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The role of decorin in collagen fibrillogenesis and skin homeostasis

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Decorin, a prototype member of the growing family of the small leucine-rich proteoglycans (SLRP's), plays significant roles in tissue development and assembly, as well as playing both direct and indirect signaling roles. This review will concentrate on decorin's function in collagen fibrillogenesis as determined through the study of mice with a disrupted decorin gene. The fragile skin and abnormal tendon phenotypes initially observed were found to be due to fundamental alterations in collagen fibers, highlighting the crucial role of proteoglycans in general and SLRP's in particular in collagen fibrillogenesis. The altered fibril formation within tissues in turn leads to observable and quantifiable changes at the organismal level. Research into certain fibrotic processes with concomitant upregulation or reduction of decorin makes interesting comparisons with the collagen malformations seen in *Dcn*$^{-/-}$ mice. Overall, decorin is shown to be a vital player in maintaining skin and tendon integrity at the molecular level, among other functions.
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Abbreviations: **SLRP, small leucine-rich proteoglycan; TGF, transforming growth factor.**

Introduction

> *For the want of a nail, the shoe was lost,*
> *for the want of a shoe, the horse was lost,*
> *for the want of a horse, the rider was lost,*
> *for the want of a rider, the battle was lost,*
> *for the want of a battle, the war was lost,*
> *for the want of a war, the kingdom was lost,*
> *and all for the want of a nail.*
>
> —Traditional

The decorin knockout ($Dcn^{-/-}$) mouse was the first example of a proteoglycan knockout animal and brought to light many fundamental observations about the role of decorin in maintaining skin integrity and collagen structure [1]. In this brief review, we highlight the major findings observed in the $Dcn^{-/-}$ mouse model, specifically those changes related to altered collagen fibrillogenesis in skin and tendon structure, and the roles played by both the decorin core protein and the dermatan sulfate side chain. Since the creation of the $Dcn^{-/-}$ mouse and the essential role decorin was proven to possess because of the model, several single and double knockout models have been created to further elucidate just how important proteoglycans are in maintaining structural integrity of skin, bone, and other connective tissues. From 1997 to today, research into proteoglycan knockout mouse models has done much to teach us about the many hats proteoglycans wear in the body—the roles they play in development, cell organization, proliferation, signaling, in addition to their myriad structural roles in membranes and tissues. A great deal of important work is being done all over the world to elucidate the details of proteoglycan function—we are already well into our journey down that path and may even have trouble looking back over our shoulders to spot where our journey to *in vivo* function began. The first step on that road to proteoglycan function through knockout models, however, was the $Dcn^{-/-}$ mouse.

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Decorin structure and function

Decorin is a member of the small leucine-rich proteoglycan (SLRP) family, a group of secreted proteins that includes biglycan, fibromodulin, lumican, and keratocan, among others [2–4]. SLRP's play major roles in collagen fibrillogenesis, growth factor modulation, and direct regulation of cellular growth [5–8].

Decorin is composed of three domains—an N-terminal region which possesses a single chondoitin/dermatan sulfate side chain and a distinct pattern of Cys residues (CX_3CXCX_6C), a central region composed of ten leucine-rich repeats which are believed to be the prime sites of interaction with other proteins, and another Cys-rich C-terminal region. The leucine-rich repeat consensus sequence (LX_2LXLX_2NXL) is well-conserved across species and is contained in several proteins of varying functions [3,9]. Known structures of leucine-rich repeat proteins are comprised of repeating parallel beta strands and more or less helical regions that form arch-shaped solenoid-like structures [10]. The concave surface formed by the beta strands is thought to form the primary surface for protein/protein interactions. Modeling of human decorin based on the porcine ribonuclease inhibitor structure [11] showed that a single collagen triple helix could conceivably fit within the area formed by the arch-shape of decorin (Figure 1A–C). The model is supported by rotary shadowing electron microscopic studies on recombinant decorin molecules [12], which have revealed an arch-shaped structure. The overall dimensions are ~7 nm (the distance between the two arms) × ~5 nm (the distance between the base of the arch and the apex), in agreement with other data obtained with SLRP's isolated from tissue [13]. Positioning of collagen in the model is consistent with other evidence that the central leucine-rich repeats comprise the high-affinity collagen-binding site [14–17]. Although the decorin's location on the collagen fibrils is still controversial [18], we believe that the most compelling evidence, including rotary shadowing electron microscopy and photoaffinity labeling, indicates that decorin likely maps to a narrow region near the C-terminus of collagen type I [12], very close to one of its major intermolecular cross-linking sites. Thus, absence of decorin (see below) might affect not only fibril formation but also fibril stability within tissues.

Recent evidence has dissected decorin's interaction with the epidermal growth factor receptor, narrowing the minimal interacting region on decorin to the inner face of a central leucine-rich repeat [19], further reinforcing the argument that the broad concave faces of these proteins allow for the myriad of protein interactions. Decorin binds to multiple collagen types, including types I [20], II [21], III [22], and VI [23], as well as transforming growth factor-β (TGF-β) [24,25], and other proteins.

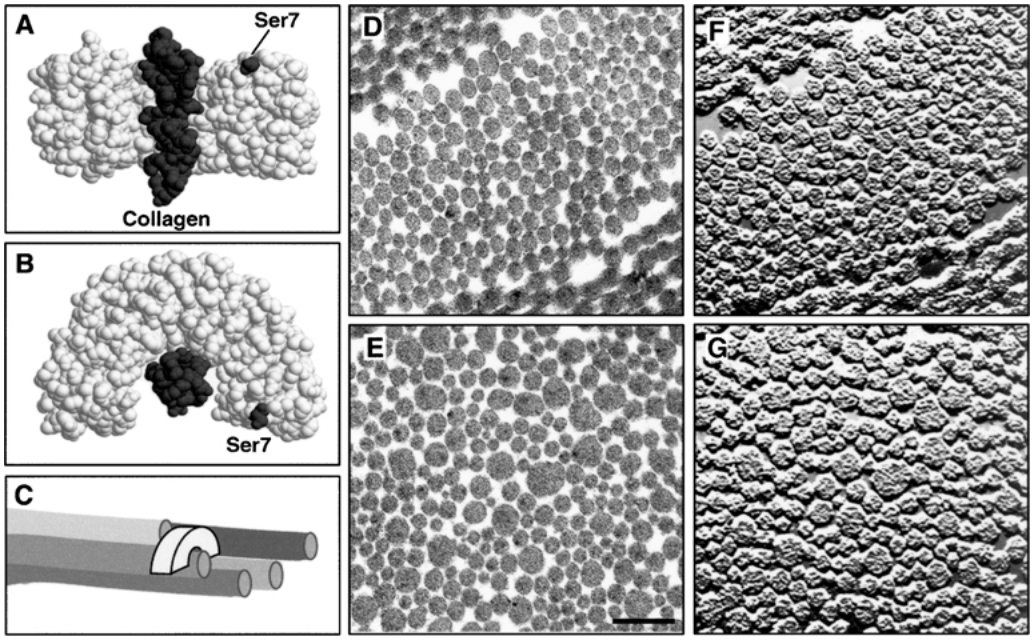

Figure 1. Decorin and its relation to collagen fibril formation. (A and B) Model structure of decorin [11] with bound collagen triple helix from edge-on (A) and top (B) views. The arch of the decorin model (white) possesses sufficient width to bind a collagen triple helix (shown in dark gray). The arch shape formed by the central leucine-rich repeats is similar to that of other leucine-rich repeat proteins whose structures have been solved [10]. Ser 7 is the site of the dermatan sulfate attachment. (C) Proposed relative binding of decorin to collagen near the C-terminus of a forming fibril. Decorin is believed to bind near the ends of individual helices and to regulate lateral fusion [12], as well as to maintain uniform interfibrillar spacing via its dermatan sulfate side chain [5]. (D) Electron micrograph of normal dermal collagen from mouse skin. Note the regularity of fibrillar spacing and the uniformity in fibril size. (E) *Dcn*$^{-/-}$ collagen exhibits none of the uniformity of the wild-type counterpart and additionally is poorly packed. Unusual fibril sizes and irregular shapes are caused by uncontrolled lateral fusion of fibrils, and lack of regular spacing could likely be the result of loss of dermatan sulfate on the collagen surface. Abnormal or reduced collagen cross-linking might also play a role in generating these abnormal structures [12]. (F and G) These two panels are the same images shown in panels D and E, respectively, following modification with Adobe Photoshop 6.0 to improve visualization of the fibril contours. Bar = 500 nm.

It has been shown repeatedly to cause growth inhibition of a variety of cell types [26–30], and to inhibit tumor cell-mediated angiogenesis [31]. Moreover, decorin has been successfully utilized to treat human tumor xenografts in a gene transfer model [32]. However, in normal cells, decorin can enhance cell survival by affecting diverse signaling pathways that still remain to be fully elucidated [33–35], and can modulate the cellular response to growth factors [36]. This review will concentrate on decorin's role in collagen fibrillogenesis in general, and skin and tendon structure, in particular.

The $Dcn^{-/-}$ mouse exhibits irregular collagen fibril formation in skin

The decorin knockout mouse model provided strong genetic evidence for the key role of this proteoglycan in collagen fibrillogenesis [1]. The $Dcn^{-/-}$ mice were healthy and completely viable. They showed no gross anatomical abnormalities, were of normal size compared to their $Dcn^{+/+}$ and $Dcn^{+/-}$ littermates, and had no skeletal anomalies or deficiencies. No significant alterations in either murine biglycan or lumican (other SLRP's), as determined by Northern blotting, were detected in the decorin knockout. The first and most salient feature observed in $Dcn^{-/-}$ mice was an unusually lax and fragile skin with dermal thinning visible upon microscopic examination. Further study of skin sections from $Dcn^{-/-}$ mice revealed loose connective tissue in the dermal and hypodermal layers of the skin. Of note is that heterozygous knockouts did not show any skin fragility or dermal thinning—both alleles had to be knocked out for the fragile phenotype to manifest, indicating at least a partial compensation by the single active decorin gene. Decorin levels in heterozygotes were found, as might be expected, to be slightly reduced, although no abnormal phenotype was associated with heterozygotes.

Transmission electron micrographs of dermal collagen from $Dcn^{-/-}$ and $Dcn^{+/+}$ mice show striking differences. Mice lacking decorin exhibited collagen fibrils of highly irregular diameter and abnormal fibrillar organization (Figure 1E and G) as compared to the wild type (Figure 1D and F). Perhaps the single most obvious change observed in collagen structure in the skin of the $Dcn^{-/-}$ mice is the irregularity of fibril diameter, thought to be caused by uncontrolled lateral fusion of thin and thick fibrils. While the mean fibril diameter was similar in $Dcn^{+/+}$ and $Dcn^{-/-}$ mice (116 nm and 119 nm, respectively), the range of observed sizes varied greatly in the knockout model. Maximum diameter of knockout collagen fibrils in skin was almost 150% greater than that observed in normal littermates. The packing of the fibrils showed a loose and irregular distribution, quite different from standard collagen structures. Other serious defects in fibril structure included irregular, bulging outlines likely due to the uncontrolled lateral fusion and scalloped edges, while $Dcn^{+/+}$ skin had fibrils that packed in a tight and uniform distribution and showed none of the defects present in the knockouts. A recent paper on collagen fibrillogenesis hallmarked the importance of proteoglycans in fibril fusion control [37]. Early collagen fibrils were found to have increased levels of proteoglycans on fibril shafts, but lesser amounts near the tips. This is consistent with a moderating effect of proteoglycans on fibril fusion in a controlled, tip-to-tip manner [37]. It is easy to envision a scenario where reduced proteoglycan levels along fibrils result in abnormal fusion events, especially if the reduction and/or absence occurred over an organism's entire development. Lack of decorin's dermatan sulfate side chains would also result in subtle changes in hydration of the environment immediately around the forming fibrils. While other SLRP's are still present in the $Dcn^{-/-}$ model, no rescue or compensation has been observed to occur in skin, and this change could, over time, be a causing factor in the profoundly altered collagen structure of the decorin knockout.

The decorin knockout mouse model showed a significantly reduced skin tensile strength, that is, the ability of skin to resist stretching [1]. Freshly isolated portions of dorsal skin from $Dcn^{+/+}$ and $Dcn^{-/-}$ animals were subjected to constant slow loading until failure. Mean failure loads of 7 N $\pm$ 2 were observed for $Dcn^{-/-}$ mice, and 21 N $\pm$ 5 for skin from $Dcn^{+/+}$ mice. Lack of decorin resulted in skin with only one-third of the tensile strength of normal murine skin. Clearly, the abnormal collagen fibrils in the $Dcn^{-/-}$ mouse result in a greatly weakened skin architecture. In accordance with the histological findings, skin from heterozygous mice behaved similarly to the $Dcn^{+/+}$ mice. Skin ductility, the ability to deform under tension, was also decreased in $Dcn^{-/-}$ mice by 35–40%. The ability to stretch and deform skin without breaking is important in medicine for closure of large skin wounds. Studies on porcine skin have shown that collagen fibers align in response to stretching in wound closure models [38]. $Dcn^{-/-}$ mice displayed both decreased ductility and tensile strength in response to skin stretching. Failure of collagen fibers to align properly could reduce inherent skin strength and ability to deform. While abnormal collagen fibril packing and lateral fusion could readily account for reduced deformability, it is also possible that the overall reduced proteoglycan content of the fibrils could play a role in decreased skin extensibility.

Interestingly, the biglycan knockout ($Bgn^{-/-}$) mouse, unlike the $Dcn^{-/-}$ mouse, showed no gross skin abnormalities but rather a reduction in bone density [39]. Thus despite high sequence identity and somewhat similar patterns of localization, decorin and biglycan are not interchangeable in function and do not have the ability to rescue each other's knockout phenotypes. Notably, $Bgn^{-/-}$ and $Dcn^{-/-}$ double knockout animals (described in detail by Young and co-workers in this issue) revealed that the effects of both gene deficiencies were additive in the dermis and synergistic in bone [40]. The lack of both genes caused a phenotype with severe skin fragility and osteopenia, resembling a rare progeroid variant of Ehlers-Danlos syndrome.

Certain abnormal fibrotic processes highlight functional differences between decorin and biglycan. Studies on keloid formation and growth have shown an upregulation of both type I

collagen and biglycan, but not decorin [41]. Lack of decorin upregulation might, however, be expected in keloid tissue. Upregulated decorin expression lends itself to smaller more uniform collagen fibril formation with limited cross-linking and fusion, while keloid tissue collagen is dense and disorganized. In addition to biglycan, lumican (a corneal proteoglycan) is expressed in skin as well. Thus, much like biglycan, lumican appears to be unable to rescue the decorin knockout phenotype, despite co-expression of both SLRP's in skin. The lumican knockout mouse [42] displays patterns of collagen fibrillogenesis and a phenotype reminiscent of the decorin knockout, including similar distribution of abnormal fibril size, and reduced skin tensile strength. Lumican's unique importance for corneal collagen structure is, however, underscored by the fact that $Lum^{-/-}$ mice display corneal opacity [42], an anomaly not observed in $Dcn^{-/-}$ mice.

Alterations in tendon structure caused by decorin knockout

Collagen fibrils from sections of tail tendon showed alterations more severe and pronounced than those in the skin. The $Dcn^{-/-}$ fibrils exhibited highly irregular outlines and tremendous variability in fibril size, with very large fibrils (660 nm), about three times the diameter of the average $Dcn^{+/+}$ tendon fibrils. The fibril organization of the knockout tendon showed poor packing and irregular distribution of thick and thin fibrils as compared to normal tendon. The additional presence of very thin fibrils (40–60 nm), observed less frequently in normal tendon, also differentiated $Dcn^{-/-}$ tendon from wild type. Mass mapping of the abnormal fibrils showed they were not uniform along their diameter, but varied in thickness along their length, indicative of lateral fusion of short fibrils with longer ones.

Patterns of proteoglycan staining on tendons have been shown to be unchanged in response to stress, raising the possibility that proteoglycans participate in transmission of force throughout the collagen network [43]. Such evidence is also consistent with another idea—a series of transient glycosaminoglycan interactions forming and breaking in a ratchet-type motion along parallel collagen fibers in response to stress [43]. This type of interaction could, at least partially, account for normal skin's ductility compared to $Dcn^{-/-}$ skin. Abnormal packing of fibers, as seen in the $Dcn^{-/-}$ model, reduces the potential for this type of contact, as would a lack of longer dermatan sulfate glycosaminoglycans along the collagen fibers. Transmission electron micrographs of longitudinal collagen fibers followed by cuprolinic blue staining showed reduced proteoglycan granules in the collagen from tendon. Less intense proteoglycan staining demonstrates that lack of decorin does indeed reduce, but not eliminate, a proteoglycan presence on fibers. The observed proteoglycan granules on tendon are likely due to keratan sulfate-contaning proteoglycans (fibromodulin and/or lumican), which appear to bind in similar locations as decorin, but lack the length of decorin dermatan sulfate glycosaminoglycan.

Manipulation of decorin levels within organized tissues and its anti-fibrotic effects

The complete absence of decorin in $Dcn^{-/-}$ mice causes abnormal collagen fibrils and overall collagen weakness similar to some connective tissue disease states. Several studies are probing how careful control of decorin expression at critical times and at specific sites in organisms can affect collagen fibril formation from an *in vivo* tissue engineering viewpoint. In a fibrous adhesion model, the addition of exogenous decorin directly at the site of adhesion formation was shown to reduce adhesion severity and to limit fibrosis [44]. Fibrotic adhesion tissue in decorin-treated animals was found to be thinner and have fewer fibers compared to controls. Decorin's protective ability in this study was found to be partly, but not completely, halted by administration of additional exogenous TGF-β, providing evidence for both TGF-β-dependent and -independent mechanisms of decorin action. Decorin, by inhibiting TGF-β activity, likely lowered collagen production in the induced fibrous adhesion, and perhaps, also played a direct role in modulating fibril formation. The anti-fibrotic properties of decorin, mediated by its ability to block or attenuate the action of TGF-β have been successfully exploited in the treatment of experimental glomerunophritis [45], and pulmonary fibrosis [46]. Thus, $Dcn^{-/-}$ mice would provide an ideal background in which to conduct further studies. Lacking decorin, TGF-β stimulation should be greater and result in the generation of fibrotic adhesions that, if left untreated, could likely be more severe than in a $Dcn^{+/+}$ background.

On the opposite side of the coin, decorin antisense DNA delivered to the site of a wounded ligament was shown to actually improve collagen fibrillogenesis and result in collagen fibers of larger diameter, closer in appearance and strength to unwounded ligament [47]. While decorin expression was not completely ablated by antisense therapy, reduction of endogenous levels locally augmented collagen fibril size in the healing ligament. Complete absence of decorin has been shown in the $Dcn^{-/-}$ mouse to result in severely abnormal collagen fibrils with many larger than normal diameters while heterozygous animals display normal collagen morphology. Therefore, it is reasonable to hypothesize that reducing decorin levels below a critical threshold could result in enlargement of collagen fibrils and allow some actual control over fibrotic processes *in vivo*.

Decorin as a mediator of fibril fusion

Recent work has demonstrated that decorin is capable of actually causing increased diameter of collagen fibrils in an *in vitro* fibrillogenesis assay [48]. This is in apparent conflict with earlier work that showed a decrease in fibril diameter in the presence of decorin [49]. The different methodologies used in the *in vitro* assays, however, underscore the ability of the local environment and conditions to change what is observed. If looked at closely, though, both results have relevance with what we see in the $Dcn^{-/-}$ mice. Decorin appears to act not as an

indiscriminate *inhibitor* of fibrillogenesis, but as a vital *mediator* of the process. Decorin may perhaps slow down lateral fibril fusion, which could, under certain conditions, result in uniformly thinner fibrils (perhaps conditions which greatly favor rapid fibril formation over decorin's ability to moderate growth and/or conditions where imperfect protofibrils are formed). It is equally possible to envision a scenario where, under correct conditions, decorin binds to and slows collagen fibril association, allowing time for optimal interactions to occur and resulting not in gigantic fibers but in fibers of a structurally idealized size (for the local conditions). While this may appear at first glance to be a description of inhibition, it is not—an inhibitor does not cause a native substrate or ligand to bind or interact in an optimal way. Just as it is possible with the correct buffer to either quickly precipitate a protein as an amorphous disorganized mass or, with a subtle alteration of buffer conditions, to initiate the growth of a well ordered and organized protein crystal, decorin may, by binding to collagen, force an ordering on the process of fusion that results in more optimal fibers for the prevailing conditions. Rate of fiber fusion, related to the concentration of collagen, pH, salts, temperature, and other factors, would all come into play to affect the final fiber size. While it is difficult or impossible to say what optimal fiber size would be with the *in vitro* systems, the $Dcn^{-/-}$ mouse demonstrates how decorin's absence can distort collagen fibrillogenesis under native conditions both towards large and small fiber diameters by altering the normal proteoglycan-mediated process of fibril fusion and growth. More rapid condensation of fibers, a possible consequence of decorin's absence *in vivo*, could result in large fibers which grow unrestrained existing side-by-side with smaller fibers which fail to grow due to improper fusion events. A brick wall built too quickly and with crooked bricks will not be a tall or strong wall; a poorly built network of collagen fibers will lack integrity in the same manner.

Other alterations in $Dcn^{-/-}$ mice

Decorin is expressed, to a greater or lesser extent, in connective tissues throughout the body, and the $Dcn^{-/-}$ mouse has been used to examine decorin's functions beyond collagen fibrillogenesis, including the parts decorin plays in several disease states. Human periodontal fibroblasts *in vitro* have been observed to secrete large amounts of decorin [50], additionally, decorin is observed in periodontal connective tissues *in vivo* [51–55]. Decorin levels have been found to decrease in periodontal tissues being broken down by inflammatory disease [52]. This raises the possibility that lower levels of decorin expression could be permissive or even encourage disease states in periodontal tissues. Careful light and ultrastructural analysis on periodontal ligaments from $Dcn^{+/+}$ and $Dcn^{-/-}$ mice showed heterogenous collagen fibrils of both large and small diameter that were irregularly packed [56]. There was also noted an increased number of fibroblasts in the $Dcn^{-/-}$ mouse periodontal ligament. The enhanced fibroblast density likely occurred due to a lack of decorin's growth inhibitory activity. The effect could arise from either direct lack of negative feedback or in response to other signals that indicate low/nonexistent decorin levels. Further studies failed to show any compensatory upregulation of biglycan in $Dcn^{-/-}$ mice in the periodontal ligament, providing evidence that the ligament is not "rescued" by increased secretion of other related proteoglycans. This study [56] has demonstrated decorin's ability to retard fibroblast growth *in vitro*, lending credence to observed increased *in vivo* proliferation because of a lack of inhibitory decorin signaling.

Decorin has also been shown to be an important mediator in kidney fibrotic disease processes. In a model of tubulointerstitial kidney damage following blockage, $Dcn^{-/-}$ animals displayed increased collagen degradation, resulting in more severe kidney atrophy [57]. Absence of decorin also appeared to result in increased apoptosis in the kidney disease model, providing strong evidence for a protective role for decorin in kidney damage due to tubulo-interstitial fibrosis by both TGF-β-dependent and -independent mechanisms [57]. The decorin knockout mouse model has also been used to study tumorigenesis and tumor permissivity. While decorin has been shown to be an effective antitumorigenic agent [27–29,31,32], loss of decorin alone is not sufficient for spontaneous tumorigenesis in the $Dcn^{-/-}$ mouse [58]. Studies of a double knockout of decorin and p53 showed that $Dcn^{-/-}\,p53^{-/-}$ mice exhibit increased spontaneous tumorigenesis, most notably thymic lymphoma. $Dcn^{+/-}\,p53^{-/-}$ mice displayed similar tumors, but had survival rates similar to $Dcn^{+/+}\,p53^{-/-}$, indicating that partial expression of decorin was sufficient to reduce severity of the phenotype. The loss of decorin appeared to be permissive for certain tumors, but was not sufficient on its own to induce actual tumor growth.

Of particular interest, $Dcn^{-/-}$ mice have been shown to be somewhat resistant to infection by *Borrelia burgdorferi*, the bacterium that causes Lyme disease [59]. Decorin is the target of a bacterial adhesin [60], and the absence of decorin in the mouse model resulted in lower rates of infection in various tissues when challenged with *B. burgdorferi*.

The road ahead

> *To work—to work! It is such infinite delight to know that we still have the best things to do.*
>
> —Katherine Mansfield

In a short time we have learned much, but there is much more to do. As we learn more about collagen fibrillogenesis in general, the story of decorin and the consequences of its absence in the $Dcn^{-/-}$ mouse will be made all the richer. A better understanding of the biological processes in which decorin is involved will, with time, give rise to new therapeutic modalities for a variety of connective tissue and fibrotic disorders. The phenotype of the $Dcn^{-/-}$ mouse lends itself to many future double knockout studies. Creating double knockouts of decorin and other proteoglycans, together with the generation of tissue

specific and conditional knockouts, will make it possible to further understand the molecular mechanisms through which decorin operates, especially in maintaining the integrity of skin and other connective tissues. In addition, this mouse can serve as a decorin null background for various signaling and tumorigenesis studies involving treatment with decorin. Additionally, fibrotic processes involving decorin have an ideal model system in the $Dcn^{-/-}$ mouse. Future therapies and improved treatment options for wound healing, tendon and ligament repair, and other conditions involving collagen deposition, control, or repair will depend on these models we as a community create and study.

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