A Novel Role of Follistatin, an Activin-binding Protein, in the Inhibition of Activin Action in Rat Pituitary Cells

ENDOCYTOTIC DEGRADATION OF ACTIVIN AND ITS ACCELERATION BY FOLLISTATIN ASSOCIATED WITH CELL-SURFACE HEPARAN SULFATE*

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Osamu Hashimoto‡§, Takanori Nakamura‡, Hiroki Shoji‡, Shunichi Shimasaki†, Yoshiiro Hayashi§, and Hiromu Sugino‡§

From the ‡Institute for Enzyme Research, University of Tokushima, Kuramoto, Tokushima 770, Japan, the §Department of Veterinary Anatomy, Faculty of Agriculture, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan, and †Department of Reproductive Medicine, School of Medicine, University of California, San Diego, La Jolla, California 92037-0674

There are two types of the activin-binding protein follistatin (FS), FS-288 and FS-315. These result from alternative splicing of mRNA. FS-288 exhibits high affinity for cell-surface heparan sulfate proteoglycans, whereas FS-315 shows low affinity. To understand the physiological role of cell-associated FS, we investigated the binding of activin to cell-associated FS and its behavior on the cell surface using primary cultured rat pituitary cells. Affinity cross-linking experiments using 125I-activin A demonstrated that activin bound to rat pituitary cells via FS as well as to their receptors on the cell surface. FS-288 promoted the binding of activin A to the cell surface more markedly than FS-315. When the cells were incubated with 125I-activin A in the presence of FS-288, significant degradation of activin A was observed, and this was dependent on the FS-288 concentration. This activin degradation was abolished by heparan sulfate, chloroquine, and several lysosomal enzyme inhibitors. Moreover, FS-288 stimulated cellular uptake of activin A, whereas chloroquine suppressed lysosomal degradation following internalization, as demonstrated by microscopic autoradiography. These results suggest that cell-associated FS-288 accelerates the uptake of activin A into pituitary cells, leading to increased degradation by lysosomal enzymes and thus plays a role in the activin clearance system.

Three gonadal peptide factors, inhibin (1–4), activin (5, 6), and follistatin (FS)† (7, 8) have been isolated and characterized by their ability to regulate follicle-stimulating hormone (FSH) synthesis and secretion by the pituitary. Inhibin and follistatin inhibit, and activin stimulates, FSH release from the pituitary both in vitro and in vivo. Inhibin and activin are structurally related and belong to the transforming growth factor (TGFB) superfamily. Both are disulfide-linked dimers; inhibin is composed of α- and β-subunits, whereas activin is a dimer of the inhibin β-subunit. Two forms of inhibin (A and B) and three forms of activin (A, AB, and B) have been isolated from ovarian follicular fluid. These forms arise because of the existence of two homologous but distinct β-subunits, called βA and βB (5, 6, 9–12).

In addition to stimulation of FSH synthesis and release, various biological roles of activins outside the reproductive system have been extensively investigated. Almost all functions associated with activins can now be interpreted by their proliferative, antiproliferative, and differentiative activities. In view of the widespread expression of activin subunits in both the embryo and the adult (13), as is also the case with activin receptors, it is not too surprising that activin effects have been noted in a multitude of diverse tissues and cell types. Activins exert their effects through specific binding to two different types of receptors, called types I (molecular mass ~53 kDa) and II (molecular mass ~70 kDa) that have recently been cloned. Types I and II receptors, which belong to the serine/threonine kinase receptor family, form heteromeric receptor complexes that are essential for signal transduction after ligand binding, but little is yet known about the events immediately following receptor activation (14).

The activity of FS resembles that of inhibin, but its structure is quite different because FS is a glycosylated single-chain protein (7). There are two forms of FS, resulting from alternative mRNA splicing and hence two different mRNAs that encode FS-315 and its carboxyl-terminal truncated homologue FS-288 (consisting of 315 and 288 amino acids, respectively) (15). Because of the widespread distribution of FS mRNA in extraglandular tissues, physiological roles of FS other than FSH suppression have been predicted (16). In line with these expectations, we previously demonstrated that FS is an activin-binding protein (17) and neutralizes the diverse activin bioactivities in various systems by stoichiometrically forming inactive complexes with activins (18). We have also shown that FS is capable of associating with cell-surface heparan sulfate proteoglycans and proposed that FS participates in the regulation of the multiple actions of activin (19). The affinities of the two FS proteins, purified from porcine ovaries, for activins were demonstrated to be essentially similar. However, the affinity of FS-288 for heparan sulfate side chains was found to be much higher than that of FS-315 (20). The widespread and similar tissue distributions of both FS and the β-subunit mRNAs imply that FS and activin proteins are produced locally and that FS acts as a local modulator of activin activity. This assumption is supported by the findings that activin binding to FS inhibits the effect of activin on granulosa (21), embryonal...
Affinity Cross-linking—The cells were washed once with binding buffer (DMEM containing 25 mM HEPES (pH 7.4) and 0.2% bovine serum albumin) and incubated on ice for 2 h with 40 ng/ml 125I-activin A in the presence or absence of unlabeled activin A in the binding buffer. After incubation, the cells were washed three times with ice-cold PBS and incubated in PBS containing 1 mg disodium phenylglycerol 20 mM sodium pyrosulfite and 0.05% CHAPS. The column was eluted with the buffer for 2 h at 37°C. After washing three times with fresh medium, 100 μl of 10% SDS was added to each well to solubilize the cells, and the radioactivity (cell-bound FS) was quantified by γ-spectrometry. To examine the association of the activin-FS complex with the cell surface, 125I-activin A (40 ng/ml) and FS-288 or FS-315 (200 ng/ml) were preincubated in DMEM containing 10% horse serum and 2.5% FBS for 1 h at 37°C to ensure complete formation of the complex. The resulting complex fraction (10 μl) was added to the pituitary cell culture (8 × 10⁴ cells/well). The amount of the cell-bound complex was determined as described above. The effect of heparan sulfate on binding was investigated by co-incubation with 10 μg/ml heparan sulfate.

Treatment of Rat Pituitary Cells with Glycosaminoglycan-degrading Enzymes (GDE)—Rat pituitary cells were grown in 96-well plates (8 × 10⁴ cells/well) in the presence or absence of FS (200 ng/ml) for 12 h at 37°C to ensure complete formation of the complex. The incubation medium was collected at various incubation times. An equal volume of 30% trichloroacetic acid solution was added to the medium. After centrifugation, radioactivity in the supernatant (trichloroacetic acid-soluble fraction), in which degraded activin was recovered, was quantified by γ-spectrometry. Degradation of the activin activity in the trichloroacetic acid-soluble fraction relative to the total radioactivity added. To examine the effects of various chemical inhibitors on the endocytotic degradation of activin by pituitary cells, the cells (8 × 10⁴ cells/well) were treated with 125I-activin A (40 ng/ml), FS-288 or FS-315 (200 μg/ml), and various concentrations of inhibitors for 24 h at 37°C in 100 μl of DMEM containing 10% horse serum and 2.5% FBS.

Determination of the FSH Release-inhibiting Activity of FS—FSH release-inhibiting activity was determined by the cultured rat pituitary cell assay (27). Cells plated into 96-well plates (8 × 10⁶ cells/well) were cultured in the presence of FS (0–100 ng/ml) for 60 h at 37°C. The culture medium was then removed and tested for FSH using a Fuji BAS1500 Bio-Imaging analyzer (Fuji Photo Film, Tokyo, Japan) and Hyperfilm (Amersham Corp.).
Untransfected COS-7 cells were also analyzed under the same conditions in the absence (200 ng/ml) and heparan sulfate (10 ng/ml), or porcine FS (200 ng/ml) (SDS-PAGE (8% gels) as described under “Experimental Procedures.”

heparan sulfate proteoglycans on rat ovarian granulosa cells that FS-288 showed a much higher affinity than FS-315 for than FS-315, which was consistent with our previous finding should be noted that FS-288 yielded a much more intense band A was observed microscopically.

UK), exposed for 5 days at 4 °C, and developed. The internalized activin receptors mRNAs have been reported to be distributed in the rat pituitary (32), but the receptor protein has yet to be identified. We attempted to analyze the pituitary activin receptor by affinity cross-linking 125I-activin A to rat pituitary cells using the bifunctional chemical cross-linker DSS. Faint but definite cross-linked bands of 80 and 100 kDa were observed (Fig. 1A), and these corresponded well with those of the activin receptors transiently coexpressed in COS-7 cells (Fig. 1B). These bands were displaced by the addition of excess unlabeled activin, demonstrating the specificity of the activin-receptor complex. The binding of labeled activin to both types I and II activin receptors was completely abolished in the presence of excess FS-288 or FS-315. These findings indicate that the activin-FS complex cannot bind to activin receptors and would account for the inhibitory effect of FS on activin-induced stimulation of FSH secretion by pituitary cells. Although formation of activin-receptor complexes was prevented by the addition of FS, broad bands with molecular masses ranging from 45 to 65 kDa and from 70 to 100 kDa were visible after treatment with either FS-288 or FS-315. These bands were not related to activin receptors, because they were also yielded by COS-7 cells that were not transfected with activin receptor DNAs. Based upon their molecular sizes, the lower band (45–65 kDa) was assumed to be a 1:1 molar complex between activin and FS and the higher band (70–100 kDa) to be a 1:2 molar complex. Labeling of these bands was completely inhibited by incubation with heparan sulfate and with excess unlabeled activin, suggesting that labeled activin is held on the cell surface by FS bound to the heparan sulfate side chains of proteoglycans. It was confirmed by binding experiments using an FS-coated microplate that heparan sulfate (10 μg/ml) had no inhibitory effect on formation of activin and FS (data not shown). It should be noted that FS-288 yielded a much more intense band than FS-315, which was consistent with our previous finding that FS-288 showed a much higher affinity than FS-315 for heparan sulfate proteoglycans on rat ovarian granulosa cells (20). To confirm that this also applied to pituitary cells, rat pituitary cells were incubated with various concentrations of 125I-FS-288 (●) or FS-315 (○) for 2 h at 37 °C. The amount of cell-bound FS was determined as described under “Experimental Procedures.” The results are expressed as means ± S.D. from triplicate experiments.

A

![Diagram A](image1)

**FIG. 1.** Inhibitory effects of FS on 125I-activin A binding to activin receptors on pituitary cells and COS-7 cells transfected with activin receptor cDNAs. A, rat pituitary cells (5 × 10⁶ cells) cultured in 24-well plates were incubated with 125I-activin A (40 ng/ml) in the absence (a) or presence (b) of unlabeled activin A (400 ng/ml), and in the presence of FS-288 (200 ng/ml) (c) FS-315 (200 ng/ml) (d), FS-288 (200 ng/ml) and heparan sulfate (10 μg/ml) (e), or FS-315 (200 ng/ml) and heparan sulfate (10 μg/ml) (f), cross-linked with DSS, and analyzed by SDS-PAGE (8% gels) as described under “Experimental Procedures.” B, COS-7 cells cultured in 6-well plates (2 × 10³ cells) and transfected with type IA and type IIA activin receptor cDNAs were incubated with 125I-activin A (40 ng/ml) A in the absence (a) or presence (b) of activin A (400 ng/ml), or porcine FS (200 ng/ml) (c), cross-linked with DSS, and analyzed by SDS-PAGE (7.5% gels) as described under “Experimental Procedures.” Untransfected COS-7 cells were also analyzed under the same conditions in the absence (d) or presence (e) of porcine FS (200 ng/ml) or porcine FS (200 ng/ml) and heparan sulfate (10 μg/ml) (f).

B

![Diagram B](image2)

**FIG. 2.** Binding of 125I-FS to rat pituitary cells. Pituitary cells cultured in 96-well plates (8 × 10⁵ cells/well) were incubated with increasing concentrations of 125I-FS-288 (●) or FS-315 (○) for 2 h at 37 °C. The amount of cell-bound FS was determined as described under “Experimental Procedures.” The results are expressed as means ± S.D. from triplicate experiments.
These data support the hypothesis that FS-288 recognizes and attaches to the heparan sulfate side chains of proteoglycans on the pituitary cell surface.

**Inhibitory Effect of Heparan Sulfate on the FSH-suppressing Activity of FS**—FS was identified as an inhibitor of FSH secretion by cultured pituitary cells, but its potency was shown to be only 10–30% that of inhibin (7, 18). The mechanism by which FS acts is unclear, but it has been suggested that it binds endogenous activin and neutralizes activin-stimulated FSH secretion. To understand the role of the interaction of FS and proteoglycans in the FSH-suppressing effect of FS, we examined the effect of heparan sulfate on this inhibitory action of FS in rat pituitary cells. FS-288 and FS-315 dose-response curves for the inhibition of basal FSH secretion into the culture medium were prepared in the presence or absence of heparan sulfate (10 μg/ml) (Fig. 4). As reported previously, FS-288 was about 6–7 times more potent than FS-315. Heparan sulfate reduced the inhibitory activity of FS-288 by about 50%, whereas the potency of FS-315 remained unchanged regardless of the presence of heparan sulfate. This suggests that cell-associated FS-288 is more positively involved in controlling activin activity on cell surfaces than FS-315.

**Binding of Activin A to Cell-Associated FS**—We then examined the binding of activin to FS associated with pituitary cell surfaces. In the presence of various concentrations of FS-288 or FS-315, rat pituitary cells were incubated with increasing amounts of 125I-activin A (0–100 ng/ml), and the cell-bound radioactivities (activin-FS complex) were determined (Fig. 5). The binding activity of activin A alone was difficult to detect, probably due to the very small number of activin receptors on pituitary cells. However, FS-288 markedly increased the affinity of activin A for cell surfaces in a concentration-dependent manner, whereas FS-315 did not enhance activin A binding to cell surfaces. These results suggest that activin A can adhere strongly to cells by forming a complex with FS-288 on the cell surface.

**Endocytotic Degradation of the Activin A-FS Complex**—We followed the behavior of the cell-associated activin-FS complex and found that it was degraded endocytotically. Rat pituitary cells were incubated with radiiodinated activin A (40 ng/ml) in the presence of increasing concentrations of FS-288 or FS-315 for various incubation periods, after which the radioactivities recovered from the trichloroacetic acid-soluble fractions (degraded activin) of the incubation media were determined using a γ-spectrometer. As shown in Fig. 6, FS-288 stimulated activin A degradation significantly in a time- and concentration-dependent manner and to a greater extent than FS-315. This stimulatory effect of FS-288 was abolished by adding heparin or heparan sulfate to the culture medium (data not shown).
Moreover, degradation was dependent on the number of pituitary cells as shown in Fig. 7; increasing their number stimulated degradation of activin A bound to the cell surfaces via FS. These degradation data were obtained by monitoring the degradation of 125I-activin A. SDS-PAGE and gel filtration of samples of the complex demonstrated that the FS component of the activin-FS complex was also degraded (data not shown).

Endocytotic Internalization of Activin A—The degradation of cell-bound activin and/or FS by pituitary cells led us to hypothesize that endocytotic internalization occurs in the cells and that the resulting endocytotic vesicles ultimately fuse with lysosomes, after which most of the vesicle contents are rapidly broken down. To explore this idea, autoradiographic experiments using radiiodinated activin A were performed. Pituitary cells were incubated with 125I-activin A at 37 °C in the presence or absence of FS, heparin, and chloroquine for 12 h, and the cells were washed with acid/salt buffer to strip 125I-activin A from their surfaces and then autoradiographed. As shown in Fig. 8, it is obvious that FS-288 markedly accelerated the uptake of activin A by pituitary cells and had a greater effect than FS-315. Heparan sulfate significantly suppressed uptake, which agreed well with the degradation data described above. Co-incubation with chloroquine, which increases the pH inside lysosomes, inhibited the degradation of activin A taken up by the cells, probably in the lysosomes, resulting in activin A accumulation within the cells. Microscopic observations supported our hypothesis that activin A bound to pituitary cell surfaces via FS-288 is taken up and packaged into endocytic vesicles, which fuse with lysosomes. This is followed by proteolytic degradation of their contents.

Inhibition of Endocytotic Degradation of Cell-associated Activin A by Lysosomal Enzyme Inhibitors—To demonstrate the participation of lysosomes in the degradation of activin A after endocytosis, we examined the effects of various types of lysosomal enzyme inhibitor on activin A degradation in rat pituitary cells. The results are summarized in Table I. Lysosomal enzyme inhibitors reduced degradation significantly, but the serine protease inhibitor aprotinin had no effect. As expected from the results shown in Fig. 8, chloroquine markedly inhibited activin A breakdown. Both heparin and heparan sulfate suppressed activin degradation significantly, strongly suggesting that degradation does not occur until FS binds to the pituitary cells. These results clearly indicate that after endo-

**FIG. 5.** Effect of FS on binding of 125I-activin A to pituitary cells. Rat pituitary cells cultured in 96-well plates (8 × 10^4 cells/well) were incubated with increasing concentrations of 125I-activin A (0–100 ng/ml) in the presence of various concentrations of FS-288 (A) or FS-315 (B) at 37 °C. After a 2-h incubation, specific binding was determined as described under “Experimental Procedures.” Concentrations of FS added were 0 (●), 25 (▲), 100 (■), and 400 (△) ng/ml. The results are expressed as means ± S.D. from triplicate experiments.

**FIG. 6.** Effect of FS on endocytotic degradation of 125I-activin A by pituitary cells. Rat pituitary cells cultured in 96-well plates (8 × 10^4 cells/well) were incubated with 40 ng/ml 125I-activin A in the presence of various concentrations of FS-288 (A) or FS-315 (B) at 37 °C. Medium was collected at the indicated time points and subjected to trichloroacetic acid treatment. The radioactivity of the trichloroacetic acid-soluble fraction (degraded 125I-activin A) was determined in a γ-counter. Concentrations of FS added were 0 (●), 40 (▲), 120 (■), and 400 (△) ng/ml. The results are expressed as means ± S.D. from triplicate experiments.

**FIG. 7.** Effect of cell density on endocytotic degradation of 125I-activin A by pituitary cells. Various numbers of rat pituitary cells cultured in 96-well plates were incubated with 125I-activin A (40 ng/ml) in the absence or presence of FS-288 or FS-315 (200 ng/ml) for 24 h at 37 °C. Degradation of 125I-activin A was determined as indicated under “Experimental Procedures.” Cell densities used were 2.4 × 10^4 (□), 8 × 10^4 (■), and 2.4 × 10^5 (▲). The results are expressed as means ± S.D. from triplicate experiments.
Follistatin-accelerated Endocytotic Degradation of Activin

Dyscytosis, activin A is hydrolyzed, probably together with FS-288, in the lysosome.

DISCUSSION

FS binds stoichiometrically to activin to form an inactive complex, which results in blockade of various activin bioactivities. However, the physiological significance of this complex formation is not fully understood. Recently, de Winter et al. (33) demonstrated that the preincubation of radioiodinated activin A with FS completely abolished binding to type II activin receptors and consequently binding to type I receptors and proposed that FS can neutralize activin bioactivity by interfering with activin binding to type II receptors. Our affinity cross-linking experiments also showed this inhibition of activin bind-

![FIG. 8. Uptake of 125I-activin A by rat pituitary cells.](http://www.jbc.org/content/225/21/13839/F11)

**TABLE I**

| Inhibitors (concentration) | Degradation |
|---------------------------|-------------|
| None                      | 100 ± 7.5   |
| Lysosomal protease inhibitors |            |
| E-64 (1 mM)               | 64 ± 6.8    |
| Leupeptin (1 mM)          | 58 ± 4.7    |
| 1,10-Phenanthroline (0.05 mM) | 68 ± 5.1    |
| Pepstatin A (1 mM)        | 89 ± 5.8    |
| Serine protease inhibitor |            |
| Aprotinin (200 μg/ml)     | 103 ± 7.2   |
| Heparin (10 μg/ml)        | 26 ± 3.8    |
| Heparan sulfate (10 μg/ml) | 40 ± 4.3    |

Signticant binding of radioiodinated activin A to pituitary cell surfaces was observed only in the presence of FS (Fig. 5). As expected, FS-288 markedly promoted this binding and had a greater effect than FS-315. Recently, Sugahara found that the smallest heparin oligosaccharides that could be recognized by FS-288 was a dodecasaccharide, suggesting that FS-288 distinguishes certain glycosaminoglycan configurations.

When incubated with pituitary cells in the presence of FS-288, activin A in the medium appeared to be trapped by cell-bound activin A. Binding to heparan sulfate side chains via FS-288 on the cell surfaces thus appears necessary for the first step of activin A degradation. This idea was further supported by our finding that activin A is not degraded in heparitinase-treated pituitary cells, to which it cannot bind. After being captured on the cell surface, activin A may, together with FS-288 and proteoglycans, be ingested by endocytotic vesicles that fuse with primary lysosomes. Most of the vesicle contents were found to be hydrolyzed into small break-

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2 O. Hashimoto, unpublished data.
3 K. Sugahara, unpublished data.
down products and secreted to the exterior. There is little doubt that activin A is broken down by such an endocytotic degradation process, because various inhibitors of each stage of this process blocked activin A degradation; the inhibitors tested included chloroquine and several lysosomal protease inhibitors. Monensin, an endosome lysosome fusion inhibitor, also almost completely inhibited degradation (data not shown). As this process was visualized (Fig. 8), activin A was collected within vesicles, which were probably lysosomes because chloroquine prevented the latter half of the degradation process. As is the case with radiiodinated activin A, the proteolytic degradation of 125I-labeled FS-288 was also observed in pituitary cells (data not shown). On the other hand, the complex between FS-315 and activin A showed low affinity for cell surfaces, resulting in the avoidance of endocytotic degradation by these complexes. Indeed, these complexes were found to be relatively stable under our incubation conditions for at least 48 h (data not shown). Activin might select FS, which adheres with difficulty to cell surfaces, as its binding partner and could therefore not shown). Activin might select FS, which adheres with difficulty for at least 48 h (data not shown). On the other hand, the complex between FS and activin A showed low affinity for cell surfaces, resulting in the avoidance of endocytotic degradation by these complexes. Indeed, these complexes were found to be relatively stable under our incubation conditions for at least 48 h (data not shown). Activin might select FS, which adheres with difficulty to cell surfaces, as its binding partner and could therefore defend itself against endocytotic internalization followed by proteolytic attack. The anterior pituitary gland consists of many different cell types, classified on the basis of size, shape, and the hormone secreted. Therefore, it is also important to identify the type of pituitary cells that undertake the degradation process.

As shown in Figs. 5 and 6, FS-288 was approximately twice as effective as FS-315 in assisting degradation of 125I-activin A, and binding of FS-288 to the cells was more than 10 times higher than that of FS-315 (Fig. 2). There is no direct evidence to explain these phenomena clearly, but there are several possible explanations. As described in our previous report (20), the majority of FS isolated from porcine ovaries is FS-303, which is thought to be derived from FS-315 by proteolytic cleavage of the 12 COOH-terminal amino acids and shows moderate affinity for cell surfaces. During incubation of FS-315 with pituitary cells, proteolytic degradation of the COOH-terminal portion of FS-315 may occur, and the resulting COOH-terminal truncated FS becomes attached to cells so that it can be degraded by endocytosis. Another possibility is that 125I-activin A may be more effectively degraded when it binds to FS-315.

The number of growth factors and cytokines discovered to bind to heparin and heparan sulfate is increasing steadily, and the list includes fibroblast growth factors (FGFs), granulocyte-macrophage colony stimulating factor, interleukin-3, pleiotrophin, hepatocyte growth factor, vascular endothelial growth factor, and midkine, among others. On the basis of our present results, we speculate that, like the activin A-FS-288 complex, these growth factors bind to cell-surface heparan sulfate proteoglycans, become internalized, and are eventually degraded in lysosomes. In fact, we found that 125I-basic FGF bound to cultured rat pituitary cells in a concentration-dependent manner and that its intracellular degradation was time-dependent. Therefore, it is conceivable that endocytotic degradation is a common mechanism for eliminating signaling molecules from cell surfaces.

It is well documented that the interaction between FGF and heparin-like molecules in the extracellular matrix is important for various biological functions, such as protection of this factor against proteolytic degradation and regulating its concentration on cell surfaces. The role of heparin-like molecules in the signal transduction of FGF is noteworthy; binding of basic FGF to its receptor requires prior binding either to the heparan sulfate side chains of cell-surface proteoglycans or to free heparin to present the ligand to the receptor (35). De Winter et al. (33) attempted to determine whether cell surface-bound FS-288 presents activin A to the activin receptors on human erythro-leukemic K562 cells and found that FS-288 and the activin A-type IIA receptor complex were not co-precipitated by an anti-type IIA activin receptor antibody, suggesting that, unlike basic FGF, cell surface-associated FS cannot present ligands to signaling receptors. Judging from these results, FS appears to be nothing more than a negative regulator for activin, its function being to form an inactive complex with activin and thereby neutralize its activity. There are some situations in which FS traps activin on the cell-surface heparan sulfate and leads to endocytotic degradation. However, taking these findings together, we hypothesize that endocytotic degradation of growth factors via cell-surface heparan sulfate is necessary to erase their signals from cell surfaces when they become excessive and thus useless. It has been established that the binding of a signaling ligand to its receptor stimulates a biological response and triggers a sequence of events leading to cellular desensitization to the ligand to regulate the responsiveness of the target cell to the ligand. We propose that, in addition to such receptor-mediated endocytosis, there must be a scavenger mechanism for clearing signaling molecules away from their target cell surfaces.

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Osamu Hashimoto, Takanori Nakamura, Hiroki Shoji, Shunichi Shimasaki, Yoshihiro Hayashi and Hiromu Sugino

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