ANTIOXIDANTS & REDOX SIGNALING
Volume 20, Number 13, 2014

DOI: 10.1089/ars.2013.5294

Discoveries

FORUM REVIEW ARTICLE

Redox-Relevant Aspects of the Extracellular Matrix and Its Cellular Contacts *via* Integrins

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Abstract

Significance: The extracellular matrix (ECM) fulfills essential functions in multicellular organisms. It provides the mechanical scaffold and environmental cues to cells. Upon cell attachment, the ECM signals into the cells. In this process, reactive oxygen species (ROS) are physiologically used as signalizing molecules. ***Recent Advances:*** ECM attachment influences the ROS-production of cells. In turn, ROS affect the production, assembly and turnover of the ECM during wound healing and matrix remodeling. Pathological changes of ROS levels lead to excess ECM production and increased tissue contraction in fibrotic disorders and desmoplastic tumors. Integrins are cell adhesion molecules which mediate cell adhesion and force transmission between cells and the ECM. They have been identified as a target of redox-regulation by ROS. Cysteine-based redox-modifications, together with structural data, highlighted particular regions within integrin heterodimers that may be subject to redox-dependent conformational changes along with an alteration of integrin binding activity. ***Critical Issues:*** In a molecular model, a long-range disulfide-bridge within the integrin β-subunit and disulfide bridges within the genu and calf-2 domains of the integrin α-subunit may control the transition between the bent/inactive and upright/active conformation of the integrin ectodomain. These thiol-based intramolecular cross-linkages occur in the stalk domain of both integrin subunits, whereas the ligand-binding integrin headpiece is apparently unaffected by redox-regulation. ***Future Directions:*** Redox-regulation of the integrin activation state may explain the effect of ROS in physiological processes. A deeper understanding of the underlying mechanism may open new prospects for the treatment of fibrotic disorders. *Antioxid. Redox Signal.* 20, 1977–1993.

Introduction

CELL ADHESION MOLECULES, such as integrins, mediate the interaction of cells with the extracellular matrix (ECM). Both the ECM and integrins are targets of reactive oxygen species (ROS). Hence, this review first highlights different redox-relevant aspects of ECM turnover, and then approaches the questions: (i) how integrin-mediated cell-matrix contacts regulate the redox potential of the cell and its environment, and in turn, (ii) how the redox potential by cell-produced ROS may affect integrin-based cell-matrix and intercellular contacts.

The Extracellular Matrix

Multicellular organisms are characterized by tissue diversification and the production of an ECM. The plethora of different ECM components are composed of a limited set of modular structures, likely arisen through gene duplication during evolution. For example, the von Willebrand factor-homologous A-domain (briefly A-domain) is found in various types of collagens and other matrix proteins (69). Another characteristic of ECM components is their propensity to (self)assemble into larger and insoluble supramolecular aggregates, such as fibrils and networks (Fig. 1) (13). For example, rope-like fibrils span the intercellular spaces of the connective tissue and bear tensile forces. The most abundant fibrillar protein is collagen (50, 132). Other matrix proteins, such as fibronectin, fibrillins, fibulins, and elastin, also form thread-like supramolecular aggregates (156). In the human body, 28 different types of collagens have been identified (50, 132). The common molecular feature of all collagens is their

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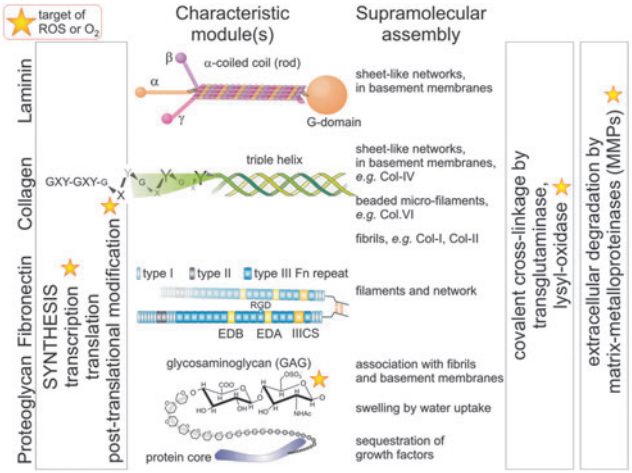

FIG. 1. Typical components of the ECM as targets of ROS. Four typical components of ECM proteins are shown with their characteristic modules. ROS and oxygen are involved in different steps of their transcription, translation and post-translational modification, such as the hydroxylation of proline residues in collagen chains, within the cells. After secretion the ECM molecules tend to assemble into higher aggregates, which possess distinct structures or functions (as named). These aggregates can be stabilized by covalent cross-linkage *via* oxidative modifications, which are catalyzed by lysyl-oxidase. Fibrils withstand tensile forces, whereas the GAG-chains of proteoglycans counteract compressive forces. Together, these forces are balanced within the matrix, similar to the tensegrity of cells, and maintain the shape of tissues and organs. The GAG chains are non-enzymatically fragmented by ROS. ECM proteins are degraded by MMPs, whose activities also depend on ROS. ECM, extracellular matrix; ROS, reactive oxygen species; MMPs, matrix metalloproteinases; GAG, glycosaminoglycan. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

triple helical structure, which consists of three collagenous α-chains with a recurring typical Gly-X-Y tripeptide sequence. Each peptide chain folds into a left-handed polyproline II conformation (Fig. 1). Three collagenous α-chains wind around each other in a right-handed manner to form the proteolytically stable triple helix. Moreover, several collagen molecules self-assemble in a staggered manner to form supramolecular fibrils (13). Type I-collagen molecules are staggered by 67 nm against each other resulting in the electron microscopically visible, classical D-banding of collagen fibrils.

Proteoglycans are matrix molecules bearing glycosaminoglycan (GAG) chains (1, 46, 71, 72), such as the basement membrane component perlecan, the small leucine-rich proteoglycans, decorin and biglycan, and several types of collagen (1, 71, 110). The GAG chains tend to swell as their numerous carboxy and sulfate groups are substantially hydrated. Thus, they withstand compressive forces and cause the interstitial tissue pressure, which is counteracted by collagen fibrils. Together, the tensile force-bearing collagen fibrils and the swellable proteoglycans act like a cushion, thereby sheltering organs. Furthermore, the GAG chains often tether growth factors and act as reservoir that releases/activates growth factors in a time- and space-regulated manner (1, 46). In recent years, proteoglycans, especially the small leucine-rich proteoglycans, have been pinpointed for their important roles in inflammation (71, 110).

In contrast to fibril-forming collagen types, type VI collagen assemble into a unique network of beaded microfibrils (3, 60, 163). It is ubiquitously expressed, but upregulated during inflammation and fibrosis (82), and associates with different ECM proteins, such as matrilins and fibrillins. Genetic deficiency of it leads to Bethlem myopathy and Ullrich congenital muscular dystrophy (89).

The network-forming type IV collagen (81, 86), but also type XV, and XVIII collagens, assemble into two-dimensional sheet-like structures, which constitute the structural basis of the basement membranes. A basement membrane defines the border between the connective tissue and any other tissue, and serves as both mechanical and signaling platform for epithelial and endothelial cells (86, 106, 170, 181). It also surrounds individual muscle cells and muscle fibers, as well as neurons and fat cells. To withstand the mechanical load, a basement membrane is anchored to the underlying connective tissue network *via* collagen VI microfibrils or *via* the collagen VII-comprising anchoring fibrils. Basement membranes are generally impermeable for cells, with the exception of leukocytes, which are able to penetrate the basement membrane for immunological tissue surveillance or for infiltration at sites of inflammation. Pathologically, tumor cells acquire the ability to penetrate basement membranes (135) and to survive in an unusual ECM environment, both hallmarks of cancer malignancy (57).

Basement membranes also contain laminins, nidogens/entactins, and the proteoglycan perlecan. Laminins are grouped into a family of about 16 members (56). Each laminin consists of three chains, α, β, and γ, which trimerize and form an α-helical coiled-coil motif, which can be visualized electron-microscopically as the long arm of the cruciform or Y-shaped laminin-molecule (Fig. 1). This so-called rod domain bears at its N-terminus the short arms of the individual laminin chains and at its C-terminus a globular (G) domain, which comprises the laminin α chain exclusively. Laminins associate homophilically *via* their N-terminal domains into a two-dimensional structure, which together with the type IV collagen-network serves an anchoring substratum for endothelial and epithelial cells. Moreover, laminins provide essential environmental signals, as, for example, mammary epithelial cells are refractory to prolactin stimulation unless they have contact to laminin (127).

Fibronectin is a dimeric molecule. Its two highly homologous chains consist of several repeats of three different modules (type I, II, and III fibronectin repeats) and are cross-linked *via* disulfide bridges at their C-terminal ends. Alternative splicing leads to about 20 different fibronectin variants in humans (160, 168). These splice variants differ in the presence of extra domains (EDs), EDA and EDB (called EIIIA and EIIIB in mouse) and of the type III-connecting segment (IIICS). They harbor cell-adhesive sites for immunologically relevant integrins ($\alpha9\beta1$, $\alpha4\beta1$, and $\alpha4\beta7$), in addition to the binding site for the classical fibronectin receptor, $\alpha5\beta1$ integrin, which is found in the 10th fibronectin type III repeat of all fibronectin isoforms (91). Plasma (p)-fibronectin, which is secreted by hepatocytes into the blood, contains only the IIICS cell adhesive site, whereas cellular (c)-fibronectin additionally contains both EDs and is expressed by mesenchymal cells, especially during embryogenesis and wound healing, in

angiogenesis along sprouting blood vessels, and pathologically, during fibrosis and in tumor tissue (42, 160, 168). Fibronectin can also assemble into a fibrillar network, especially when it is in physical contact with cellular receptors, such as integrins and syndecan-4 (148). The ECM protein tenascin-C influences the formation of the fibronectin matrix and is expressed in granulation tissue during wound healing (160). In addition, the fibronectin matrix binds numerous growth factors. The most prominent one is transforming growth factor-β1 (TGFβ1), which forms a complex with the latency activating peptide (LAP). The latent TGFβ1-binding protein-1 (LTBP-1) links this complex to the fibronectin network (148, 168). Proteolytic cleavage of LAP or tensile forces along fibronectin fibrils release and thereby activate TGFβ1 (169).

The hypoxia-driven gene expression of several matrix proteins, the hydroxylation of collagen chains and other ECM proteins, the covalent cross-linkage of ECM proteins *via* oxidative modifications of amino acid side chains, and the activity regulation of matrix-degrading enzymes by ROS are redox-relevant processes, which regulate ECM turnover.

Hypoxia-inducible factor is a transcription factor that upregulates the expression of collagens, fibronectin, fibulin-5, and other matrix proteins (32, 41, 54, 61, 75, 115), as well as the expression of enzymes, which catalyze posttranslational oxidative modifications of matrix proteins, such as collagen prolyl 4-hydroxylase and lysyl oxidase (27, 39, 65, 113, 155). These two enzymes oxidize the side chains of proline and lysine residues within collagen chains and thus, stabilize the triple helix and the supramolecular assembly of collagen (48). In contrast to the collagen proline 4-hydroxylases, which stabilize the collagen triple helix, hypoxia-inducible transcription factor (HIF) proline 4-hydroxylases mark HIF-1α in a similar proline hydroxylation reaction for ubiquitinylation and proteosomal degradation. Thus, the latter serves as oxygen sensor and is less active with decreasing oxygen supply, whereas the collagen proline 4-hydroxylase is still active under lower oxygen tension because of its higher affinity for oxygen (113, 114).

The oxidative modification of ECM proteins also lead to covalent cross-linkages, which stabilize the insoluble, supramolecular scaffold structures of the ECM. Several redox-relevant cross-linkage reactions are initiated by a copper-containing enzyme lysyl-oxidase (96). It oxidizes lysine or δ-hydroxy-lysine to the respective aldehyde derivative (136). This reactive group may react with ε-amino-groups of other lysyl residues or the thioether group of methionine in close vicinity; thus, cross-linking collagen and other matrix molecules, for example, elastin, *via* an (iso)desmosine ring structure and *via* the more recently discovered sulfilimine bond (159). In addition, non-catalyzed oxidative cross-linkage occurs between matrix molecules, especially during aging and UV-irradiation (143).

Matrix proteins, especially the triple-helical collagen, are especially resistant to proteolysis. Therefore, matrix metalloproteinases (MMPs) are specifically employed to degrade matrix proteins during tissue remodeling, regeneration, and pathologically, in tumor invasion (47, 55, 73, 135). The matrix fragments released by MMP cleavage may influence cell behavior and constitute signaling molecules of their own (111). Some of the 24 MMP family members are able to cleave even the stable collagen triple-helix, among them MMP-1, which is redox-regulated, presumably like other MMPs (84, 120, 167, 183). The expression of some MMPs, for example, MMP-1 and MMP-2, is regulated by HIF-1α under hypoxia (61, 64, 74). However, also the endogenous tissue inhibitors of MMPs, such as TIMP-1, are expressed under the control of HIF-1α (122). Moreover, the expression of MMP-1 is highly influenced by ROS at the epigenetic and transcriptional level. H_2O_2 inactivates histone deacetylase-2 (HDAC-2), and it thereby keeps the MMP-1 gene promoter accessible to transcription factors, c-Fos, c-Jun, and Ets-1, which, in turn, are activated by the redox-sensitive mitogen-activated protein kinases signaling pathway (80, 121). Consequently, the increase of the basal mRNA levels of several MMPs (MMP-2, -3, -7, -10, and -12) in fibrosarcoma cells cultured in the presence of the H_2O_2-generating enzyme superoxide dismutase-2 (SOD-2) is accompanied by an enhanced metastatic potential (120). Restoring the redox balance by increasing the ratio of reduced to oxidized glutathione abolishes this effect in intestinal myofibroblasts (134). At the protein level, the catalytic activity of MMP-1 and other MMPs (for example, MMP-2, -7, and -9) is enhanced by oxidative activation of an internal inhibitory thiol group (53, 80, 130). Therefore, changes in the cellular redox-status may modulate both expression and enzymatic activity of MMPs. Redox-regulated matrix degradation is an important step in aging, cancer, and fibrotic diseases (26), and it is influenced by the redox-status of the cells within the connective tissue. This can partially be influenced by dietary antioxidants found in green tea leaves or the skin of red wine grapes (118, 123).

ECM Regulates ROS Production

Cell anchorage to the ECM influences the cellular metabolism, including the production of ROS. Among these partially reduced oxygen species, hydrogen peroxide (H_2O_2) is the most persistent ROS, which is able to diffuse through lipid bilayer membranes (44, 107, 139).

Deprivation of matrix contacts triggers ROS production in many adherent cells. Two underlying pathways have been pinpointed in epithelial cells (Fig. 2) [recently reviewed by Grassian *et al.* (51), see also references therein]. Loss of matrix contacts decreases the uptake of nutrients (glucose, glutamine) into the cell. Additionally, the absence of ECM components and growth factors decreases the activities of phosphatidyl-inositol-3-kinase (PI3K) and protein kinase Akt (52, 138). This leads to increased expression of pyruvate dehydrogenase kinases (PDKs), all four different isoforms (PDK1–4) of which inhibit pyruvate dehydrogenase (PDH) (149). Since PDH catalyzes the gate reaction for the citric acid cycle, its inactivation turns down catabolic glucose utilization and ATP generation *via* this metabolic pathway. This effect is further aggravated by decreased glucose uptake by the cell (51). Moreover, a diminished activity of the pentose phosphate pathway leads to a drastic lack of reducing equivalents, especially NADPH molecules, which are usually transferred to glutathione to eventually scavenge hydrogen peroxide and other ROS. As a consequence of abolished cell-matrix contact, fatty acid oxidase (FAO) in peroxisomes metabolizes fatty acids to provide the cell with the necessary energy and therefore, is a potential source of ROS (142, 164). The peroxisomal lipid oxidation releases the by-product H_2O_2, which due to the lack of reducing equivalents accumulates within the cells and diffuses through the cell membrane. Thus, detached cells raise their intra- and extracellular redox potential,

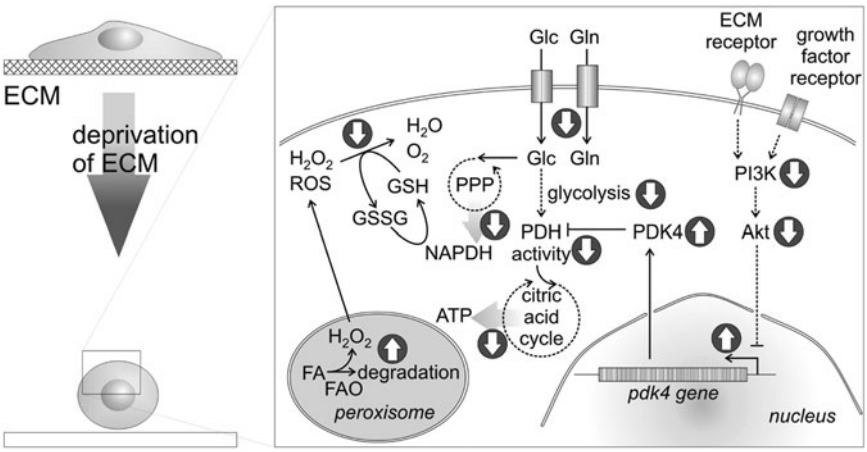

FIG. 2. Cell contacts with the ECM affect the production of ROS. Detachment of cells from their ECM substratum and ECM deprivation, along with a lack of growth factors, decreases the activity of PI3K and consequently of protein kinase Akt. Transcription factors, repressed by Akt, are activated and upregulate the expression of PDK, especially PDK4, which phosphorylates/inactivates PDH. Together, with a diminished uptake of nutrients, glucose (Glc) and glutamine (Gln), this exacerbates fuel deprivation for the citric acid cycle and hence, a shortage of ATP. Likewise, lack of fuel for the PPP reduces the concentration of NADPH, which serves as reducing and scavenging agent for H_2O_2 and other ROS. To compensate the low levels of ATP and NADPH, peroxisomal FA degradation by FAO is likely turned on with a concomitant release of additional H_2O_2, which further increases the redox potential of ECM-deprived cells. The metabolic effect of ECM deprivation resulting in increased ROS production has recently been reviewed by Grassian *et al.* (51). PI3K, phosphatidyl-inositol-3-kinase; PDK, pyruvate dehydrogenase kinases; PDH, pyruvate dehydrogenase; PPP, pentose phosphate pathway; FA, fatty acid; FAO, fatty acid oxidase.

which underlines the fact that cell-matrix contacts are able to regulate cellular ROS production (51).

During adhesion, the generation of ROS is not homogenously distributed throughout the whole cell. By indirect measurement of ROS-driven cysteine thiol oxidation in proteins indirectly *via* dimedone chemistry (18, 126), we observed that high concentrations of ROS oxidize proteins in membrane protrusions at sites where a cell establishes new contacts to its surrounding matrix, presumably *via* integrins (29). We named these subcellular areas redox hotspots (Fig. 3). Redox hotspots are not only spatially restricted but also temporarily reversible. They return to the nonoxidized state within minutes after

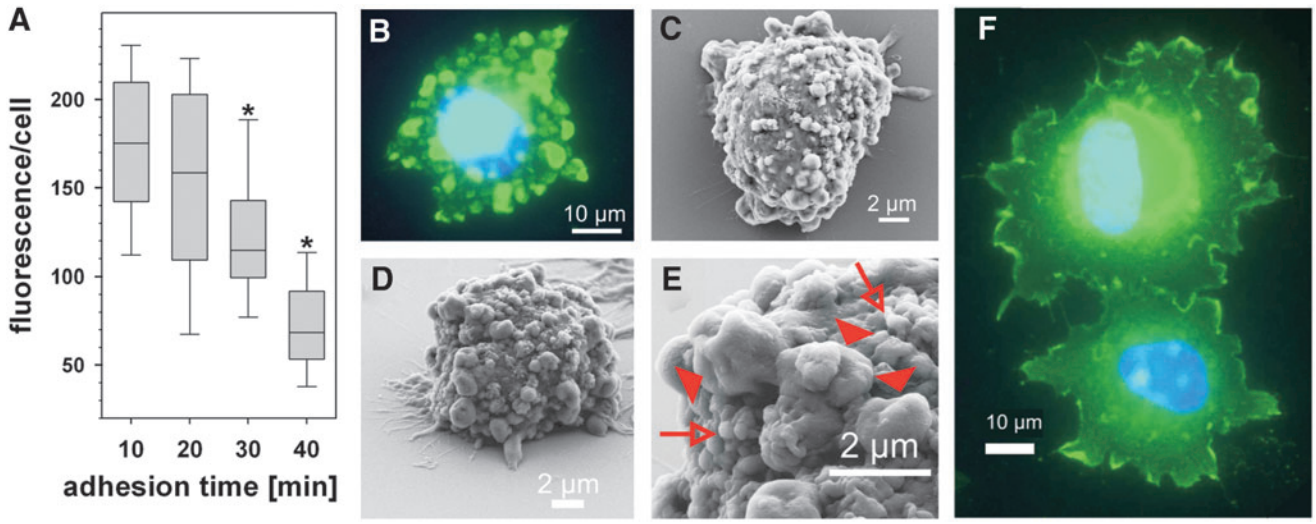

FIG. 3. ROS production is influenced by cell adhesion in a temporally transient and spatially restricted manner. Rat aorta smooth muscle cells were seeded onto laminin-111 and the thiol-based protein oxidation was monitored by immunofluorimetric detection of dimedone adducts of cysteine sulfenic acid groups. Protein oxidation decays during the course of adhesion **(A)**. Protein oxidation does not occur homogeneously across the cell but in spot-like areas, as seen about 10–20 min after cell-matrix contact **(B)**. These cell areas, named redox hotspots, represent membrane protrusions, as visualized by scanning electron micrographs **(C–E)**. The larger protrusions **(**filled *red arrowhead* in **E)**, but not the smaller membrane wrinkles **(***open arrows* in **E)** are the sites of protein oxidation. After the cell has fully spread, protein oxidation is only detected at the lamellipodia **(F)**. The figure is adapted from the authors' recent publication (28). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

the initiated cell-matrix contact has been established (Fig. 3). Thus, ROS-production and concomitant protein oxidation is regulated in an adhesion-dependent manner (29). These studies were performed with vascular smooth muscle cells, which are able to secrete H_2O_2 in a physiological range of submillimolar concentrations (139). Recently, these data have been corroborated by live cell imaging of fibroblasts, in which a fluorescent redox probe was directed to focal adhesions *via* a paxillin tag. Thereby, it was demonstrated that a platelet-derived growth factor (PDGF)-elicited ROS signal is reduced in $\alpha V\beta 3$ integrin-containing focal adhesions of fibroblasts attached onto fibronectin (94).

Leukocytes are also well-known for their capacity to release substantial amounts of ROS, especially in the respiratory burst, while they attack pathogens (28, 125). This ROS production in leukocytes is a regulated process. While extravasating and migrating towards the site of inflammation, leukocytes temporarily produce less ROS when in close contact with the matrix. This also indicates that cell-matrix contacts regulate ROS synthesis in a suppressive manner (185).

Tumor cells are generally characterized by increased ROS production as compared to normal tissue cells (43). Consequently, studies carried out with tumor cells, showed enhanced ROS production upon cell-matrix interaction. Using various inhibitors against different ROS-producing enzymes, Taddei *et al.* demonstrated an increased mitochondrial ROS-production in a fibroblastic cell line during the initial phase of cell attachment. This mitochondrial ROS production peaks at 10 min, while a second peak after 45 min is likely caused by 5-lipoxygenase (152). Recently, in invadopodia protrusions of invasive and malignant tumor cells yet another redox-relevant regulatory pathway has been identified (31). Src family members are essential signaling molecules during cell adhesion and interact with tyrosine kinase substrate (Tks) proteins-4 and -5 (Tks-4 and Tks-5). These, in turn, stimulate Nox family members to produce ROS within invadopodia (23, 30). As ROS also affect cell attachment, this may lead to a temporary stimulation of adhesion-mediated src-activation [see also the article by Giannoni and Chiarugi (47a) in this Forum].

Tissue Oxygen Tension and ROS Influence ECM Production and Deposition

Conversely, ROS and oxygen influences the production of ECM proteins at both transcriptional and (post)translational levels (115). Several genes, that code for ECM proteins, such as various collagen α-chains, fibronectin, and elastin, are transcriptionally controlled by HIFs, and hence, are preferentially expressed by cells under low oxygen tension (8, 32, 41). Among the enzymes, which post-translationally modify nascent matrix proteins, lysyl oxidase is even one of the most strongly hypoxia-regulated genes (39, 40). In addition to the hydroxylated proline and lysine residues, distinct cysteine residues within ECM-proteins are targets of ROS. Their redox modifications lead to rearrangement of inter- and intramolecular disulfide bridges and thereby to activity-relevant conformational changes (20).

While a balanced ECM production is essential for development, remodeling and regeneration, an overshooting ECM production causes excess tissue stiffness and tension. It is typical of the pathologic process of fibrosis, which can be elicited by ROS. These ROS are secreted by leukocytes after having infiltrated a site of inflammation (28, 92). Moreover, resident mesenchymal cells at the inflammation site are able to produce ROS (6).

In the classical model, developed by Gabbiani (45) and modified by Hinz (62), fibroblast differentiation into myofibroblasts is the key process of fibrosis (Fig. 4). Fibroblasts or

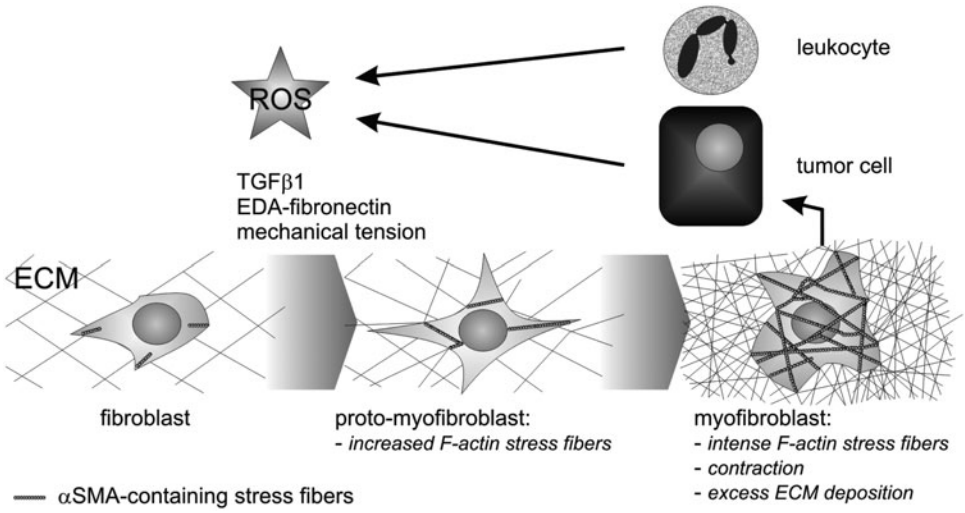

FIG. 4. ROS stimulates myofibroblast differentiation and fibrosis. TGFβ1, the EDA-splice variant of fibronectin and mechanical tension are well-known stimuli for myofibroblast differentiation, in which fibroblasts differentiate first into proto-myofibroblasts and further into myofibroblasts, which are characterized by increased expression of αSMA with intense formation of stress fibers, and by an excess synthesis and deposition of ECM, respectively. This fibrotic reaction is typical of inflammation elicited by leukocytes *via* ROS. Pathologically, the fibrotic environment of desmoplastic tumors supports tumor cell progression. Tumor cells also produce ROS, which stimulate the myofibroblast differentiation of additional fibroblasts. Thus, ROS act as an alternative stimulus for fibrosis, including the characteristic upregulation of ECM production. TGFβ1, transforming growth factor-β1; αSMA, α-smooth muscle actin; EDA, extra domain A.

other mesenchymal cells, for example, adventitial fibroblasts, pericytes, endothelial cells, and epithelial cells after epithelial-mesenchymal transition differentiate initially into a proto-myofibroblast with typically strong and contractile stress fibers. In a subsequent step, final differentiation into a myofibroblast goes along with abundant expression and deposition of ECM proteins (62, 63, 82). Key factors for myofibroblast differentiation are TGFβ1 and other growth factors (63), the cellular fibronectin isoform harboring both EDA and EDB domains (140, 157, 168), as well as enhanced mechanical load onto the matrix (157, 158). Markers for myofibroblasts are α-smooth muscle actin (αSMA), smooth muscle myosin heavy chain, desmin, caldesmon, and fibroblast-specific protein (FSP-1, or synonymously S100A4), although the latter is neither exclusively nor indispensably linked to the myofibroblast phenotype. According to these markers, myofibroblasts resemble smooth muscle cells.

Additional factors which stimulate myofibroblast differentiation are often released from immune cells infiltrating an inflamed or damaged tissue region (63). Some of these factors, such as PDGF, insulin-like growth factor, and angiotensin-II, are also known stimuli for cellular ROS production (93, 102). ROS can also elicit and influence fibrosis and ECM production at concentrations typically found at sites of inflammation (6). Hallmark matrix proteins for myofibroblast-associated fibrosis are type I and IV collagens. Type III and VI collagens, as well as tenascin-C, are also excessively deposited into the fibrotic matrix, which resembles a ontogenetically early matrix (82). ROS was reported to directly stimulate collagen synthesis in lung fibrosis (93). After ROS-stimulated expression, the alternatively spliced c-fibronectin isoform with both EDs, EDA and EDB, further stimulates myofibroblast differentiation and exacerbates fibrosis. Therefore, ROS may be considered as profibrotic agent.

Members of the nicotinamide adenine dinucleotide phosphate oxidase (NADP-oxidase, Nox) family seem to be the major source of ROS during myofibroblast differentiation. One isoform of this family, Nox4 was pinpointed as an essential player, as silencing of Nox4 expression abolishes myofibroblast differentiation and lung fibrosis (10, 17, 58). Nox4 stands apart from the other members of the Nox family [see also the article by Schröder (138a) in this Forum]. Nox proteins generally transfer single electrons from NADPH *via* an internal electron transfer chain to molecular oxygen, thereby converting it to the superoxide anion $O_2^{\bullet -}$. In contrast, Nox4 seems to transfer two electrons onto molecular oxygen, thereby producing H_2O_2. The latter has a longer lifetime and longer diffusion path. Because it diffuses through biological membranes, it serves as both intracellular and paracellular messenger molecule (44, 139). All Nox family members consist of several subunits, of which two subunits span the plasma membrane: the isoform-specific Nox subunit (*e.g.*, Nox1, gp91^{phox} = Nox2, Nox3, Nox4) and the common p22^{phox}-subunit shared by all Nox members. Several additional, cytosolic subunits associate with the Nox complexes and regulate their enzymatic activities. However, some of these regulatory subunits are missing in the Nox4 complex. This may explain why the enzymatic activity of Nox4 is not or just poorly regulated in comparison with the other Nox members. Nevertheless, it is assumed that the constitutively active Nox4 enzyme is controlled at its expression level, whereby the translation of its mRNA into the active protein seems to be an essential step. TGFβ1, the major driver of myofibroblast differentiation, increases the expression of Nox4, and silencing Nox4 expression by siRNA technology abrogates myofibroblast differentiation (12, 58). After binding to its cognate heterodimeric receptor, TGFβ1 mainly signals *via* the Smad pathway (99, 100), triggering the transcription of αSMA. In addition, TGFβ1 also increases the expression of Nox4 and the p22^{phox} subunit (12). Consequently, ROS production is enhanced and influences the Smad pathway of TGFβ1 signaling. However, the interrelation of the TGFβ1 and ROS signaling pathways has still remained elusive and may be cell-type specific (6, 12).

Fibrosis with excessive ECM production is not only triggered by ROS-secreting leukocytes. Tumor cells also secrete ROS and induce the differentiation of stromal fibroblasts into myofibroblasts (62, 124). These cancer-associated fibroblasts support the progression of such fibroblastic/desmoplastic tumors (Fig. 4).

Redox Potential and ROS Affect Cell Adhesion and Migration

In a seminal work, Go and Jones demonstrated that the extracellular redox potential in human serum varies with different (patho) physiological conditions, such as age and cigarette smoking (49). Moreover, an increased extracellular redox potential increases the expression of cell adhesion molecules, such as intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), as well as P- and E-selectins (49). This is of great importance for leukocytes to extravasate to an inflammation site, which is one of the initial triggers of inflammatory fibrosis (92). It comprises a cascade of subsequent steps: leukocytes first interact with endothelial cells, then adhere to the basement membrane underneath the endothelial cells and eventually penetrate it to migrate into the interstitial matrix. Cell adhesion molecules of the integrin family mediate such cell-cell and cell-matrix contacts. They consist of two subunits, α and β. Eighteen α- and eight β-subunits have been reported so far, which combine to form 24 functional receptors for ECM proteins or cell membrane-anchored adhesion molecules. During diapedesis, the production of ROS in leukocytes is suppressed in an ECM-dependent manner (185). After having reached the site of inflammation, leukocytes produce ROS in a Nox2-dependent manner, also known as the respiratory burst, which destroys pathogens (28). In addition, these ROS, including the far-reaching hydrogen peroxide, affect the infiltration of additional leukocytes, by increasing the expression of the leukocyte-adhesive molecules on endothelial cells, such as ICAM-1 and VCAM-1 (101). Moreover, the ligand-binding activity of the respective receptors, $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins, is increased in a thiol-dependent manner (21). The protein disulfide isomerase inhibitor bacitracin inhibits these two integrins and thus, affects leukocyte diapedesis (112). Free cysteine thiol groups within $\alpha 4\beta 1$ (90) and other integrins (104) were identified as targets for ROS on the leukocyte cell surface by protein chemical means and by proteomics.

Platelet aggregation and hemostatic thrombus formation are further examples of how adhesion is redox-regulated at the integrin level. The platelet integrin αIIbβ3 plays a crucial role in this process (9). Redox-sensitive free cysteine thiol groups within this integrin receptor and also an inhibitory

effect of bacitracin on platelet aggregation support the hypothesis of a thiol-based redox-regulation of platelet activity (88, 97, 133). In fact, platelet aggregation is influenced by reducing agents, such as glutathione (4). An extensive body of literature underlines the importance of redox-regulation in integrin-mediated platelet activation, which is discussed in more detail in the article by Murphy *et al.* (112a), in this Forum.

Integrins as Cellular Receptors for ECM Proteins

At the molecular level, cell-matrix contacts are mediated by various cell adhesion molecules. Integrins are the most numerous and versatile family of these cell adhesion molecules [recently reviewed in Refs. (5, 68)]. Both integrin subunits, α and β, span the plasmalemma once, as type I transmembrane proteins, thereby linking various components of the ECM or cellular counter-receptors [Table 1, and reviewed in Humphries *et al.* (66)] with the intracellular cytoskeleton. Thus, they mediate cell-matrix and cell-cell contacts. Due to their bidirectional signaling capabilities, integrins also serve as perceptive molecules which sense their surrounding matrix and convey these environmental cues into the cell. Integrins do not signal by themselves as they lack any kinase domain. However, they recruit adaptor and signaling molecules into adhesion-dependent organelles, called adhesomes (79, 171). As key elements of adhesomes, integrins also serve as force-transducing receptors. They are docking sites for cell-spanning actin stress fibers, which together with myosins form intracellular molecular motors. Mechanical force transmission is necessary for cell anchorage to the ECM and cell migration, as well as for tissue/matrix remodeling. This is highly relevant for fibrosis as integrin-mediated forces applied to the matrix release TGFβ1, thereby accelerating the fibrotic process (169). The physiological importance of most integrins has also been shown by genetic ablation models in mice (67, 70).

Some of the integrin recognition sites within the ECM proteins have been elucidated for the last three decades. The best-known recognition site is the linear peptide sequence arginine-glycine-aspartate (RGD) located at the tip of a peptide loop (128, 137). The RGD-bearing peptide loop within the fibronectin type III repeat 10, together with the synergy binding site within the adjacent fibronectin type III repeat 9, is the binding site for the "classical" fibronectin receptor, integrin $\alpha5\beta1$ (91). Originally discovered in fibronectin, this RGD peptide has been found to be the recognition site for several other integrins, now grouped into the subset of RGD-dependent integrins. The RGD-dependency of the αV-containing integrins, together with the key role of $\alpha V\beta3$ integrin in tumor-induced angiogenesis has spurred the development of pharmaceutical compounds mimicking the RGD motif (98). Naturally occurring hemorrhagic toxins of snake venoms also utilize RGD-containing peptide loops to block the RGD-dependent platelet integrin $\alpha IIb\beta3$, which usually binds to fibrin(ogen) after platelet activation and thus, forms a hemostatic thrombus. These disintegrins differ in their domain structure from a fibronectin type III repeat but share the very effective RGD-containing peptide loop (16). The pharmaceutical imitation of this structure has led to the new generation of antithrombotic drugs, which are in clinical use nowadays (24, 25).

Integrins $\alpha9\beta1$, $\alpha4\beta1$, and $\alpha4\beta7$ recognize the EDA-module of c-fibronectin, and hence, are good candidate receptors to elicit the c-fibronectin-induced myofibroblast differentiation and the concomitant ROS-production (168). Interestingly, the α4-integrins, along with the β2 integrins, fulfil immunological functions. They recognize an LDV or IET-sequence within a typical immunoglobulin fold (D-domain) of their cognate ligands. Both the immunologically relevant β1 integrins, $\alpha4\beta1$ and $\alpha4\beta7$, and the β2-integrins ($\alpha L\beta2$, $\alpha M\beta2$, $\alpha X\beta2$, and $\alpha D\beta2$) are all expressed in leukocytes. Interestingly, the expression of β2 integrins is upregulated by ROS (117). Integrin $\alpha4\beta1$ interacts with its ligand VCAM-1 on endothelial cells. This is an essential step for extravasation and attainment of the inflammation site (162). Due to their medical importance in inflammation, $\alpha4\beta1$ integrin and the leukocyte integrin $\alpha L\beta2$ are targets for pharmaceutical integrin antagonists, which are in clinical trials to treat immunological disorders (24).

An indispensable requirement for collagens to be recognized by their cognate integrin receptors ($\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$, and $\alpha11\beta1$) is their triple-helical quaternary structure, which presents the integrin binding motif as a special array of amino acid side chains to which all three collagen chains contribute (33–35, 59, 83, 184). The homotrimerically presented GFO-GER-sequence (O = hydroxy-proline) was identified as a prototypic integrin recognition site in collagens (83). This RGD-independent collagen-binding is achieved by an additional A-domain of the integrin α-subunit and is accompanied by substantial conformational changes within the A-domain (38). Mediating the interaction of fibroblasts with the collagen-rich stroma, the collagen-binding integrins play an important role in fibrosis (36).

Least is known about the molecular recognition site of laminin-binding integrins. These integrins bind to the globular G-domain of laminins; however, only if the neighboring α-coiled-coil laminin rod allows a binding-competent conformation (119). The laminin-integrin interaction cannot be inhibited by RGD peptides.

Integrin Structure

Both integrin subunits comprise several modules, whereby the N-terminal domains of both subunits join to form a common head, which harbors the ECM ligand interaction site (Fig. 5). Two individual stalks (or legs) of each subunit stretch out from the head domain and connect it with a single membrane-spanning motif anchored within the plasmalemma. The common head region contains a propeller domain of the α-subunit, as well as a plexin-semaphorin-integrin (PSI) domain, a hybrid domain and a βA-domain of the β-subunit (116, 174–177). The propeller domain consists of seven blades of β-sheets and is stabilized by divalent cations complexed to the ligand-distal face of the propeller domain. Between blades 2 and 3, an additional A-domain is inserted within the α-subunits of the collagen-binding β1 integrins and the leukocyte β2 integrins. This A-domain, like the A-domain of the β-subunit, is homologous to the von Willebrand factor (vWF)-A domain and folds into a typical Rossman fold (37, 129, 131). Upon ligand binding, the A-domains undergo substantial conformational changes (38), which eventually are conveyed through the entire molecule into the stalk regions; hence, mediating signal transduction through the integrin molecule (2, 141).

The stalk of the β-subunit is made of four EGF-domains and one small β-tail domain. They are rich in disulfide-engaged

Table 1. Ligand Specificity of the Known 24 Integrins

	β1[a]	β2	β3	β4	β5	β6	β7	β8
α1[b]	Collagens: Col-IV, Col-I laminins (low affinity)							
α2	Collagens: Col-I, Col-IV, laminins (low affinity), E-Cad, C1q, CLRPs							
α3	Ln-332, Ln-511, Inv							
α4	Fn (EDA, IIICS), ICAM-1 (D1/D2), VCAM-1, MAdCAM-1, Opn, TSP						MAdCam-1, VCAM-1 (D1/D2), Fn (EDA, IIICS), Opn	
α5	Fn (III9+10), Inv							
α6	Laminins, *e.g.*, Ln-111 (E8), Inv			Laminins, *e.g.*, Ln-332 in hemi-desmosomes				
α7	Laminins, *e.g.*, Ln-111 (E8), Inv							
α8	Fn, Vn, Ten-C, Opn, nephronectin							
α9	Fn (EDA), VCAM-1, Opn, tenascin							
α10	Collagens							
α11	Collagens							
αE							E-Cad (D1)	
αD		VCAM-1, ICAM-3						
αL		ICAM-1–5 (D1/D2)						
αM		ICAM-1,-2,-4 Fb, iC3b, factor X						
αX		ICAM, Fb, iC3b, collagens						
αIIb			Fb, Fn, Vn, vWF, TSP					
αV	Vn, Fn, Opn, LAP-TGFβ		Vn, Fn, Fb, vWF, Opn, BSP, TSP, tenascin, Fib, Fbu, gelatin, LAP-TGFβ		Vn, Opn, BSP	Fn, Opn, LAP-TGFβ		Vn, LAP-TGFβ

Possible combinations of α- and β-subunits are indicated. Their cognate ligands are indicated in the cells of the table. This list of proposed integrin ligands is incomplete and only best-validated integrin ligands are shown. The color coding of the ligands represents the potential integrin recognition motif.

Absence of grey shading indicates integrin ligands using the peptide sequence RGD for integrin binding.

Light grey shading marks the LDV or IEV sequence within an immunoglobulin fold (D-domain), which is recognized by the immunological relevant integrins.

Dark grey shading highlights the collagen and laminin ligands, which require a characteristic three-dimensional structure (such as the collagenous triple-helix) to be recognized by their respective integrin receptor.

[a]First row indicates the different integrin β-subunits.

[b]First column indicates the different integrin α-subunits. Alpha-subunits that contain an A-domain are underlined.

Potential integrin ligands: BSP, bone sialoprotein; CLRP, C-type lectin-related protein (found in snake venom); Col, collagen (Roman numerals indicate the type of collagen); D, D-domain of an immunoglobulin fold (with Arabic numbers specifying its position within the molecule); E-Cad, E-cadherin; EDA, EDB, extra domains A and B, respectively, of fibronectin; Fb, fibrin(ogen); Fib, fibrillin; Fbu, fibulin; Fn, fibronectin; iC3b, immobilized complement factor 3b; ICAM, intercellular cell adhesion molecule; Inv, invasin; LAP-TGFβ, complex of latency activating peptide and TGFβ; Ln, laminin; MAdCAM, mucosal addressin cell adhesion molecule; Opn, osteopontin; Ten-C, tenascin-C; TSP, thrombospondin; VCAM-1, vascular cell adhesion molecule-1; Vn, vitronectin; vWF, von Willebrand factor; IIICS, fibronectin type III connecting sequence; RGD, arginine-glycine-aspartate; TGFβ, transforming growth factor-β.

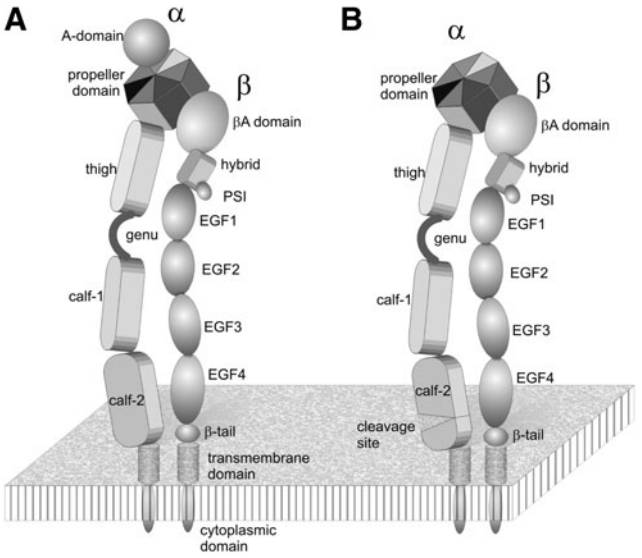

FIG. 5. Domain structure of integrins, containing (A) and lacking (B) an A-domain within their α-subunits. The propeller domain and A-domain of the α-subunit, together with the PSI, hybrid, and Aβ-domain of the β-subunit form the joint headpiece of the integrin, which binds to the extracellular ligand. Two separated stalks/legs point out of the headpiece and contain different modules lining up like pearls on strings: the thigh, genu, and the two calf domains of the α-subunit and the EGF1–4 and β-tail domain of the β-subunit. Both subunits are anchored within the cell membrane with a single-span α-helical transmembrane domain. The short cytoplasmic C-terminal domains of the two subunits interact with different cytoskeletal, adaptor, and signaling molecules, which enable the integrin to link the ECM to the cytoskeleton across the plasmalemma and to convey environmental cues by outside-in and inside-out signaling. PSI, plexin-semaphorin-integrin.

cysteine residues (76, 109, 172), whereas the stalk of the α-subunit is shaped of three β-strand-rich domains, a thigh and two calf-domains. The α-subunit leg contains only a few cysteine residues. The two calf domains share a homologous domain structure, each consisting of two β-sheets of five and four β-strands (Fig. 6). The calf-2 domains of those integrin α-subunits which lack an A-domain contain a cleavage site, the proteolysis of which results in a heavy and a light chain. They are covalently cross-linked *via* a disulfide-bridge between β-strands 6 and 7 (Fig. 6B). This disulfide bridge might not be necessary as the β-strands of heavy and light chains interdigitate and the numerous noncovalent interstrand bonds stabilize the two β-sheets of the calf-2 domain. The proteolytic cleavage of the integrin α-subunit is structurally interesting with respect to the newly generated, floppy N-terminus of the light chain, which allows an increased flexibility of the N-terminal β-strand 7 within the calf-2 domain (Fig. 6).

The genu region within the leg of the integrin α-subunit is of special importance for integrin activation. It acts as pivot around which the head domain and the two stalk domains can rotate relative to each other and thus, change the conformation of the integrin ectodomain between a bent and an extended/upright conformation (Fig. 7). The genu peptide of the integrin α-subunits consists of a short peptide loop, which is cross-linked by an intrachain disulfide-bridge.

As opposed to the cysteine-poor stalk domains of the α-subunit, the stalk domains of the integrin β-subunits are rich in cysteine residues. For example, there are eight cysteine residues in each EGF-domain and several ones in the β-tail domain (76, 109, 172). However, the cysteine residues within the stalk of the β integrin subunit are engaged in disulfide bridges, which stabilize the stalk domains. According to our studies, the cysteine side chains of the β1 subunit stalk are not targeted by hydrogen peroxide and hence, they cannot be redox-regulated by oxidizing agents (29).

Conformational Changes and Thiol-Based Redox-Regulation of Integrins

Both the ligand-binding head domain and the two legs play pivotal roles in integrin activation and regulation (2, 141, 145, 146, 153, 173). The head domain apparently is not susceptible to redox-regulation with the exception of one long-range disulfide bridge within the integrin β-subunit. Among the two stalks, only the leg of the integrin α-subunit, but not of the β-subunit seems to contain cysteine residues, which are sensitive to oxidizing agents. Several studies identified redox-relevant cysteine bridges *via* their susceptibility to reducing agents (15, 76, 77, 85). In contrast, ROS and other oxidizing agents are able to not only establish disulfide bridges, but also to prevent their formation when two vicinal disulfide bridges are concomitantly oxidized to cysteine sulfenic acid groups (29, 109). This may explain the observed different effects of ROS on thiol-based modulation and activation of integrins in comparison to studies, which employ the reductive cleavage of intramolecular disulfide bridges.

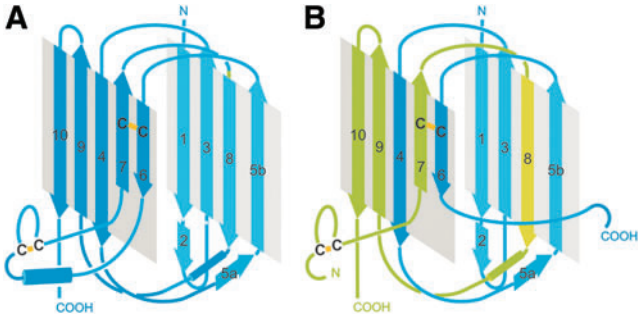

FIG. 6. Structure of the calf-2 domain of the integrin α-subunit, either with (A) or without a proteolytic cleavage site (B). Five and four antiparallel β-strands (numbered 1, 3–10) form two β-sheets, which fold into the stable tertiary structure of the calf-2 domain. **(A)** In α-subunits, which contain an A-domain, the peptide chain within the calf-2 domain is not cleaved. **(B)** The integrin α-subunits lacking A-domains are generally cleaved in a loop between β-strands 6 and 7 of the calf-2 domain, resulting in a heavy and light chain of the α-subunit (marked in blue and green, respectively). Although there is an interchain-disulfide bridge between β-strands 6 and 7, the strong interdigitation of the β-strands is likely to hold the heavy and light chains together after the proteolytic cleavage. The proteolytic cleavage generates a new N-terminus of the light chain which allows a higher flexibility of β-strand 7. The secondary structure elements are sketched in **(A, B)** according to the crystal structure of the integrins, αXβ2 and αVβ3, respectively (174, 175, 176). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

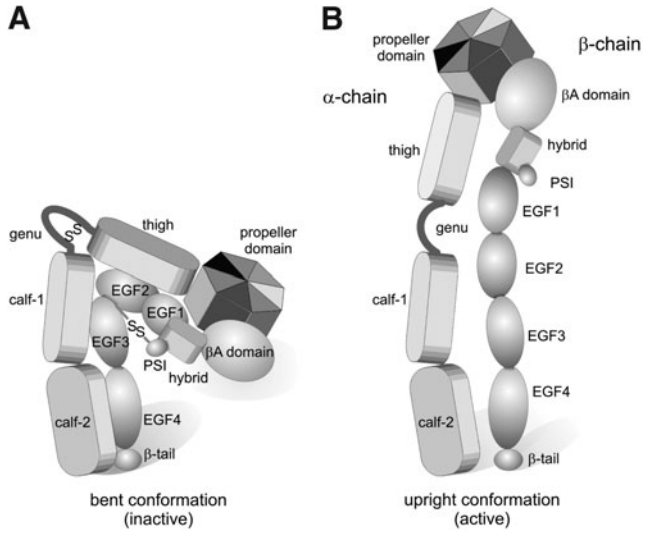

FIG. 7. The two major conformations of the integrin ectodomain: bent (A) and upright/extended (B). The genu region acts as a pivot around which the head domain and the stalks of the integrin ectodomain can hinge against each other, leading to a bent **(A)** and an upright **(B)** conformation. Low and high binding activities are ascribed to the bent and upright conformations, respectively. A disulfide bridge within the genu region of the α-subunit and the long-range disulfide bridge between the PSI and the EGF-domains of the β-subunit are indicated. Their redox-regulated cleavage is likely involved in the conformational transition of the integrin ectodomain.

Experimental works on conformational changes within the head domain mainly highlight the A-domains within the β-subunits (βA) and the α-subunits (αA) of the collagen-binding and leukocyte integrins. While there is no evidence for activation-related conformational changes within the propeller and PSI domains of the integrin α and β-subunits, the A-domains of both integrin subunits undergo major conformational changes. The structure-function relationship of an A-domain has been unraveled by Emsley *et al.* (38). They demonstrated that collagen-binding to the integrin α2 A-domain causes unfolding of an αC-helix and eventually results in a translational shift of α-helices 7 and 1 against each other by almost 10 Å, a tremendous movement in molecular scale. Although A-domains from the leukocyte integrin α-subunits lack this αC-helix, they also undergo such a conformational shift of homologous α-helices (22, 147). A similar change is envisioned for the Aβ-domain of αVβ3 upon RGD-binding (177). The translational shift of the two piston-like α-helices against each other is potently amplified through a hinge between the Aβ domain and hybrid domain and leads to the transition from the bent into the upright conformation of the integrin ectodomain (2, 141, 145, 146, 153). Springer *et al.* (145, 146, 174, 180) proposed a mechanism of conformational signal transduction within the αA-domain-containing integrins. According to such a rope belt/pull spring mechanism, the conformational change of the αA-domain after ligand binding induces the exposure of a glutamic acid side chain at the ligand-distal face of the αA-domain, which then serves as intrinsic ligand for the Aβ-domain. Similar to ligand occupancy, this triggers the conformational change of the Aβ domain and leads to a bent-to-upright transition of the integrin receptor and to the separation of the two legs.

Despite its importance in ligand binding, there is little evidence that the head domain is redox-regulated at the level of thiol groups. The head domain, including the αA-domain, contains several cysteine residues. Yet, it does not seem to be affected by reducing agents or ROS. Moreover, mutation of cysteines within the Aβ-domain of the β3 subunit did not show any effect on the ligand-binding activity of the platelet integrin (19). An exception to this redox insensibility of the head domain is a long-range disulfide bridge between the PSI and a peptide sequence within the EGF-domains 2 and 3 of the β-subunit stalk. Its importance for activation of the platelet integrin αIIbβ3 was proven by protein-chemical, mutational and crystallographic studies (15, 144, 150, 178, 179), which demonstrated how this disulfide bridge constrains the integrin in its inactive bent conformation (Fig. 7). Such a long-range disulfide bridge of similar physiological importance also exists in the β2 subunit (182) and likely in other β integrin subunits as well (7, 144, 154). Its redox-dependent cleavage is a prerequisite for the integrin's transition into the active upright position (144, 150).

Redox-dependent conformational changes occur within the integrin legs. The high number of cysteine residues within the stalk of the β-subunit makes this region highly likely for redox-regulation (76, 109, 144, 154). However, instead of having a regulatory role, these cysteine residues are probably engaged entirely in structure-stabilizing disulfide bridges within and between the EGF-domains, although conformation-stabilizing and regulatory effects are sometimes difficult to distinguish from each other. Mutations of two individual cysteines within the EGF-domains 1 and 3 of β3 reduced the expression of αIIbβ3 integrin in Glanzmann thrombasthenia patients (105), whereas mutation of the most membrane-proximal disulfide-bridge within the β-tail-domain leads to a constitutively active platelet receptor (14). Redox-regulation by a few distinct cysteines is indicated by the work of Mor-Cohen *et al.* (109), as the mutation of disulfide bridged cysteine residues only affect integrin activity when performed with single cysteine residues, but not with an even number of cysteines, indicating that free thiol groups at certain positions within the leg EGF-domains may act as redox-regulated entities. However, there might be differences in the redox-regulation of EGF-domain-based cysteines between the two integrins αIIbβ3 and αVβ3, although they share the same β3 integrin subunit (108). This indicates that the partnering α-subunit also affects thiol-based redox regulation within the β integrin leg domains.

Thiol-based redox-regulation occurs within the stalk region of the integrin α-subunits. Our own work on the laminin-binding α7β1 integrin, in which we analyzed redox-modifications of this β1 integrin with hydrogen peroxide, highlighted six cysteine residues as selective targets for ROS. They are located at two sites exclusively within the integrin α7 subunit: the genu and the calf-2 domain (29). The genu region of the integrin α-subunit consists of a peptide loop, which contains a disulfide bridge. Opening this endogenous intrachain cross-link allows a higher flexibility of this pivot. This permits the integrin ectodomain to hinge from an inactive/bent to an extended/active conformation. In line with this, introducing a clamping disulfide bridge adjacent to the genu loop of the αIIb subunit yields an inactive αIIbβ3 integrin (11, 77). Although

the cysteine residues within the α-subunit calf-2 domain are limited to a number of four, protein-chemical and crystallographic analysis revealed some differences in the disulfide bonding within this domain (85). These discrepancies might not only be due to methodological limitations, for example, by the fact that the integrin ectodomains are crystallized in their bent conformations (174, 176), but may reflect the disulfide-based conformational flexibility of the calf-2 domain. Therefore, we postulate the existence of three different conformations of the calf-2 domains, which differ in cross-linkage of a cysteine residue within the β-strand 6 of the heavy chain with one of the first three cysteines of the N-terminal end of the light chain (29) (Fig. 8). Newly generated by proteolytic cleavage, the N-terminus of the light chain is flexible and likely facilitates the existence and/or transitions between these conformations. As the N-terminal sequences of the α-subunit light chains flanking the three different cysteine residues mostly support β-strand folding, the conformational changes could be envisioned as sliding of the β-strands 6 and 7 against each other within the calf domain. Due to the anchoring disulfide bridge, conformational changes would require free thiol groups, which after transition can be fixed again rendering either all four cysteine residues engaged in disulfide bridges again (conformation I and III) or partially unlinked as free thiol groups (conformation II). These conformations not only vary in the length of the free N-terminal end of the light chain, but also in the size of the peptide loop between β-strands 7 and 8, which could cause far-ranging conformational transitions, including the extension of the bent to the activated integrin. Interestingly, the calf-2 domain of the integrin α-subunit forms an interface with the EGF4-β-tail domain of the β-subunit (174, 176), which is relevant for integrin activation (77, 78, 165).

The equilibrium between free thiol groups of cysteine residues and disulfide bridges within proteins is the basis for a reversible redox-regulation and is shifted by reducing agents, oxidants and even photolytically by UV-C light (161). Reducing agents, such as the physiological glutathionine, cleave disulfide bridges and thus, unlock cystine-stabilized tertiary structures. Oxidants, such as the membrane-permeable hydrogen peroxide and other ROS, oxidize cysteine thiolate reversibly into sulfenic acid groups or into disulfide bridges, if two cysteine residues are in close vicinity. However, concomitant oxidation of such vicinal cysteine residues into two sulfenic acid groups also prohibits disulfide cross-linkage. Despite the even number of cysteines able to pair entirely into disulfide bridges, free thiol groups have been detected in integrins under physiological conditions, indicating such a redox-relevant equilibrium between free and bridging cysteine residues (97, 104, 133). In addition to noncatalyzed redox reactions, protein disulfide isomerase and redox-relevant chaperones have been reported to associate with integrins on the cell surface, thereby regulating integrin activity (87, 151). However, the correlation between redox state and activity of integrins has not been studied conclusively yet. Moreover, other redox-related modifications of integrins, such as nitrosylation, need yet to be analyzed.

So far, the experimental data provide compelling evidence, that integrins are redox-regulated. Cysteine residues within the genu and calf-2 domain of the integrin α-subunit, as well as a long-range disulfide bridge between the head domain and the leg of the β integrin subunits have been pinpointed to be the redox-regulated sites within the integrin ectodomain. Their redox-modification influences the transition between a bent/inactive and extended/active conformation of the integrin ectodomain. Consequently, these conformational transitions within the head domain induce the physical separation of the two stalks and lead to changes within the transmembrane and cytoplasmic domains (166, 186). This restructuring then transduces a signal onto integrin-associated molecules (95, 103, 141). Thus, the redox-regulated conformational rearrangements of the integrin structure are translated into cell-matrix

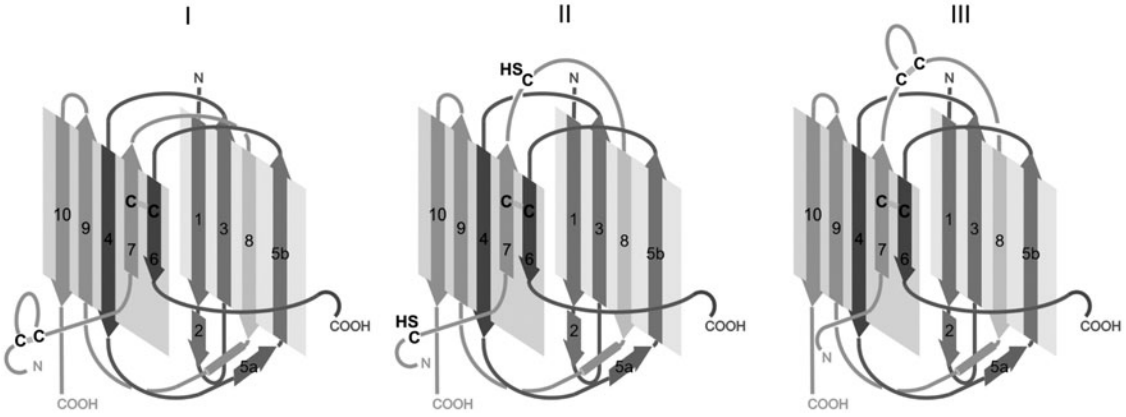

FIG. 8. Disulfide bridge-dependent conformation of the calf-2 domain. The calf-2 domain of the cleaved integrin α-subunits consists of a heavy and a light chain, marked in dark and light grey. It contains four cysteine residues. With the assumption that the cysteine residue of β-strand 6 is considered as the anchor point, it can be cross-linked to the three cysteine residues of the light chain in three different ways, resulting in conformations I–III. Interestingly, the amino acids neigboring the light chain cysteines can support β-strand folding in most α-subunits. Moreover, structural analyses of different integrin ectodomains crystallized in the bent conformation (174, 176) showed the calf-2 domain in conformation I, whereas protein chemical analyses of integrins in solution (85) indicated other disulfide patterns within the calf-2 domains, which comply with conformations II and III. This suggests that thiol-based redox-regulation may affect the conformation of the calf-2 domain, which as part of the alpha subunit leg affects the entire structure, and hence, activity of the integrin.

contact-modulating signals that eventually influence diverse physiological and pathological processes.

Concluding Remarks

In the last decade, it has been demonstrated that hydrogen peroxide and other ROS, at physiological concentrations, are crucial for cell signaling and redox-regulation, instead of just causing oxidative stress and damage. Cell-matrix interaction and ECM turnover are redox-regulated processes. While the ECM is modified by ROS, it also affects the cellular ROS production. Integrins are pivotal in cell-matrix cross-talk and are modified and thus, modulated in their activity by ROS. Conformational changes in the integrin structure suggest a mechanism of this redox-regulation of integrin activity. In future, treatment of diseases in which redox-regulated processes are involved, such as fibrosis and cancer, may benefit from understanding the redox-regulation of integrin-mediated cell-matrix interaction.

Acknowledgments

This work was financially supported by the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007–2013/under the REA grant agreement nr. 316610 (CAFFEIN) and by Deutsche Forschungsgemeinschaft (DFG, SFB815, project A6). We thank Patricia Niland, Stephan Niland, and Shaughn Daniel for proofreading the manuscript. We apologize to all experts in the field whose publications we could not cite due to the restricted number of references.

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Date of first submission to ARS Central, March 11, 2013; date of final revised submission, August 29, 2013; date of acceptance, September 16, 2013.

Abbreviations Used

IIICS = fibronectin type III connecting sequence
αSMA = α-smooth muscle actin
A-domain = von Willebrand factor A-homologous domain
BSP = bone sialoprotein
CLRP = C-type lectin-related protein
Col = collagen
E-Cad = E-cadherin
ECM = extracellular matrix
ED = extra domain
FAO = fatty acid oxidase
Fb = fibrin(ogen)
Fbu = fibulin
Fib = fibrillin
Fn = fibronectin
FSP = fibroblast-specific protein
GAG = glycosaminoglycan
HDAC-2 = histone deacetylase-2
HIF = hypoxia-inducible transcription factor
ICAM-1 = intercellular cell adhesion molecule-1
iC3b = immobilized complement factor 3b
Inv = invasin
LAP = latency activating peptide
Ln = laminin
LTBP-1 = latent TGFβ1-binding protein-1
MAdCAM = mucosal addressin cell adhesion molecule
MMP = matrix metalloproteinase
Nox = nicotinamide dinucleotide phosphate (NADPH)-oxidase
Opn = osteopontin
PDGF = platelet-derived growth factor
PDH = pyruvate dehydrogenase
PDK = pyruvate dehydrogenase kinase
PI3K = phosphatidyl-inositol-3-kinase
PPP = pentose phosphate pathway
PSI = plexin-semaphorin-integrin
RGD = arginine-glycine-aspartate peptide sequence (*one-amino-acid-code*)
ROS = reactive oxygen species
SOD-2 = superoxide dismutase-2
Ten-C = tenascin-C
TGFβ1 = transforming growth factor-β1
Tks = tyrosine kinase substrate
TSP = thrombospondin
VCAM = vascular cell adhesion molecule
Vn = vitronectin
vWF = von Willebrand factor