Comment

Syndecan-regulated Receptor Signaling

Alan C. Rapraeger

Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, Wisconsin 53706

The syndecans are transmembrane heparan sulfate (HS) proteoglycans expressed on all adherent cells (Bernfield et al., 1999; Rapraeger and Ott, 1998). A family of four, they have diverse functions ranging from participation in cell–cell adhesion, regulation of the signaling of HS binding growth factors, and organization of cell–matrix adhesion and signaling. A paper published in this issue of *The Journal of Cell Biology* (Iba et al., 2000) provides novel information on the specificity of syndecans in carrying out the latter function.

Address correspondence to Alan C. Rapraeger, Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, 1300 University Avenue, Madison, WI 53706. Tel.: (608) 262-7577. Fax: (608) 265-3301. E-mail: acraprae@facstaff.wisc.edu

The syndecan core proteins have several important domains, although much remains to be learned about their respective functions (Fig. 1; Bernfield et al., 1999; Rapraeger and Ott, 1998). The syndecans may function with several types of receptors. They are expressed at cell–cell adhesion sites (Fig. 2 A), e.g., syndecan-1 on epithelial cells and syndecan-2 in neuronal synapses. Here, they are expressed with the PDZ protein CASK and the cytoskeletal protein 4.1, and β-catenin linked to cadherins (Cohen et al., 1998; Hsueh and Sheng, 1999). All three of these cytoplasmic proteins have nuclear functions and CASK binding to syndecans has been shown recently to alter its nuclear targeting (Hsueh et al., 2000). This suggests that coregulation of cadherins and syndecans may have important outcomes in the nucleus.

HS-binding growth factors (FGFs, VEGF, HGF, etc.)

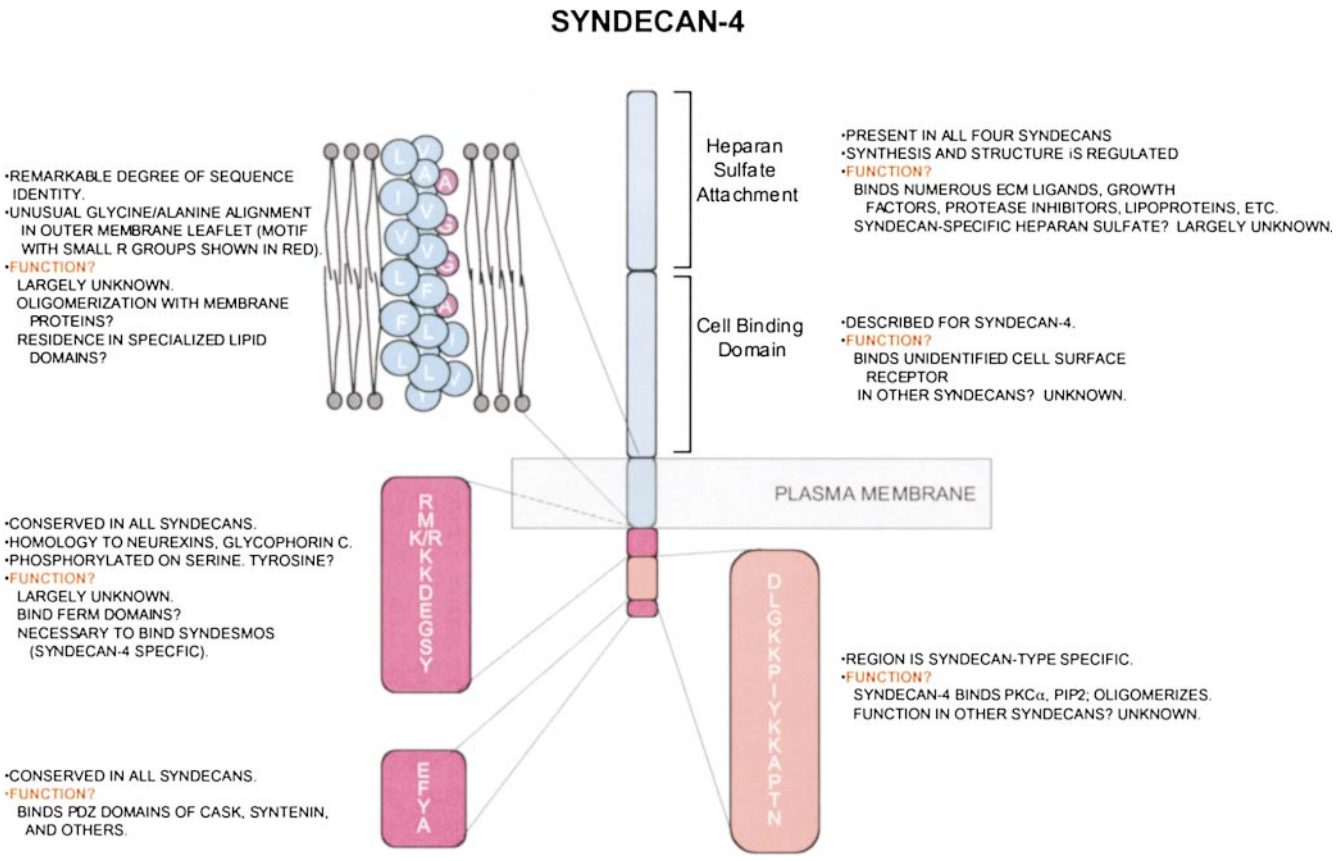

Figure 1. Syndecan functional domains. The extracellular, transmembrane and cytoplasmic domains of the syndecans contain important features, but the exact roles of these regions and how their function may be regulated remains uncertain.

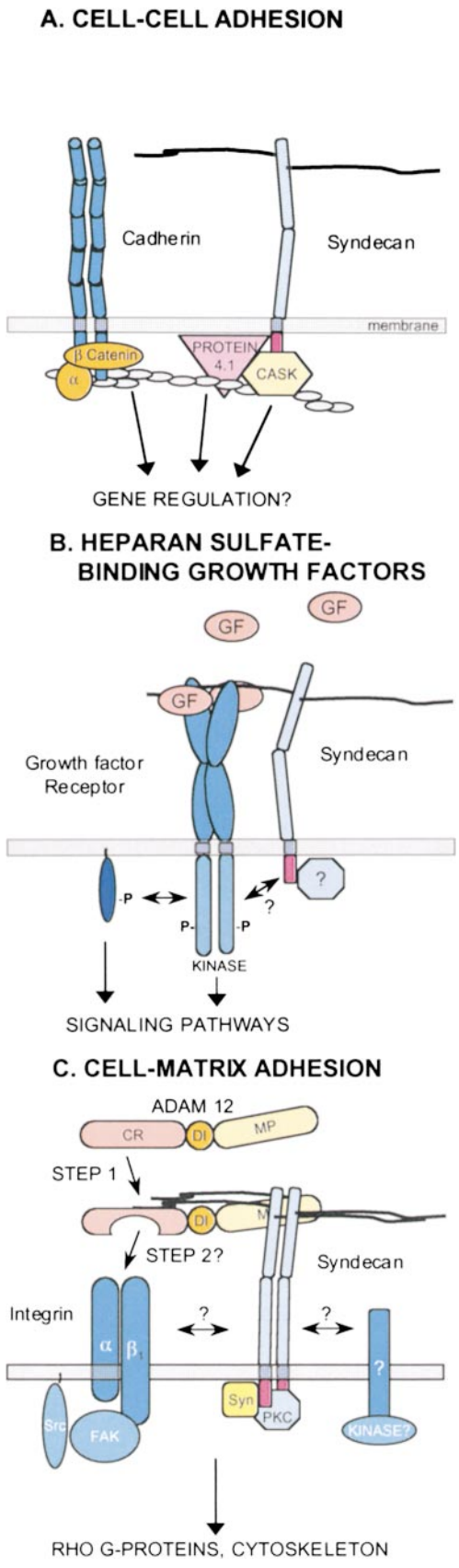

Figure 2. Syndecan-regulated signaling. Speculative examples of signaling mechanisms regulated by syndecans. (A) Cell–cell adhesion. Syndecan localized to sites of cell–cell adhesion (epithelial adherens junctions, neuronal synapses) may regulate the distribution of cytoskeletal/nuclear proteins CASK, protein 4.1 and β-catenin. (B) Signaling by HS-binding growth factors. Syndecan HS binds growth factors (GF) and growth factor receptors, regulating their assembly (positively or negatively) into signaling complexes. (C) Cell–matrix adhesion. Syndecans (syndecan-4) participate with integrins in focal adhesion assembly. Here, binding to ADAM 12 (step 1) may trigger syndecan core protein interactions with β1 integrins or unidentified signaling partners, leading to integrin activation. Alternatively, HS binding to the ADAM 12-cys region (CR) may alter CR domain conformation (step 2), exposing a cryptic binding site for β1 integrin binding and activation. This activation leads to focal adhesion and stress fiber formation, suggesting the participation of syndecan-4 and its associated syndesmos (Syn) and protein kinase C-α (PKC). DI, disintegrin domain; MP, metalloproteinase domain; FAK, focal adhesion kinase).

are highly regulated by HS, perhaps reflecting the ability of an HS proteoglycan to harbor specific binding sites within the architecture of its chains (Lindahl et al., 1998). In the case of FGF, the HS binds not only the growth factor but also the receptor, thus forming a ternary complex that includes the HS chain (Fig. 2 B; Rapraeger, 1995). The role of the core protein in this signaling is almost wholly unknown. However, direct interactions with the growth factor receptor or altered interactions of the core protein with adhesion receptors or signaling components (e.g., as shown in Fig. 2, A and C) are possibilities.

A third scenario for syndecan-mediated regulation is shown for cell–matrix adhesion (Fig. 2 C) and reflects the work by Iba et al. (2000) in this issue. The adhesion involves ADAM 12 (a disintegrin and metalloproteinase). Iba et al. (2000) show that a cysteine-rich domain (ADAM 12-cys) binds HS and serves as a substratum for cells bearing cell surface HS proteoglycans. Using the ADAM 12-cys domain as an affinity matrix, the authors isolate syndecan-4 from cell lysates of rhabdomyosarcoma cells that also express syndecans-1 and -2. Participation of syndecan-4 in this process is not surprising, as the cells form focal adhesions and stress fibers on the ADAM 12-cys domain, a process in which integrins and syndecan-4 cooperate (Couchman and Woods, 1999). Indeed, integrins are involved, as spreading on ADAM 12-cys does not occur if β1 integrins are absent or inactivated. However, it is surprising that syndecan-4 would emerge from a screen relying on HS rather than core protein binding. This raises several questions. Is the syndecan's sole interaction with ADAM 12-cys through its HS chains? Is this specific for syndecan-4 to the exclusion of other syndecans and other HS proteoglycans?

There is scant evidence to date that HS is syndecan-type specific. Such evidence awaits further progress in the difficult arena of HS sequencing. Confirmation of HS binding ADAM 12-cys is shown by Iba et al. (2000) using syndecan-null ARH77 myeloma cells, which can be transfected with native or mutant syndecans. Adhesion to ADAM 12 is clearly HS dependent, but is seen with cells expressing either syndecan-4 or -1. This casts doubt on the strict specificity of syndecan-4 binding, as the authors acknowledge, although leaving open the possibility that the HS specificity may not be preserved in the ARH77 cells.

Syndecan core protein participation is also an important issue. The adherence of the ARH77 cells expressing syndecans provides more information, as cells expressing native syndecan-1 or -4 adhere but do not spread, and cells expressing syndecan-1 with a truncated cytoplasmic domain fail to adhere to ADAM 12-cys altogether. This contrasts with Raji lymphoid (Lebakken and Rapraeger, 1996) and ARH77 (Sanderson, R.D., personal communication) cells expressing syndecan-1 and adhering to other ligands, e.g., fibronectin or anti-syndecan antibodies, where the syndecan mediates cell spreading with or without a truncated cytoplasmic domain. This signaling mechanism, in which the syndecan transmembrane or extracellular domains presumably interact with an active but unknown signaling partner (Lebakken and Rapraeger, 1996), may be an important aspect of the cell's response to the ADAM 12 protein. Why does this fail in the ARH77 cells binding ADAM 12-cys? The affinity of the binding may be low, suggesting that the syndecan cytoplasmic domain may cluster or position the syndecan to strengthen the adhesion. However, the failure of the ARH77 cells to spread, whether expressing either native or truncated syndecans remains a puzzle, particularly as they express β1 integrin. Is it possible that a component is missing in the ARH77 cells? Or does the failure trace to their origin as tumor cells?

A final question focuses on how the syndecan works in concert with the β1 integrin. A crucial point from previous work is that mammary carcinoma cells bind to ADAM 12-cys, but fail to spread unless β1-integrins are artificially activated (Iba et al., 1999). This points to an important difference between normal and tumorigenic cells. Is the integrin activation regulated by the syndecan? If it is, how might this occur? A model proposed by Iba et al. (2000) is that HS binding to the ADAM 12-cys protein exposes a cryptic site for integrin binding (Fig. 2, steps 1 and 2). If true, this places additional importance on understanding the potential syndecan (and its HS) specificity in the interaction and raises questions about altered HS specificity in carcinoma cells. Another possibility is that a syndecan binds to the ADAM 12 protein and provides signals that activate the integrin (step 1 alone) without the integrin binding the ADAM 12-cys domain. Of course, a combination of these events is also a possibility. How might the syndecan signal? The range of possibilities is dictated by whether this is syndecan-type specific. If the binding is specific for syndecan-4, then the interactions include oligomerization of syndecan-4 with PIP2, PKCα and syndesmos, which are known to promote focal adhesion and actin stress fiber formation (Couchman and Woods, 1999; Baciu et al., 2000) and potential interactions between signaling receptors and the syndecan transmembrane and/or extracellular domain.

Regardless of the mechanism, Iba et al. (2000) describe an important regulation of integrin activity by syndecans that poses questions about HS specificity, the function of individual syndecan core proteins, and the manner in which the syndecan HS chains and core proteins act in unison to regulate a signaling mechanism. As is the case for most intriguing papers, the work raises numerous questions for each that it answers and suggests new avenues of investigation for workers in the field.

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