and nutrients, the nurtured functionality of resident and visiting cells, and local physical integrity.[4,5] *De novo* synthesis of tissue is often inadequate as evidenced by scars and the shut-down in elastin synthesis after childhood.[6] When there is diseased or damaged tissue, surgery and repair can restore function if components are available. However, when functional components are unavailable, science has turned to tissue engineering, which is pursuing a long-term strategy to deliver reliable, commercially available spare parts. Elastin is a natural choice as an integral component of reliable three-dimensional elastic tissue, as it is responsible for tissue elasticity, and is the dominant protein in large arteries, where it serves essential roles in both the mechanics and cell interactions of the arterial wall.[7] Although other proteins are present, elastic tissue is predominantly made of one protein, tropoelastin,[8] which dominates molecular contributions to the architecture and function of elastin.[9,10] For this reason, the present review considers the perspective of tropoelastin.

Elastic Tissue is Needed for Elasticity

Elastic tissue is integral to the extracellular matrix of vertebrate tissues such as blood vessels, lungs, and skin, where it provides the structural integrity and elasticity required for mechanical stretching of these tissues during normal function.[7] Elastin is confined to vertebrates and is distinct from invertebrate elastomers. Biological demands for elastin's three-dimensional architecture reflect its physical environment and biological demands: tubes carry blood in the vasculature, the lung expands and contracts with each breath, and fibres in the dermis facilitate skin recoil. Elastin is typically arrayed in the form of fibres, the dominant component of which is the elastin polymer. Elastin is one of the most durable human proteins as it lasts as long as the host,[11,12] pointing to an impressive ability to withstand repeated mechanical cycles of loading such as in the elastic vasculature, where elastin encounters over 2 billion expansion and contraction events.

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Tropoelastin is the Dominant Molecular Component of Elastin: Molecular Features

Elastin is assembled by aggregating its soluble precursor, tropoelastin. Elastin is decorated (<10 % w/w) by microfibrillar proteins. We discovered that tropoelastin has a defined monomer shape in solution that is needed for assembly,[9,10,13] but also displays a large percentage of flexible, disordered regions needed for molecular elasticity.[14,15] The tertiary structure of human tropoelastin represents an ensemble of elastic conformers,[10,16] yet occasional conserved sequence elements hint at requirements for functional demands in one or more key parts of this molecule.[17–19] Tropoelastin is a 20-nm-long asymmetric protein monomer with a slightly curved nanospring shaft that extends from its N-terminus to a bifurcating hinged foot region towards the C-terminus.[10] Small-angle X-ray (SAXS) and neutron scattering (SANS), combined with antibody access, assembly, and cell interaction data reveal that tropoelastin has defined shape requirements, yet operates as a kinetically dynamic molecule. The protein's dynamics can be described as an ensemble of conformational microstates that are intrinsically accessible to the molecule under physiological conditions. Physically, its modes represent deformations that are least energetically costly on a multidimensional energy landscape, where mode frequency represents the curvature of the free-energy landscape. Importantly, these motions are tied to the overall tropoelastin solution shape rather than specific interatomic interactions. This model of tropoelastin dynamics describes a characteristic scissors-like motion between the hinge and foot regions, as well as a twisting motion in the N-terminal coil region, in which the N-terminus experiences highest displacement.[9,10,13,20] The geometric distribution of the tropoelastin molecular volume intrinsically prompts a cohesive motion pattern between the upper and lower regions of the molecule, giving rise to local regions of conformational flexibility thought to be responsible for elastic deformation, while maintaining a defined tertiary shape with distinct functional segments for protein and cellular interactions. Despite the intrinsic flexibility of tropoelastin as a supposedly disordered elastomeric protein,[21] local changes to its native shape, manifested by a perturbed hinge region in human tropoelastin, can have a substantial impact on function, including its assembly into larger-scale structures.[13]

This presents a structural paradox: tropoelastin is flexible, yet it maintains a defined solution shape to fulfil its competing structural requirements for both elasticity and assembly. These dual competing requirements are unusual for studied proteins, so we have begun the process of identifying new principles for this class of elastic protein, by reconciling the dual requirements for structural flexibility for elasticity and structural order for regional cooperative demands to facilitate efficient protein self-assembly into elastic fibres.[13] Elastic tissue assembly is poorly understood, yet provides the opportunity to elucidate the key molecular interactions and temporal sequence, because tropoelastin is predominantly a self-assembling system. To emphasize the extraordinary versatility of this protein monomer, tropoelastin is encoded by a single gene but is found in multiple structures and locations in all vertebrates except the cyclostomes.[22–24]

Tropoelastin Assembly

Elastin formation occurs in a stepwise process involving tropoelastin association, massive molecular deposition, and crosslinking.[23] In contrast to the complex assembly of collagen type I, which relies on multiple proteolytic processing steps of the associating heterotrimer units,[25] tropoelastin is more amenable to study because its unprocessed monomers assemble on the path to elastin.[26] Multiple laboratories[27,28,29] have elegantly explored how microfibrils contribute to the distribution of elastin in vivo and that low-abundance molecules such as fibulins are needed for elastic fibre organization in vivo.[30][31] Over the past few years, we and others have found that, in vitro, these molecules are not needed for the spatial and temporal elastin assembly of tropoelastin.[9,15,20,26,32,33] This may be due to the fact that elastin is over nine times more abundant than microfibrillar components, and therefore tropoelastin–tropoelastin interactions dominate.[34]

The observed tropoelastin and aggregate structures are similar to those found in natural tissues, as evidenced by electron microscopy, which supports the concept of an aggregation unit corresponding to tropoelastin assemblies.[35,36] Unlike other fibrous proteins such as collagen, there are no good X-ray images for elastin due to anisotropic protein distribution needed for multidirectional elasticity. These data and the order of molecular assembly, combined with the similarity of the physical properties of crosslinked tropoelastin with elastin, indicate other elastic tissue components are not needed for templating to a native arrangement and so allow sufficient strain within a crosslinked network.[37]

By exploring the functional roles of domains by systematically introducing mutations at selected sites across tropoelastin, in concert with SAXS and allied methods including SANS, NMR, and molecular dynamics on these constructs, we have solved the solution structures for the normal and a panel of mutant forms of tropoelastin.[9,10,20,26] In each of the mutant cases, there is altered assembly. This has facilitated mapping of these effects onto specific parts of the molecule, together with comparison of the assembly performance of wild-type and mutant forms in vitro and in concert with elastogenic cells. This approach has helped to define the core rules of assembly between tropoelastin molecules and in models of elastogenesis. These approaches have led to a testable model of assembly that proceeds sequentially: it starts with a temporally coordinated head-to-tail assembly with propagated polymerization,[9,10,20] then in concert with the side-by-side association of tandem multimers to dimensionally expand into complex architectures that encompass fibres, sheets, and tubes.

Concluding Comments

Engineered tropoelastin constructs interact with cells through the elastin receptor[38] and integrins $\alpha v\beta 3$[39] and $\alpha v\beta 3$,[40] which makes them attractive for tissue augmentation and repair. Furthermore, tropoelastin reduces blood clot formation[41] which is a useful feature for vascular design but this feature's functional role has not been assigned to specific part(s) of the molecule.[42] The benefit of low thrombogenicity appears to be concentrated in a region from the N-terminus to domain 10 whereas endothelialization appears to be conferred by two autonomously functional regions: the central[43] and the C-terminus of tropoelastin.[44] Given the transient nature of tropoelastin in vivo before crosslinking into elastin,[27,45] these effects are presumably a feature of elastin or subregions within elastin. Tropoelastin elicits potent tropoelastin-promoted neoangiogenesis in mice and sheep and enhanced wound closure through accelerated epithelialization[46] but it is not yet known which part(s) of tropoelastin contribute(s) this effect, although endothelial cells recognize elastin by upregulating nitric oxide

production by endothelial nitric oxide synthase through a phosphatidylinositol-4,5-bisphosphate 3-kinase pathway.[47] Research into this is worth pursuing, given the slow repair rates of current tissue,[48] because accelerating the appearance of blood vessels, neodermis, and epithelialization can deliver enhanced skin repair.[49]

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