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Molecular composition and function of integrin-based collagen glues—Introducing COLINBRIs ☆

Cédric Zeltz [a], Joseph Orgel [b], Donald Gullberg [a,*]

[a] Department of Biomedicine and Centre for Cancer Biomarkers, Norwegian Centre of Excellence, University of Bergen, Jonas Lies vei 91, N-5009 Bergen, Norway
[b] Departments of Biology, Physics and Biomedical Engineering, Pritzker Institute of Biomedical Science and Engineering, Illinois Institute of Technology, 3440 S. Dearborn Ave, Chicago, IL 60616, USA

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ABSTRACT

Background: Despite detailed knowledge about the structure and signaling properties of individual collagen receptors, much remains to be learned about how these receptors participate in linking cells to fibrillar collagen matrices in tissues. In addition to collagen-binding integrins, a group of proteins with affinity both for fibrillar collagens and integrins link these two protein families together. We have introduced the name *COLINBRI* (*COL*lagen *IN*tegrin *BRI*dging) for this set of molecules. Whereas collagens are the major building blocks in tissues and defects in these structural proteins have severe consequences for tissue integrity, the mild phenotypes of the integrin type of collagen receptors have raised questions about their importance in tissue biology and pathology.
Scope of review: We will discuss the two types of cell linkages to fibrillar collagen (direct- versus indirect COLINBRI-mediated) and discuss how the parallel existence of direct and indirect linkages to collagens may ensure tissue integrity.
Major conclusions: The observed mild phenotypes of mice deficient in collagen-binding integrins and the relatively restricted availability of integrin-binding sequences in mature fibrillar collagen matrices support the existence of indirect collagen-binding mechanisms in parallel with direct collagen binding *in vivo*.
General significance: A continued focus on understanding the molecular details of cell adhesion mechanisms to collagens will be important and will benefit our understanding of diseases like tissue- and tumor fibrosis where collagen dynamics are disturbed. This article is part of a Special Issue entitled Matrix-mediated cell behaviour and properties.

1. Introduction

The view of the extracellular matrix (ECM) has changed dramatically in the past three decades. From being regarded as merely a structural framework where cell–cell contacts were thought to be the main organizers of functional organ units, the ECM is taking more and more of a centre stage in modern cell biology. We know today that the ECM in addition to functioning as a structural framework has many other roles including: acting as storage depots for growth factors and cytokines [1,2], regulating stem cell fate [3], elaborating stem cell niches [4] and thus affecting many aspects of a cell's life. Still, a major role of the ECM is to act as tissue glue. On the anecdotal side, the word "collagen" itself derives from the Greek κόλλα/*kolla*, "glue", from the practice of boiling animal skins in ancient times to make glue. In the current review we will mainly discuss collagens as molecular glue when present in the body as native collagen fibrils.

Collagens are the most abundant proteins in vertebrate organisms. Although composed of 28 different proteins [5], a few family members (i.e. collagens I–III) of the fibrillar collagens (collagens I, II, III, V, XI, XXIV and XXVII) [6] dominate quantitatively and form the framework of connective tissues in the bone, cartilage, tendons and soft interstitial matrices.

The starting point for the changed view of the matrix (not just a structural component but a dynamic regulatory compartment in tissues) was the identification of matrix receptors [7,8]. In recent years, our knowledge of how these receptors signal and cross-talk with other receptor groups has increased. A major group of receptors for collagens are found in the integrin family [7,9,10]. In addition to collagen-binding integrins, syndecans, discoidin domain receptors (DDRs), GpVI, LAIR [9], OSCAR [11] and GPR56 [12] have been implied to be collagen receptors. Interestingly, recent data suggest that DDRs can act by activating collagen-binding integrins [13–15], while blocking DDR–collagen interaction leads to reduced integrin-mediated cell adhesion.

Most of the detailed knowledge about how integrins and other receptors work has been collected from experiments performed with

Abbreviations: DDR, discoidin domain receptor; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; FN, fibronectin; LOX, lysyl oxidase; M4/5, monomer 4/5; MMP, matrix metalloproteinase; PN, periostin; SLRP, small leucine-rich proteoglycan/protein; TSP, thrombospondin; VN, vitronectin; vWF, von Willebrand factor

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* Corresponding author. Tel.: +47 55586332.
E-mail address: donald.gullberg@biomed.uib.no (D. Gullberg).

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defined purified matrix proteins *in vitro*. Much less is known about the molecular composition of the actual linkages in the tissues; from the cell attachment points to the ECM based fibrils and networks (tissue glue function). We will discuss how fibrillar collagen is arranged in the tissues and how cells stick to fibrillar collagen matrices; direct binding via collagen receptors and indirect binding via bridging molecules. We use the name "*COLINBRI*" (*COL*lagen *IN*tegrin *BRI*dging) for a class of non-collagen molecules with the potential to bridge the binding of integrins to fibrillar collagens [16].

2. Collagen

Collagens come in many different forms and in the current review we will focus on the interaction of connective tissue cells with the major fibrillar collagens. Collagens have as a major function to serve as a structural scaffold in forming tissues—being the mold onto which tissues grow. In normal adult tissues, collagens fulfill the function of maintaining tissue structure, but during fibrosis, excess collagen synthesis may cause tissue dysfunction. Since fibrillar collagens are large molecules existing in complex environments, they also have the capacity to interact with secreted proteins in the microenvironment, which can impose a greater adhesivity to the matrix scaffold. This alloy of collagen – COLINBRIS – and cell receptors constitute the components of this molecular tissue glue.

The mechanical properties of this molecular glue can be changed either by crosslinking of collagen fibrils, or by crosslinking of secreted proteins onto the fibrillar collagen matrix, thereby changing the stiffness of the collagen-based matrix. Accordingly, this will change the ability of cells to respond to the collagen-based tissue glue. Lysyl oxidases (LOX) are important enzymes in this regard. Periostin (PN) is an interesting matricellular ECM molecule and COLINBRI has been suggested to change the stiffness of the collagen matrix [17,18]. Transglutaminases are another group of molecules with the potential to change the properties of matrix proteins, including collagens [19]. Furthermore, the composition of proteoglycan (PG) protein cores and their glycosaminoglycan chains affects matrix stiffness, as does age and diabetes associated non-specific crosslinking [20–22].

In cancer cells, the term tensional homeostasis has been introduced to describe the intense relationship between intracellular tension and extracellular tension, but this term can also be applied to normal cells in interstitial matrices, and we predict that in years to come, the importance of tissue stiffness for cell function will be increasingly recognized [23–25].

Since collagens are major building blocks, one would expect that a major function of receptors that directly link cells to collagen would be to maintain the structural integrity of the tissues. On the other hand, proteins bound to the collagen network might impose additional functions to the ECM that are not related to the structural integrity, and thus add functional flexibility. Intriguingly, analyses of mutant mice lacking integrin type collagen receptors have failed to replicate the severe effects that are seen when fibrillar collagens are mutated or absent in mice. We will later discuss the possible explanations for this anomaly and speculate on how our picture of collagen receptors might change in the future.

3. COLINBRIs and COLINBRI-mediated cell binding

We define COLINBRIs as molecules that form a bridge between fibrillar collagens and integrins on cell surfaces. In this review we will list and discuss some prototypical ECM proteins that could act as COLINBRIs, but we make no claims to report a complete list of COLINBRIs. We will thus focus on three relatively abundant, well-characterized proteins that have the greatest potential to serve as intermediary linkers in the indirect collagen–cell interactions: fibronectin, vitronectin and periostin. We will also briefly mention von Willebrand factor as a COLINBRI of special importance for platelets. A schematic illustration of the indirect and direct binding to collagen fibrils is shown in Fig. 1. The domain structure, the collagen-binding sites and the integrin-recognizing sites of three prototypical COLINBRIs are shown in Fig. 2.

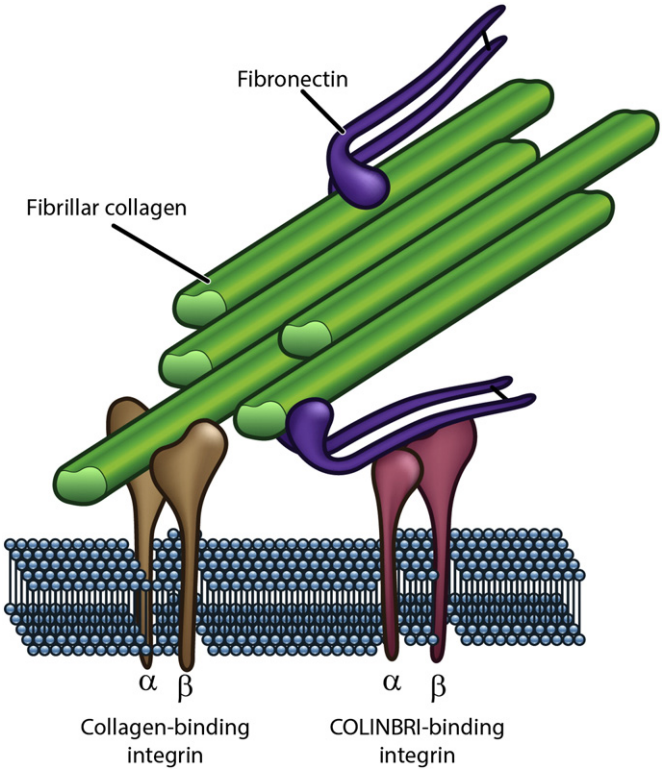

Fig. 1. Schematic illustration of direct (collagen-binding integrin-mediated) and indirect (COLINBRI-mediated) binding to collagen fibrils. In the direct cell-binding mechanism, collagen-binding integrins (brown) directly interact with the fibrillar collagen (green) through their αI domain to provide cell adhesion. In the indirect way, cell binding involves COLINBRIs like fibronectin (purple) represented here. The COLINBRI molecule is anchored to collagen and provides cell attachment by interaction with the COLINBRI-binding integrins (magenta), which are lacking αI domain.

3.1. Fibronectin

Fibronectin (FN) is a ubiquitous extracellular matrix glycoprotein that promotes cell adhesion, cell migration and cell differentiation [26]. Mouse embryos lacking FN die at E8.5, presenting a severe failure in vascular development [27]. FN harbors multiple adhesion sites for other molecules in the microenvironment including proteoglycans, TGF-β and collagens [28].

The collagen-binding domain was the first FN domain to be isolated [29]. The domain is located near the N-terminus and is composed of the repeats ^{6}FNI$^{1-2}$FNII$^{7-9}$FNI [30]. Blocking of the collagen-binding domain demonstrated a role of the FN–collagen interaction in collagen organization and deposition [31]. FN binds to both native and denatured collagen (gelatin), but it presents greater affinity for gelatin *in vitro* [32], suggesting that the FN–collagen interaction occurs with higher affinity in remodeling processes like wound repair and tissue growth. In collagen I the FN-binding site overlaps with the matrix metalloproteinase (MMP)-1 cleavage site, which has been reported to "breathe" and locally melt, which hence enables FN binding under some conditions [33].

So far, 11 integrin heterodimers, including all members of the αv subfamily, have been reported to interact with FN (for reviews, see [34,35]). The recognition of the Arg-Gly-Asp (RGD) motif located in the ^{10}FNIII domain [36] is central for cell adhesion although other sequences are also involved in this interaction [37–39]. The prototypical "FN receptor" – integrin α5β1 – the first integrin to be characterized [40], is widely expressed on different cell types. Integrin α5-deficient mice present some similarities with the FN-null mice [41], although

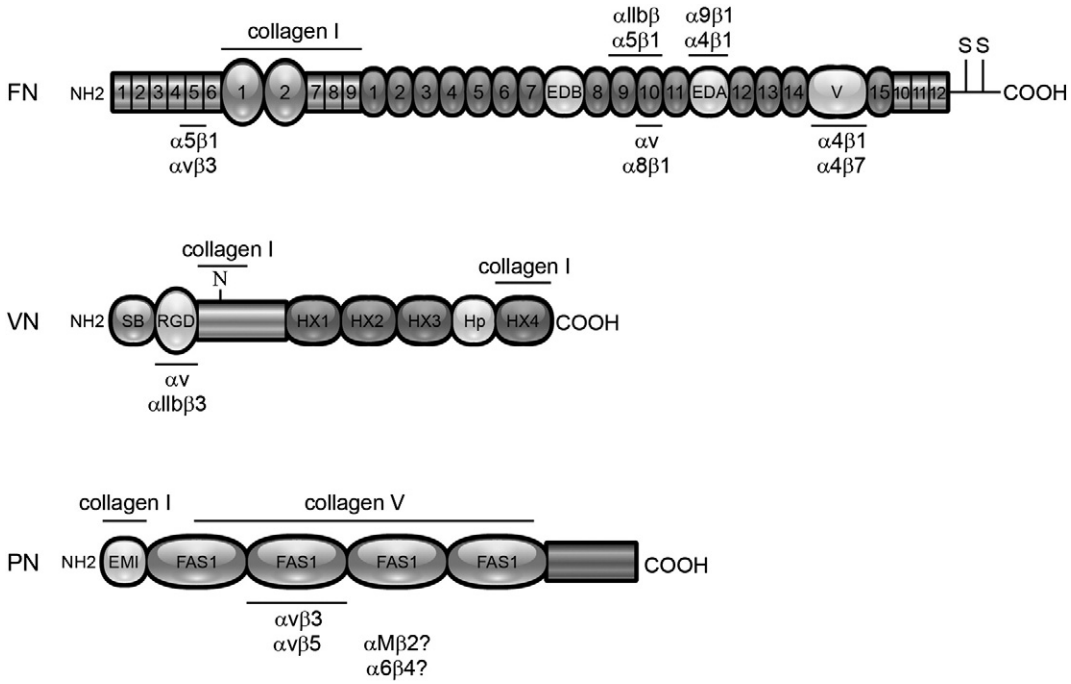

Fig. 2. Collagen- and integrin-binding sites on COLINBRIs. The domain structures of the three COLINBRIs fibronectin (FN), vitronectin (VN) and periostin (PN) are schematized. Fibronectin is composed of the $^{1-9}$I domain (light gray rectangles), the $^{1-2}$II domain (light gray ellipses) and the $^{1-15}$III domain (dark gray ellipses). The variable spliced domains are represented in white (EDA/B: extra domain A/B, V: variable). Fibronectin dimerizes through two disulfide bonds present at the C-terminal. Fibronectin interacts with collagen *via* the repeats ^{6}FNI$^{1-2}$FNII$^{7-9}$FNI. The RGD sequence is localized in the ^{10}FNIII domain, but other domains are also involved in integrin-mediated cell adhesion. The cell-binding site of vitronectin (RGD module) is located near the somatomedin B domain (SB). Collagen interaction with vitronectin can be modulated by its *N*-glycosylation (N). Another collagen-binding site is located in a Hemopexin repeat (HX) in the C-terminal, close to the heparin-binding site (Hp). Collagen I interacts with the EMI domain of periostin, whereas collagen V binds to the Fasciclin 1 domain (FAS1). The binding sites of integrins αvβ3 and αvβ5 are located in the second FAS1 domain whereas the cell-binding site(s) of α6β4 and αMβ2 integrins have not been characterized.

less severe. The α5β1 integrin, similarly to the platelet integrin αIIbβ3, binds to the RGD sequence of FN, but high affinity binding for both integrins are dependent on the synergy site PHSRN located to the ^{9}FNIII [39,42]. The integrins α4β1 and α4β7 interact with the variable domain of FN through LDV and REDV sequences [37,43]. The α4β1 and α9β1 integrins also both bind to the alternatively spliced extra domain A (EDA) in FN [44].

The presence of at least one of the alternatively spliced extra domains, EDA or EDB, is essential during development, since deletion of both regions leads to embryonic lethality [45]. In adult tissues, these FN splice variants are only expressed at low levels but are upregulated during wound healing, where the FN-EDA variant is important in the organization of the granulation tissue [46]. It has been suggested that FN-EDA is necessary for TGF-β-induced myofibroblast differentiation [47] and increased levels of FN-EDA have also been reported during tumor angiogenesis, epithelial-to-mesenchymal transition (EMT) and fibrosis [48–50].

FN receptor integrins show overlapping functions during embryogenesis and FN assembly [51]. Four FN-binding integrins, α4β1, α5β1, α9β1 and αvβ3, are known to participate in FN assembly. It is important to recognize that the effects of integrin blockage depend on the total integrin repertoire. In one study, embryonic cells depleted in either α5β1 or α4β1 displayed no defects in the FN matrix assembly; this function is presumably assumed by αv integrins in these conditions [52], although other studies have suggested αvβ3-mediated FN assembly to be less efficient [53]. In a detailed study on the role of FN receptors, α5-deficient endothelial cells showed reduced FN assembly, whereas double α5/αv-null endothelial cells failed to assemble a FN matrix altogether, suggesting a cooperation of both integrins in endothelial cell-mediated FN assembly [54]. In contrast to their overlapping functions in FN assembly, FN receptor integrins also have distinct roles as supported by the distinct phenotypes of FN receptor deficient mice [41,51].

The ability of FN to bind both fibrillar collagens and multiple RGD-dependent and RGD-independent integrins, expressed on a variety of cell types, qualifies FN as a major COLINBRI to have important functions in a variety of developmental and pathological processes.

3.2. Vitronectin

Vitronectin (VN) is an adhesive glycoprotein present in the blood, from where it was first identified as the "serum spreading factor" [55], but it is also present in the ECM [56] and in the α granules of blood platelets [57]. VN promotes cell adhesion and cell migration and is involved in fibrinolysis, immune defense and hemostasis [58,59]. Initial studies of VN-deficient mice revealed viable and fertile mice, which did not display any obvious phenotype compared with wild-type littermates [60]. Later studies attributed a role of VN in response to tissue injury, where VN-null mice displayed delayed wound healing and decreased angiogenesis [61].

VN is anchored to the ECM via its interaction with collagens and thus promotes cell adhesion and migration, defining this protein as COLINBRI. VN interacts with several collagen types, including fibrillar collagens, but unlike FN, VN shows greater affinity for native triple-helical collagen [62]. Convincing data have shown that VN can inhibit FN binding to collagen I, suggesting that these proteins both interact at a similar site, or sites, on collagen I. Conversely, the collagen-binding site of VN is located toward the amino terminus, near the cell-binding site [63]. However, a second binding site has been identified toward the carboxy terminus, near the heparin-binding domain of VN [64]. It is interesting to note that collagen binding of VN can be modulated by its glycosylation status [65,66]. Thus, it has been shown that the presence of *N*-glycans covalently linked on VN decreases its binding to collagen, whereas de-*N*-glycosylation of VN enhances collagen interaction. This suggests that modulating the VN–collagen interaction regulates VN-mediated cell adhesion and migration in the tissues.

Cell interaction with VN is, like FN, mediated *via* an RGD motif, which is mapped to the amino terminus of VN. Integrin αvβ3 was the

first identified cell receptor shown to bind VN and was initially defined as the "VN receptor", since it at first seemed to be specific to this protein [67]. In addition to αvβ3, integrins αvβ1, αIIbβ3, αvβ5, αvβ6, and α8β1 have been described to interact with VN [68–70]. VN can also induce cell migration and cell signaling, RGD-independently, through its interaction with the urokinase plasminogen activator receptor [71]. Furthermore, the plasminogen activator inhibitor-1 is known to bind to the somatomedin B domain of VN, modulating the RGD-dependent cell interaction [72,73]. Another regulation of the VN–cell interaction is mediated *via* the heparin-binding domain, and it has been suggested that oligomerization of VN in ECM through this domain can enhance fibroblast adhesion and spreading [74]. The main integrin VN receptor, αvβ3, is expressed on endothelial cells and plays an important role in vascular cell biology [75,76]. Since VN is not the only one ligand of αvβ3, the different biological roles of αvβ3 are not systematically linked to the VN functions. However, VN has also been suggested to have roles in angiogenesis [61], by regulating endothelial function [77] or by modulating MMP expressions [78].

Although VN-deficient mice display delayed wound healing and VN is enriched at the sites of injured tissues, the exact role of VN in tissue repair is not completely clear [61,79]. VN appears to be an important ECM substrate for epithelial and smooth muscle cell migration in response to injury, which involves αvβ3 and αvβ5 integrins [80,81].

3.3. Periostin

Initially called osteoblast-specific factor 2, this member of the fasciclin I family has been renamed periostin (PN) to reflect its high expression in periosteum and periodontal ligament [82]. PN is expressed in additional tissues including the bone, heart and skin [83–85]. The PN null mice show a variable penetrance of a postnatally lethal phenotype related to cardiovascular failure. Surviving PN-deficient mice display dwarfism, defective periodontal ligaments and an incisor eruption defect [86,87]. It is interesting to note that α11 integrin deficient mice [10] also have a periodontal ligament phenotype, although it is restricted to the incisors. However, direct interaction between α11β1 and PN has not been reported so far and the similar periodontal ligament phenotypes are presumably more related to their respective collagen interactions.

PN co-localizes with collagen *in vivo* and binds directly to collagens I and V *in vitro* [88,89]. The type I collagen seems to interact with the EMI domain of PN [18], whereas collagen V has been found to interact with the fasciclin-1 domains of PN [89]. Since PN-deficient mice showed altered collagen fibrils, a role of PN in fibrillogenesis has been suggested [88,90]. During this process, PN has been suggested to act indirectly in collagen crosslinking by increasing the activation of LOX via interaction with BMP-1 and FN [91] and to protect against MMP-mediated proteolysis [92]. Other data suggests that a dimeric form of PN could crosslink collagen fibrils [17].

PN is an adhesive protein that thus promotes cell adhesion and migration. The integrins αvβ3 and αvβ5 were the first candidate PN receptors to be described, promoting ovarian epithelial tumor cell motility [93]. The YH motif contained in βigH3, a protein structurally close to PN, was found to be the ligand for both αvβ3 and αvβ5 [94,95]. This motif located in the second FAS1 domain of PN was later demonstrated as a cell-binding site [96]. Integrins α6β4 and αMβ2 have also been described to bind to PN, however for these integrins the cell-binding sites have not been characterized [97,98].

It has become increasingly clear that PN is involved in tissue repair and remodeling [99,100]. In wound healing studies, PN-deficient mice exhibit delayed wound healing [101]. It appears that during wound repair, PN is expressed at day 7 in the granulation tissue and co-localizes with α-SMA-expressing cells [85]. Furthermore, PN null mice display reduced α-SMA expression in the granulation tissue, and fibroblasts isolated from these mice present a defect in collagen gel contraction, which can be rescued by adding recombinant PN. These data demonstrate that PN is involved in the regulation of myofibroblast differentiation [102]. It is also interesting to note that PN can be upregulated by TGF-β in dermal fibroblasts [103]. PN is expressed in the heart, and could contribute to cardiac development and repair and it has been shown to be upregulated after vascular injury [104–106]. However, the role of PN in angiogenesis seems to be restricted to pathological processes [107,108].

3.4. Von Willebrand factor

Von Willebrand factor (vWF) is a multimeric glycoprotein that plays a crucial role in hemostasis by promoting platelet adhesion at sites of vascular injury with high shear stress [109,110]. Collagen binding occurs via the vWF A1 and A3 domains and the platelet integrin αIIBβ3 can bind via the RGD sequence in vWF C1 domain [111], qualifying von Willebrand factor as a COLINBRI molecule for platelets. Deficiency or mutations of vWF lead to von Willebrand disease, a bleeding disorder [112].

3.5. Matricellular proteins

In addition to PN, which has been described as a matricellular protein, the matricellular proteins thrombospondins (TSP), SPARC and osteopontin (for review see [113]) have also the potential to act as COLINBRIs, since they all directly interact with fibrillar collagens and with integrins. However, TSP-1, -2 and SPARC are known to induce cellular "de-adhesion" characterized by the loss of focal adhesion and stress fibers [114], which would be in contrast with the molecular tissue glue concept.

3.6. SLRPs

The small leucine-rich proteoglycan/protein (SLRP) family is composed of 17 ECM proteins that include decorin, biglycan and lumican [115]. A major role of the SLRPs, determined using SLPR-knockout mice, is to regulate the collagen fibrillogenesis [116,117]. Recently, it has been shown that SLRPs can interact with different cell receptors to regulate cell behavior [118]. Decorin and lumican have been described to interact with the collagen integrin α2β1, but with low affinity compared to α2β1–collagen I interaction [119,120]. The fact that lumican increases cell adhesion [121] and strongly inhibits cell migration [122] could limit its role in some biological processes, but it could act as COLINBRI in the integrin-based collagen glues.

4. Collagen-binding integrins

Before collagen receptors were isolated, a widely held belief in the cell adhesion field claimed that cells did not interact directly with collagen, but *via* "nectins" [123,124]. From *in vitro* studies we now know that cells do interact directly with native collagens *via* the four collagen-binding integrins α1β1, α2β1, α10β1 and α11β1 [125]. We will in the next sections focus on the integrin family and will start our discussion by summarizing what is known about the ligand specificity of the integrin type collagen receptors α1β1, α2β1, α10β1 and α11β1 (Table 1).

4.1. Ligand specificity

Most of the cell adhesion studies have been performed with the most abundant fibrillar collagens I–III, whereas not so much is known about the newest members of the collagen family. To study direct physiological interactions with collagens, it is essential to ensure that the collagen used is triple helical and appreciate if the interaction is to be interpreted within the context of an intact fibril-bundle, fibril, damaged fibril structure with developing fibers or with collagen monomers. Interactions with these structures may or may not be analogous. For instance, a mature fibril is a structure that by design would be expected to be somewhat robust. Structural data has indicated that the fibril exterior

Table 1
Ligand specificity of collagen-binding integrins.

Integrin	Recognition of the fibrillar form of collagen	Collagen specificity	Recognition sequence specificity in monomeric collagen	References
α1β1	*No*	Collagen IV > collagens I, II, and III	GFOGER (in coll. I, II, IV and more) GVOGEA (in coll. II) GLOGEN (in coll. III)	[205–207]
		Collagen IX	?	
		Collagen XIII	?	
		Collagen XVIII	?	
α2β1	*Yes*	Collagen I > collagen IV	GFOGER (in coll. I, II, IV and more) GMOGER	[206,207] [130]
		Collagen IX Collagen XXIII	?	
α10β1	*No*	Collagen IV/VI > collagen II	GFOGER (in coll. I, II, IV and more)	[173,206,207]
		Collagen IX	?	
α11β1	*Yes*	Collagen I > collagen IV Collagen V	GFOGER (in coll. I, II, IV and more)	[153,207,208]
		Collagen IX	?	

is 'tough', resistant to proteolysis and cellular attachment [126,127]. With minor, directed proteolytic modification or through damage to the matrix, sites of increasingly direct and strong cell interaction can be exposed by 'pealing' away layers of the collagen hierarchical structure (Figs. 3–5). It has also been suggested that bending of the microfibril exposes cell-binding sites [128].

In vitro studies have most often been performed using the monomeric triple-helical form of the major fibrillar collagens I–III. Using libraries of overlapping collagen peptides (collagen toolkits), the GFOGER sequence has been identified as a major cell-binding site for collagen-binding integrins [129,130]. Studies using the fibrillar form of collagens indicate that α1 and α10 bind poorly to the fibrillar form, suggesting that these two integrins interact with higher affinity to the native collagen ligands that belong to collagen subfamilies such as the network-forming collagens (collagen IV for α1β1 expressing cells and collagen VI for the cartilage integrin α10β1) or the FACIT collagens (collagen IX for α10β1) [131,132]. *In vivo* [133], the interaction between integrin α1β1 and collagen IV occurs on some epithelial, smooth muscle and endothelial cells in contact with basement membranes. Based on ligand affinity studies *in vitro*, α1β1 expressed on mesenchymal cells like fibroblasts, would mainly bind to immature or reorganizing monomeric form of fibrillar collagens. Fibroblasts that are in close vicinity of collagen IV-containing basement membranes could possibly access collagen IV *via* α1β1. Collagen XIII is another possible ligand for α1β1 [131] and recent studies have suggested a role for the α1β1-mediated binding to collagen XVIII during liver injury [133].

4.2. Availability of fibrillar collagens to integrin binding

The fibrillar collagens are the most abundant members of the superfamily. Although each shares significant similarity (D-period, triple-

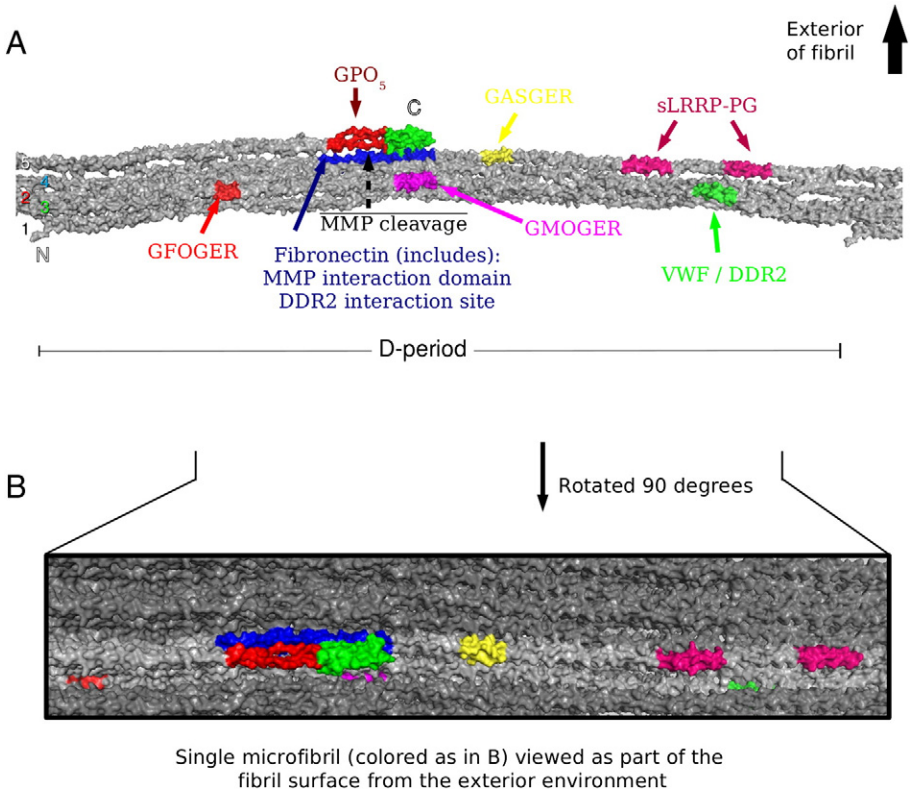

Fig. 3. Accessibility of integrin-binding sequences in context of collagen microfibril. A) One D-period of the type I collagen microfibril, composed of 5 molecular collagen segments (numbers 1–5 are labeled). The Hodge–Petruska scheme describes the D-period structure of collagen packing by dividing the collagen molecule into 5 units (collagen monomers 1–5: M1, M2….M5), of length 1D except M5, which has a length of 0.46D. The collagen monomers marked M1–M5 are segments of neighboring collagen monomers but if they were stacked end on end in order, they would form a complete collagen molecule in terms of sequence and structure [138]. It is shown outside of the collagen fibril in A and within the collagen fibril surface in B. Orientation of the microfibril relative to the exterior fibril surface is indicated (black arrow). Functional sequences are marked with patches of color: GFOGER, GMOGER, GASGER are integrin- (direct) binding sequences of descending affinity, MMP cleavage/interaction domain (in common with the fibronectin-binding domain, the C-terminal region of which may also be a DDR2-binding site), von Willebrand's Factor (vWF) (which is partially in common with a major DDR2-binding site), the imino rich repeat sequence, GPO (5) which also may promote hemostasis on the fibril surface, small leucine-rich proteoglycan/protein (sLRRP-PG) high affinity binding sequences in the e and d microscopy bands, and the C-terminal telopeptide (in green) which may regulate access to the more sensitive cell-interaction sites including the MMP cleavage site. B) Part of the microfibrillar D-period is viewed from the fibril exterior (opposite point of black arrow in A). The single microfibril is light gray while the sequences colored in A are shown as before. This perspective gives some prospective of the relative accessibilities of these ligand-binding sites in the unmodified fibril surface (see Fig. 2).

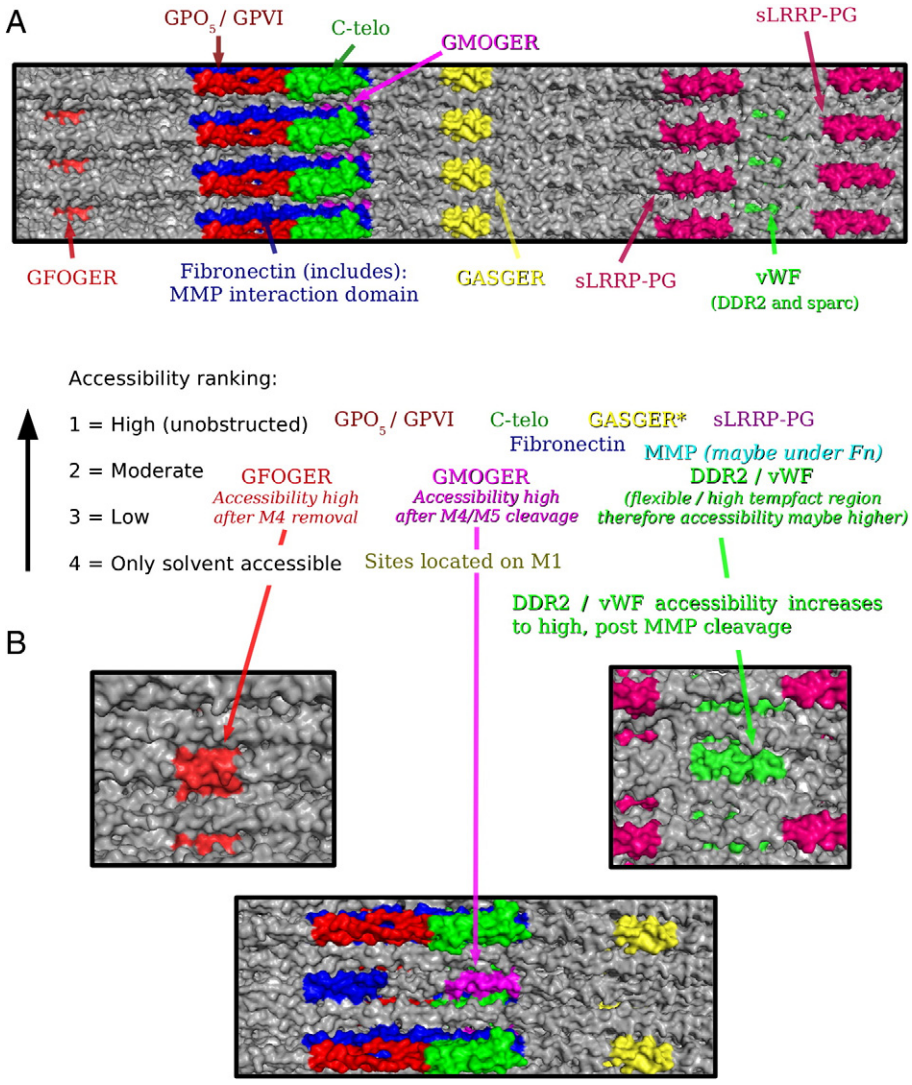

Fig. 4. Relative accessibilities to binding sequences and functional domains in the native and 'functionalized' (bent or proteolyzed) fibril. A) Key functional domains of collagen are marked on the four microfibrils modeling the fibril surface, viewed from the fibril's exterior. A qualitative molecular 'accessibility' ranking of binding sites was determined for the intact and following MMP cleavage and removal of Monomer 4 from a single microfibril. B) View of accessibility of vWF/DDR2-binding site following removal of the ¼ segment from MMP cleavage (partial Monomer 4 and all of Monomer 5), right and bottom. The DDR2/fibronectin site might facilitate the removal to then expose vWF/DDR2 in the gap region and GMOGER at the overlap/gap interface. Removal of the remaining collagen fragment, especially the remaining part of Monomer 4, gives clear access to the high-affinity integrin-binding site, GFOGER. Abbreviations are as Fig. 3 legend, except for: Glycoprotein VI (GPVI), which binds to imino acid repeat sequences such as GPO5 and C-telopeptide (C-telo).

helical domain, sequence, and aspects of telopeptide structure), they are subtly distinct in their basic structure from ligand-binding sequences to their molecular packing and fibrillar structures, leading to different properties and therefore emphasis and roles within tissues [127,134]. It is believed that the fibrillar collagens form microfibrils with a rope-like assembly of collagen triple-helices and that these microfibrils concentrically assemble to form fibrils. Well-defined structures for the microfibril and the fibril surface for type I collagen have been obtained [135,136]. The microfibrils are bound within the fibrils by lysyl crosslinks between the telopeptides and key triple-helical sites on neighboring D-staggered (where D = 67 nm in hydrated native tissues) as well as extensive side–side packing interactions including water and protein based hydrogen bonding, electrostatic and hydrophobic interactions. Fibrils are decorated with PGs to construct much larger fibers, which may be capable of fusing and certainly are found to enable the construction of a variety of variations of a theme in terms of collagen fiber organization [137]. The fiber organization varies from long parallel arrays in tendons to the felt-work like organization found in dermis. Fig. 3 shows how this fibrillar organization effects the hierarchically available cell interaction and matrix-binding sites. The part of the fibril surface most exposed to the extracellular environment is the most densely packed, the C-telopeptide region of the D-period [138]. As such, and due to the presence of immino rich regions in the C-terminal sequence of the monomer, it may act to stabilize this exposed region. Underneath this protective layer of collagen monomers are several key amino acid sequences that are involved in fibril assembly and stability [139,140] (Figs. 3–5). One of these sequences, GPO5 [137] is the one known to bind the platelet glycoprotein VI [140] and is found clearly exposed on the fibril surface (Figs. 3–4) as are the putative SLRP core protein interaction sites in the microscopy D and E band regions on Monomer 4 (M4) [22,141]. Due to the large area that the PG covers of the fibril-bundle, it is likely that they exclude the binding of other ECM ligands in this region when present. Due to their key role in conveying structural continuity in most connective tissues, this seems likely to be the case in most mature connective tissues. This in turn suggests that the majority of the fibril surface in the mature form is structural, unless it undergoes some interaction with cells. Limited proteolysis or cell associated torsion and bending will expose key binding sites in the fibril [128,136].

The molecular packing of the collagen monomers in the organization of the type I fibril and as far as can be inferred due to their smaller size,

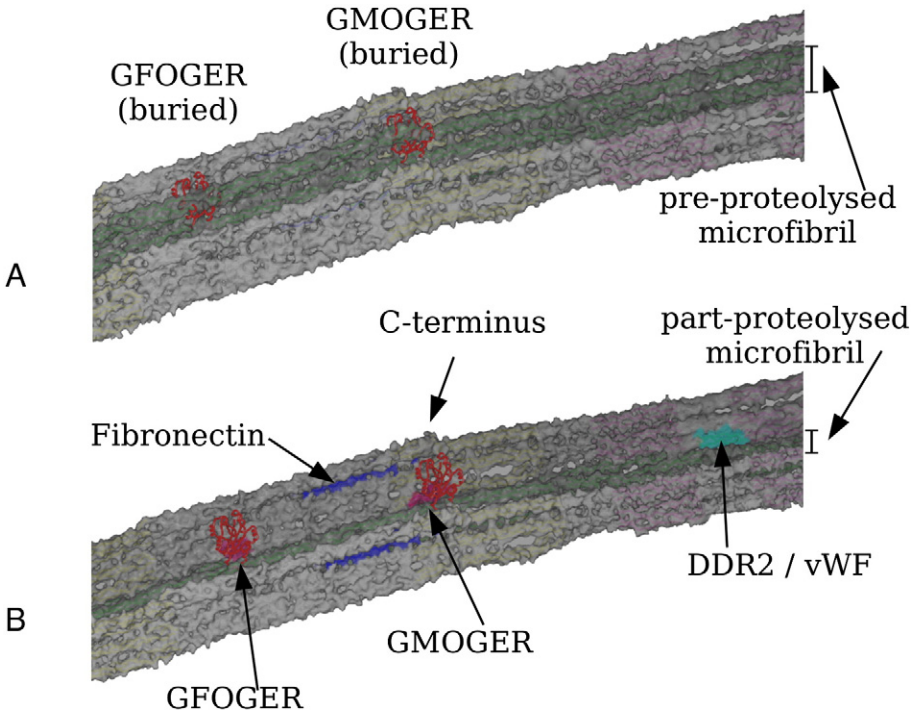

Fig. 5. Unmasking of integrin-binding sites during partial proteolysis of collagen microfibril. A) View of the unmodified surface of a mature fibril, 3 microfibrils shown. B) View of a partially proteolyzed microfibril. The docked positions of the integrin I domain are shown for the GFOGER and GMOGER sites. The Monomer 5 and Monomer 4 molecular segments obscure these binding sites, as does Monomer 4 for DDR2 and Monomer 5 for fibronectin. Removal of the C-terminal telopeptide gives partial access to the fibronectin site. Removal of the ¼ segment (most of Monomer 4 and all of Monomer 5) gives clear access to GMOGER, fibronectin and DDR2. Removal of another small portion of what remains of Monomer 4 opens the GFOGER site for unrestricted binding also. Abbreviations are as for Fig. 3.

to a lesser extent types II and III collagen fibrils, restricts what cell-binding interaction sites are freely available at the fibril surface. In the simplest of terms, this translates to the Monomer 5 (M5) and M4 molecular segments obscuring the principal integrin-binding sites (GFOGER) as does M4 for DDR2 and M5 for FN-binding site. Removal (or absence) of the C-terminal telopeptide gives partial access to the FN site. Removal of the ¼ segment of collagenase cleavage (most of M4 and all of M5 monomers) gives clear access to GMOGER, FN and DDR2. Removal of yet another small portion of what remains of M4 opens the GFOGER site for unrestricted binding.

A possible exception to the burying of integrin-binding sequences is the GASGER sequence (Figs. 3 and 4). However, it is weak binding and its accessibility may be significantly modulated by fibril-associated PGs or mineralization [142].

Lastly, all of these above mentioned sites are relatively available for interaction in individual microfibrils, for instance, those at the edge of a region of fibril construction during fiber polymerization. That being said, starting with weak integrin interaction/indirect integrin interaction sites being the most accessible, leading to the strongest direct integrin collagen-binding sites being the most inaccessible (before the removal of M4). This could suggest a possible process of progressively stronger COLINBRI-dependent cellular interaction with mature fibril modifications. In the case of tissue repair, the damaged fibrils at these sites close to the fibril surface would be quickly recognized and adhered to by cells, which could be part of the process of damage sensing and the repair response. Other than the observations made from the molecular structure of the fibril surface, there are no direct studies to support this theoretically based hypothesis, but there are some observations that could fit with it.

4.2.1. Observations supporting interactions of collagen-binding integrins with immature collagen matrices

Integrin α11β1 is highly expressed in the mouse incisor periodontal ligament [10,125,143], which is the tissue in the vertebrates with the highest turnover of collagen I. It is possible that α11β1 in the periodontal ligament is an organizer of collagen assembly.

α10 expression includes growing epiphyseal cartilage, the ossification groove of Ranvier and the meniscus [125,144–146], all sites with dynamic collagen turnover, suggesting that α10β1 might be an organizer of cartilage collagens.

Collagen V seems to play a central role in cell-assisted collagen fibrillogenesis [147–149]. The molecular composition of the nucleating complex at the cell surface has not been characterized yet. If collagen-binding integrins take part in this process they would mostly bind to immature collagen protofibrils to nucleate fibrillogenesis [150].

4.2.2. Observation supporting interactions of collagen-binding integrins with mature collagen fibrils

Both α2 and α11 I domains bind to collagen I fibrils with measurable affinity *in vitro* [132]. Furthermore, integrin antibodies can inhibit cell- and platelet-binding to fibrillar collagen I.

Intriguingly, structural modeling shows directive restraint in the docking of integrins (and other ECM ligands) to the binding of collagen fibrils (see Figs. 4 and 5 as illustration of the point). However, 'opportunistic' binding to areas of the fibril surface more loosely organized (such as at bends induced by cell action or damage) and/or directed proteolysis readily and straight-forwardly opens the collagen fibril surface to strong integrin binding (Fig. 4). The nature of the hierarchical organization and the placement of cell interaction sites within it might hint at a directive process for increasingly strong cell adhesion culminating in the high-affinity cell-binding site GFOGER. This site itself being located within the densely packed overlap zone within the middle of the microfibril (and hence somewhat harbored) while the less strong and more indirect interaction sites are located within the overlap zone (DDR2) and at the overlap/gap interface (e.g. GMOGER, FN-binding sites) and are much closer to the fibril surface. Under these conditions integrins are able to directly interact with collagen fibrils.

β1 integrins efficiently mediate the reorganization of floating and restrained fibrillar collagen matrices *in vitro* [151–153]. These fibrillar matrices have formed by self-assembly and are different from the mature fibrillar matrices seen *in vivo*. MMPs facilitate collagen reorganization *in vitro* and this process can also occur independently of MMPs, although less efficiently [154,155]. With time, COLINBRIs will be secreted in these matrices and also contribute to the reorganization. One could argue that the polymerized collagen used *in vitro* to study the collagen reorganization, in the collagen gel contraction model, contains monomeric collagen molecules that would coat the collagen fibrils, but most likely this cannot account for the majority of the rapid and robust collagen remodeling that occurs in these matrices.

In summary, it is likely that collagen-binding integrins can bind to both monomeric and fibrillar forms of collagen in physiological settings. We postulate that in assembling matrices (development, high tissue turnover and regeneration) the availability of integrin-binding sites is high. In situations where integrins are in contact with mature, highly crosslinked complex collagen matrices, current structural data indicates that the availability of direct integrin-binding sites might be a limiting factor and instead the availability of DDR2-binding site or indirect COLINBRI-binding sites would be much higher. DDR1 has been reported to regulate integrin-mediated interactions with collagen [14] and can cooperate with integrins in collagen I-mediated EMT [156]. In contrast, Zhang et al. recently showed that DDR2, independently of integrins, sustain the EMT phenotype and facilitate breast cancer invasion [157]. It is possible that at this early stage, before reorganization of collagen at the tumor boundary, in accordance with the hierarchical availability of collagen receptor binding sites, DDR2 would be the first receptor to bind to the collagen fibrils. The DDR2-mediated collagen remodeling would then give access to the integrin-binding sites and allow cancer cell invasion to occur more efficiently.

5. Collaborative role of integrins in fibrillogenesis

We predict that the roles of collagen-binding integrins in collagen fibrillogenesis will be re-evaluated in the years to come. Whereas substantial data has been produced on the role of α5β1 and αvβ3 integrins in FN assembly [158–160], much less effort has been put into analyzing the role of collagen-binding integrins.

5.1. Fibronectin matrix assembly—a cell driven process

Seminal papers on the role of integrins in FN assembly have demonstrated a preferred role for α5β1 over αvβ3 in mesenchymal cells [53]. Under artificial conditions where α5β1 is ablated, small FN fibrils can form in an αvβ3-dependent manner. Surprisingly, when the RGD motif of FN is mutated *in vivo* to become non-functional, αvβ3 can rescue assembly by binding to a distinct site [159]. In platelets the αIIbβ3 integrin can take part in FN matrix assembly during thrombus formation [161]. Separate experiments have demonstrated that also α4β1 [162] and α9β1 [163] can take part in RGD-independent FN assembly in specialized cells. Part of the increased effectiveness of α5β1 over αvβ3 in assembling FN matrices has been demonstrated to be due to enhanced capacity to activate Rho mediated contractility and signaling by α5β1 [164]. In this context it is interesting to note that in some cells there is a cross-talk between integrins so that high β1 levels lead to lower β3 integrin levels due to mRNA destabilization [165]. In a recent report, knockdown of β1 integrins in malignant mammary epithelial cells elicited a robust compensatory expression of β3 integrins, but not in their normal counterparts, suggesting that in this system the cross-talk is correlated with the malignant phenotype [166]. The cell type specific integrin cross-talk can thus influence integrin levels, and the data also suggests that integrin signaling might affect other important factors involved in FN assembly.

5.2. Spontaneous assembly and cell-driven collagen fibrillogenesis

Multiple studies have suggested that FN assembly drives collagen fibrillogenesis, which can be regarded as the dogma in this field (reviewed in [150]). Whereas cell driven assembly is a prerequisite for FN assembly, collagens under appropriate conditions can form an imperfect fibrillar matrix, by self-assembly. Several elegant experiments have shown that collagen containing telopeptides contribute to a more homogenous 3D fibrillar structure under some conditions [167]. The telopeptides are not just vital for stabilizing crosslink formation, the C-telopeptide in particular may regulate access to key ligand-binding sites (MMP cleavage, FN binding, GFOGER and GMOGER) (Figs. 3 and 4). Hence the presence of the telopeptides represents key elements in the assembly (or disassembly) of collagen fibrils.

It is becoming increasingly clear that collagen fibrillogenesis *in vivo* needs to be organized and that it occurs at cell surface [150]. For collagen fibrillogenesis *in vivo*, collagens V and XI, seem to have a nucleating function for assembly that starts at the cell surface [149]. Convincing data shows that mice lacking collagen V have severely disturbed collagen I fibrillogenesis [147,168]. Since collagens form complex fibrils, assembled from multiple collagens into heterotypic fibrils coated with FACIT collagens at the fibril surface, collagen fibrillogenesis is more complex than the FN type of matrix assembly. Molecules involved in fibrillogenesis include tenascin-X, TSP-2, SLRPs and COMP at later stages of the assembly (reviewed in [150]). PN can increase the stiffness of collagen matrices, suggested to occur *via* increased collagen crosslinking, but an effect of PN on enhanced fibrillogenesis has not been excluded [17].

Whereas a number of studies suggest that the collagens are assembled on top of FN networks [160], other studies suggest that collagen receptors enhance collagen assembly [169] and others have shown that FN assembly is dependent on a preformed collagen matrix [147,170]. Especially compelling are studies using human and mouse fibroblasts isolated from Ehlers–Danlos patients lacking collagen V, showing that these fibroblasts failed to assemble collagen and FN matrices [171,172]. Collagen mutations have additional effects such as changed synthesis of the ECM components, which complicate the picture further.

The conclusion we draw from these studies is that FN assembly and collagen fibrillogenesis are tightly connected. When studying these processes at the cell level it seems essential to first of all characterize the cell repertoire of FN and collagen receptors. We postulate that in cells with high levels of FN receptors like α5β1 (strongly promoting FN assembly), and that at the same time express low levels of collagen receptors, will be prone to assemble an extensive FN matrix, which will guide collagen fibrillogenesis. Conversely, in cells that express high levels either of the collagen receptors α2β1 or α11β1 combined with low expression levels of FN receptors, we suggest that integrins will primarily nucleate collagen fibrillogenesis and incorporate FN onto these collagen fibrils, in a manner dependent on the availability of the FN-binding sites.

Studies performed *in vitro* present a number of limitations that are important to remember when interpreting the results in the context of our current knowledge about the assembly process of FN. For example, when using GD-25 -β1 cells, which are embryonic cells of unknown origin that lack collagen receptors, it is quite possible that some intra-or extracellular factors related to collagen receptor functions are also missing [169]. When these cells are transfected with cDNA encoding either α2 or α11 integrin chain, it is thus possible that FN-mediated assembly of collagens is favored over collagen receptor-mediated assembly, contributing to a biased view of how the assembly process occurs [169].

Most published studies on integrin-mediated collagen fibrillogenesis have been performed without considering the more recently identified collagen receptors α10β1 [173] and α11β1 [174]. However, since α10β1 is mainly expressed on chondrocytes and seems to prefer non-fibrillar collagens, we predict that the major collagen receptors involved

in collagen fibrillogenesis on fibroblasts are α2β1 and α11β1. α2β1 is more widely expressed than α11β1, which is restricted to subsets of fibroblasts [10,143,153]. More systematic studies on the relative importance of these integrins for collagen fibrillogenesis together with studies addressing whether these receptors show different abilities to nucleate and direct collagen fibrillogenesis remain to be performed.

In summary: Compared to FN assembly, collagen assembly is more complex. At the cell surface level, fewer integrin types might be able to directly nucleate collagen fibrillogenesis compared to FN assembly, but the subsequent collagen fibrillogenesis steps contain more components, which all can vary in a cell specific manner.

For future studies of FN and collagen fibrillogenesis, it will be essential to characterize FN and collagen receptor repertoires, as well as the isoforms of small Rho GTPases and their regulators present in cells and shown to be involved in collagen remodeling [175–177]. Only then can one begin to understand the molecular mechanism of matrix assembly in the context of different mesenchymal cell types such as osteoblasts, chondrocytes and fibroblasts.

6. Lack of expected phenotypes in mouse models: role of collagen-binding integrins?

6.1. Limitations of mouse as animal model

The phenotypes observed in mice with defects in the collagen-binding integrins are summarized in Table 2. The broadly expressed α1β1 and α2β1 integrins have been studied extensively, whereas substantially less has been published on the collagen-binding integrins α10β1 and α11β1. Interestingly both α10β1 and α11β1 show a remarkable cell specificity in their cell expression pattern in the tissues. During normal development α10β1 is restricted to chondrocytes and a subset of junctional fibroblasts [144,146]. α11β1 is restricted to fibroblasts in dense connective tissues but display a transitory expression in odontoblasts [10,143]. From *in vitro* studies, α11 has been shown to be induced upon cell culture of mesenchymally-derived cells not normally expressing α11 *in vivo* (chondrocytes, myoblasts, and endothelial cells) ([178], unpublished data). Cultured mesenchymal stem cells also express high levels of α11 [179]. Since collagen is the most abundant protein family in vertebrates, and multiple disease-causing mutations in collagens have been described (Table 3), one would also expect severe defects when individual or multiple collagen receptors are lacking. Whereas mutations in fibrillar collagens have revealed severe defects in the bone, cartilage, skin, tendon, blood vessels, an analysis of collagen-binding integrins at this stage does not reveal the corresponding phenotypes, with one exception, the α10 integrin cartilage phenotype (Table 3).

This is unlike the situation for some other integrin–ligand pairs, like integrins–laminins, integrins–FN [180], where mutations of integrins give rise to similar phenotypes as mutations of ligands and hence confirm a major contribution of integrins to the integrated biological function of these ligands. The reason for this lack of correlation between integrin and ligand phenotypes can be manifold in the case of fibrillar collagens:

1. The structural defects seen when collagen is lacking or mutated, might not primarily be dependent on the interaction with cells. Fibrillar collagens more than any other ECM protein form the armament that reinforces the basic ECM structure.
2. In addition to direct linkages to collagen, indirect linkages mediated via COLINBRIs contribute to attaching cells to the ECM network under normal conditions. The fibrillar collagen phenotypes would in some instances be reflected in the phenotypes from several integrins, some of which will include COLINBRI-binding integrins.
3. Mild phenotype in genetic models could be due to integrins having overlapping roles. Further analysis when multiple collagen-binding integrins are inactivated in advanced compound mouse models will reveal whether they act together and have overlapping roles.
4. In genetic models, compensation mechanisms by other collagen-binding integrins, COLINBRI-binding integrins or both collagen-binding and COLINBRI-binding integrins might compensate for loss of collagen-binding integrins, which would explain the mild, restricted phenotypes observed when individual integrins are inactivated.
5. Finally, using small animals the existing data so far for individual knockouts suggest that collagen-binding integrins have a limited role during embryonic development and in normal adult tissue homeostasis. One recent unexpected recent finding in dogs, suggests that cartilage might be the first tissue where a collagen phenotype corresponds to a collagen-binding integrin mutant phenotype (Table 3). Dogs with mutations in α10 integrin develop a severe canine chondrodysplasia [181], whereas α10 mutations in mice only lead to very mild cartilage phenotype [146]. A number of mutations in mouse show important different manifestations compared to corresponding mutations in humans [182]. The different physiology of small rodents and an upright-walking human is expected to result in differences in phenotypic manifestations, especially in the muscoskeletal system. Since several collagens are present in the cartilage matrix it is unclear what the *in vivo* ligand(s) for α10β1 are, but analysis of collagen deficient mouse phenotypes would suggest that collagen II and collagen XI are likely candidates.

Interestingly, the DDR-deficient mice have more severe phenotypes than any of the collagen-binding integrin deficient mice, indicating a role of these cell surface proteins during development [183,184]. It is however unclear if these phenotypes in $DDR^{-/-}$ mice reflect defective cell–collagen linkages or if the observed defects are related to the indirect effect of impaired cell signaling originating from these enigmatic tyrosine kinase receptors. DDR2 mutation in human also leads to a skeletal disorder, a rare form called pondylo-meta-epiphyseal dysplasia [185].

In summary: Although we do not fully understand the role of collagen-binding integrins, in our current understanding they have limited roles during embryonic development and in normal adult tissue homeostasis. The finding that integrin α10-deficiency causes a severe skeletal phenotype when analyzed in larger animals suggests that also for the other collagen-binding integrins, the mouse phenotype might not reflect the role of these receptors in larger mammals, where mutations in cells forming the skeleton manifest a more severe phenotype.

We believe that COLINBRIs serve as an important link *in vivo* to mature crosslinked collagen fibrils and offer one explanation to why the phenotypes of the ligand – receptor pair collagen – collagen receptors do not correlate to the same degree as for other ligand–integrin pairs. However, to resolve these issues, more complex compound integrin deficient mouse models are needed.

6.2. Integrin type collagen receptors in pathology

Challenging of the α1 and α2 deficient mouse models have revealed phenotypes in inflammatory conditions, wound healing, tumor metastasis and bone physiology (Table 3). Similar studies in the α10 and α11-deficient mouse models remain to be performed.

In a recent review article the role of collagen-binding integrins are discussed in the context of stem cells/stem cells niche in skeletal muscle and adipose tissue in connection with obesity [186]. The background for these suggestions goes back to the finding that the stiffness of the matrix seems to direct stem cell fate [187]. Volloch and Olsen [186] tried to relate the fate of stem cells in fat and muscle tissues to the type and number of collagen-binding sites for α1β1/α2β1 (native collagens I and IV) and αvβ3 (denatured collagens I and IV). It is suggested that a distinct α1β1 integrin-binding site present in the non-collagenous domain of collagen IV determines adipogenic cell fate and prevents stem cells in fat tissue to differentiate along a non-adipogenic pathway

Table 2
Phenotypes of mice deficient in collagen-binding integrins.

Integrin subunit	Distribution	Knockout phenotype	Phenotype of challenged knockout mice
α1	Endothelial cells, smooth muscle cells, fibroblasts and more cell types [209]	Normal development [209], hypocellular dermis [210,202], isolated cells display defect in collagen IV cell attachment	Reduced inflammation [211], reduced psoriasis [212], prevention of contact hypersensitivity [213], reduced tumor vascularization [214], reduced bone fracture healing [215], exacerbated diabetes-induced kidney disease [216], accelerated age-dependent osteoarthritis [217]
α2	Platelets, epithelial cells, endothelial cells [218], mesenchymal stem cells [179], fibroblasts and more cell types	Mild mammary gland phenotype, otherwise normal development [219,220], cell attachment defect to collagen I of isolated platelets [221], needed for thrombus stabilization [222]	Defective inflammatory response to listeria infection [223], augmented vascularization in wound healing [200,224], improved bone density aging mice [225], reduced inflammation and cartilage destruction in rheumatoid arthritis model [226], reduced glomerular injury [227], increased tumor metastasis [228]
α10	Chondrocytes and subsets of junctional fibroblasts [144,173].	Mild cartilage phenotype [222]	ND[a]
α11	Subsets of fibroblasts[143,153,174], cancer associated fibroblasts [229], increased levels on myofibroblasts [230], developmental expression in odontoblasts, mesenchymal stem cells [179], induced in cultures of mesenchymally-derived cells including myoblasts (do not express α11 *in vivo*) [178]	Defective incisor eruption [10], dwarfism [231], increased mortality	ND

[a] Not determined.

under mechanically stressed conditions, such as during exercise. The review is interesting, but similar comparison should include an analysis of possible α11β1 integrin involvement, and experimental approaches to support the suggestion that type and availability of integrin-dependent collagen-binding sites determine stem cell differentiation should be undertaken.

To understand the gene function in the context of human physiology it will also be interesting in the years to come to analyze the effect of integrin mutations in rare human genetic disorders. Gene linkage analysis, single-nucleotide polymorphism analysis and gene network analysis have been used so far in studies implying collagen-binding integrins in disease. A limited study has indicated roles for *ITGA1* polymorphisms in predisposing Korean women to osteoporosis [188]. Numerous reports have implicated platelet *ITGA2* expression in the control of hemostasis [189,190]. The oldest report of integrin α2 function in platelets refers to one patient with a bleeding disorder and lack of GPIa [191]. The role of α2β1 in human platelets might thus be different from its role in mouse platelets. More recent studies suggest *ITGA2* polymorphism as a predisposing factor in stroke and myocardial infarction [192–195].

The recent identification of *ITGA10* mutation as the causative mutation in severe canine chondrodysplasia [181] suggests that human *ITGA10* mutations might also have more severe phenotypes than the mild cartilage phenotype seen in *Itga10* deficient mice [146]. Finally, analysis of susceptibility modules for coronary artery disease using a genome wide integrated network analysis approach suggests that *ITGA11* might be involved in vascular disease [196]. Since α11 expression to our knowledge is restricted to fibroblasts, the possible involvement of α11 in cardiovascular disease will be interesting to follow up.

With the advancement of whole genome sequencing we are likely to obtain a clearer picture of the role of collagen-binding integrins in human disease in the years to come.

Table 3
Comparison of phenotypes in mice deficient in collagens or collagen-binding integrins.

Ligand			Receptor		
Fibrillar collagen	KO phenotype in mouse	KO phenotype in human	Putative collagen receptor(s) *in vivo*	Correlation KO phenotypes collagen/receptor in mouse	KO phenotype in human/dog
I	Mov13 mice [232]: embryonic lethality E12-14, major blood vessel rupture	EDS[a] VIIA, EDS VIIB, OI[b], osteoporosis, joint hypermobility	α2β1 α11β1	Not in single integrin mutant strains	?
II	Perinatal lethality [233,234] short long bones, rudimentary vertebral arches, lack of inter-vertebral discs, notochord defect	Lethal achondrogenesis II, osteochondrodysplasia, osteoarthrosis	α1β1 α2β1 α10β1	α10 integrin mutation [146], mild cartilage defect β1 integrin [197], severe cartilage defect	Chondrodysplasia in dogs, integrin α10 mutation [181], severe cartilage phenotype
III	Neonatal lethality [235], 5% survival with shorter lifespan, intestinal defect, skin lesions, arterial rupture	EDS IV, arterial aneurysms	α2β1 α11β1	?	?
V	Embryonic lethality E10–11 [147], cardiovascular insufficiency, lack of collagen fibrillogenesis	EDS I, EDS II	α2β1 α11β1	?	?
XI	Cho mice: perinatal lethality by asphyxia [236], weak tracheal cartilage, short snout and mandible, cleft palate, short limbs, externally rotated distal portion of hindlimbs	Schmid chondrodysplasia, non-syndromic hearing loss, osteoarthrosis	α2β1 α10β1 α11β1	α10 integrin mutation [146], mild cartilage defect β1 integrin [197], severe cartilage defect	Chondrodysplasia in dogs, integrin α10 mutation [181], severe cartilage phenotype
XXIV	?	?		?	?
XXVII	Mutant transgene[237]: perinatal lethality, lung defect, chondrodysplasia	?	α2β1 α11β1	?	?

[a] Ehlers–Danlos syndrome.
[b] Osteogenesis imperfecta.

7. Cell–collagen interactions in biological processes: involvement of COLINBRIs?

In tissues like the periodontal ligament with high collagen turnover, the direct collagen interaction appears to be essential (supported by knockout phenotype of α11 integrin deficient mouse [10,125]), but the absence of the COLINBRI molecule PN in the periodontal ligament likewise leads to a severe phenotype [86], supporting an important role of both direct and indirect linking mechanism of cells to fibrillar collagens in the periodontal ligament (Fig. 6).

Conditional deletion of β1 integrins in the cartilage has indicated an important role of integrins for chondrogenesis [197] and probably also collagen II assembly, but individual integrin α-chain phenotypes have so far failed to replicate the β1 integrin deficient phenotype in mice. Cartilage might thus be another example where collagen-binding and COLINBRI-binding integrins cooperate during embryogenesis (Fig. 6).

In vitro experiments have suggested distinct roles of α5β1 and αv-containing integrins on endothelial cells during angiogenesis [54], whereas *in vivo*, compound αv/α5 deficient mice do surprisingly well up till midgestation, unlike mice deficient in β1 integrins on endothelial cells [198,199]. Independent studies have implied collagen receptors in angiogenesis [200,201]. This suggests the involvement of other FN-or collagen receptors on endothelial cells that possibly in a COLINBRI-mediated mechanism could contribute to angiogenesis (Fig. 6).

During wound healing integrin on dermal fibroblasts are thought to mediate collagen reorganization to close the wound and contract the granulation tissue. In this process also, mice defective in individual collagen receptors [200,202,203] have failed to reproduce the phenotype in β1 integrin-defective mice [204], suggesting a cooperation between indirect and direct mechanisms for cellular interactions with the granulation tissue collagen.

In pathological situations with high turnover of both FN and collagens the chances for both mechanisms of cell adhesion are high. We predict parallel roles of collagen-binding and COLINBRI-binding integrins in wound healing and fibrosis. In this context the changed stiffness of the contracting collagen matrix might be sensed by COLINBRI-binding integrins, which in turn could contribute to TGF-β activation.

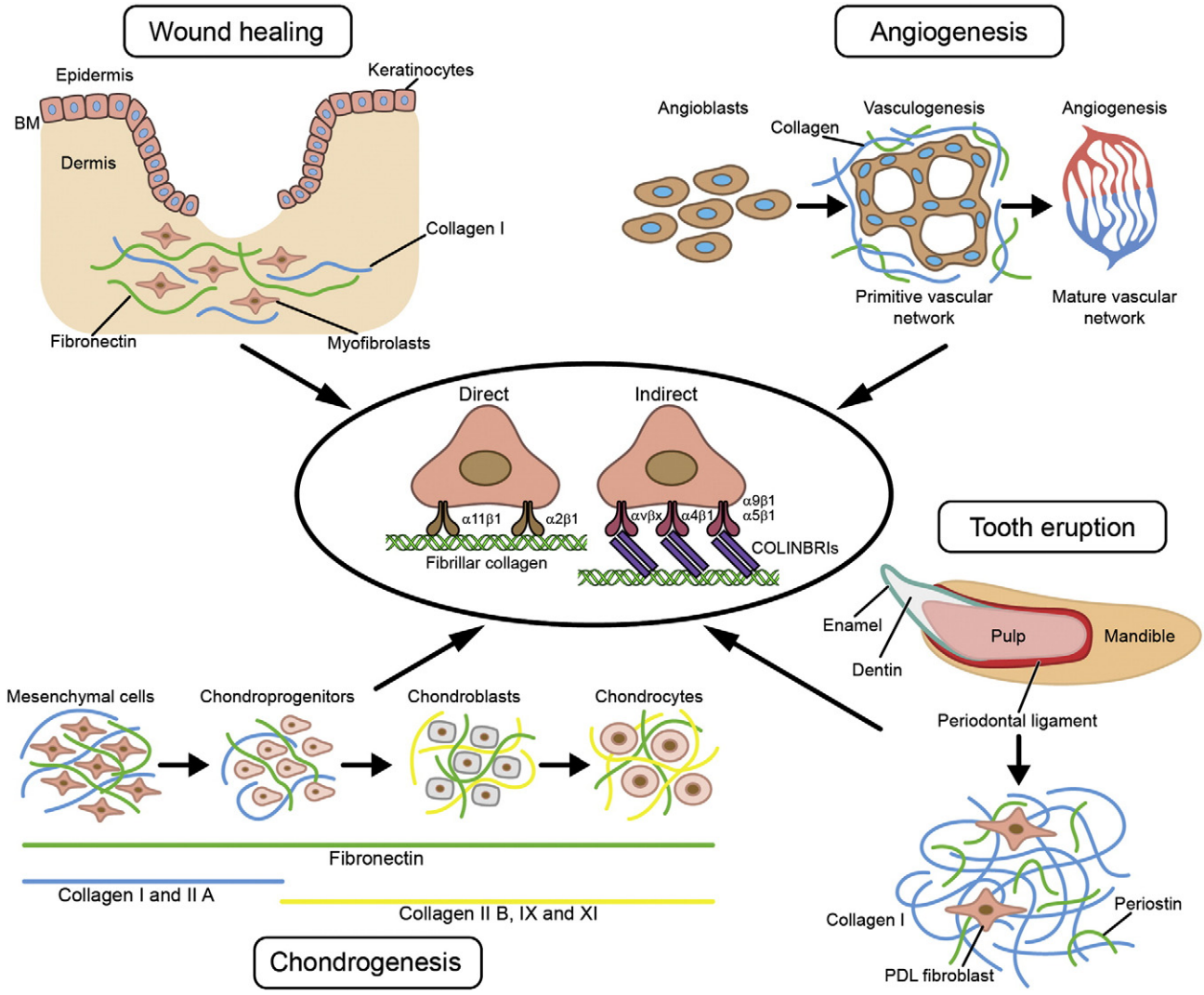

Fig. 6. Schematic illustration of some biological processes where direct and indirect binding to collagen cooperate. The cooperation between direct and indirect cell-collagen-binding mechanisms in biological processes is predicted from phenotype data obtained with integrin knockout mice. During wound healing, dermal fibroblasts reorganize collagen to close the wound and contract the granulation tissue. We suggest a cooperation between indirect and direct mechanisms for cellular interactions with the granulation tissue collagen. During tooth eruption, both direct and indirect cell-collagen interaction could play an essential role as supported by knockout phenotypes of α11 integrin- and periostin-deficient mice. We also predict collaboration between collagen- and COLINBRI-binding integrins in chondrogenesis, where mesenchymal cells differentiate to chondrocytes in a fibrillar collagen- and fibronectin-rich environment. Angiogenesis is supported both by the "fibronectin, vitronectin and periostin"-COLINBRI-binding integrin and collagen receptors.

8. Concluding remarks

We have tried to highlight some recent dilemmas in the collagen-binding integrin field. Whereas collagens are the major building blocks in tissues and defects in these structural proteins have severe consequences for tissue integrity, the mild phenotypes of the integrin type of collagen receptors have raised questions about their importance in tissue biology and pathology. We have tried to offer some explanations for the observations obtained so far, including the suggestion that indirect mechanisms for anchoring cells into collagen fibrils exist in parallel with direct collagen binding. We furthermore suggest that mutations in integrins, expressed in the muscoskeletal system, are biased towards a mild phenotype in mice but will be more strongly manifested in larger vertebrates. We thus offer alternative interpretations for the observed mild phenotypes, and if proven correct in the analyses of new compound mouse models and genetic diseases in larger animals/humans, we believe that the collagen-binding integrins will come into the limelight of connective tissue biology.

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