Identification of the Basic Fibroblast Growth Factor Binding Sequence in Fibroblast Heparan Sulfate

(Received for publication, March 2, 1992)

Jeremy E. Turnbull¶§¶, David G. Fernig*¶¶, Youquiang Ke*¶¶, Mark C. Wilkinson*¶¶, and John T. Gallagher†

From the ¶Clinical Research Department and §Cancer Research Campaign Medical Oncology Department (University of Manchester), Christie Hospital, Wilmslow Road, Manchester M20 2BX and the **Cancer and Polio Research Fund Laboratories, Department of Biochemistry, University of Liverpool, Liverpool L69 3BX, United Kingdom

The structural properties of fibroblast heparan sulfate (HS) that are necessary for it to bind strongly to basic fibroblast growth factor (bFGF) have been investigated using bFGF affinity chromatography. Specific enzymic and chemical scission of HS, together with enzymic and chemical scission of HS, together with

In Fibroblast Heparan Sulfate*

parent molecule. Heparinase treatment of this fraction was investigated using bFGF affinity chromatography. Specific enzymic and chemical scission of HS, together with enzymic and chemical scission of HS, together with

in HS by treatment with heparitinase led to the identification of an oligosaccharide component (oligo-H), seven disaccharides in length, with a similar affinity for bFGF as the parent molecule. Heparinase treatment of this fraction abolished the high affinity binding to bFGF. Analysis of oligo-H indicated that 74% of the disaccharide units had the structure IdoA(2-OSO₃)₆, 4GlcNSO₃; the remainder comprised N-acetylated and N-sulfated units, the majority of which were devoid of O-sulfate groups. Oligo-H was fully degraded to disaccharides by treatment with nitrous acid. These results indicate that the sequence of oligo-H is as shown below.

$$\Delta$$Glca₁₋₄GlcNSO₃₋₅(1₋₄)IdoA(2-OSO₃)₆, 4GlcNSO₃

Sulfated oligosaccharides of similar size but with a lower affinity for bFGF had a reduced concentration of IdoA(2-OSO₃) but significant quantities of GlcNSO₃(6-OSO₃) and GlcNAc(6-OSO₃). The data indicate a primary role for contiguous sequences of IdoA(2-OSO₃)₆, 4GlcNSO₃ in mediating the high affinity binding between fibroblast HS and bFGF.

Basic fibroblast growth factor (bFGF) has an important role in processes such as embryonic development, wound repair, and tumor growth (1). It delivers its signal to cells by binding with specific cell surface tyrosine kinase receptors ($K_a = 10-500 \text{ pM}$), for example the product of the gene $f_{\text{g}}g$ (2), which generate intracellular signals. However, the mode of action of bFGF is complex and also appears to involve an interaction with cell-surface heparan sulfate proteoglycans (HSPG) ($K_a = 5-50 \text{ nM}$) (3-7). Moreover, recent work has shown that the $f_{\text{g}}g$ receptor of cells which are deficient in heparan sulfate (HS) synthesis will not respond to bFGF, but that addition of HS or heparin could restore responsiveness (8, 9). It was suggested that the polysaccharides induce a conformational change in bFGF which is a prerequisite for binding to the signal transducing receptor. A model invoking a dual-receptor mechanism for the action of bFGF has been proposed (8, 10).

These novel observations raise the important question of the nature of the bFGF binding site in HS. HS is probably the most complex mammalian glycosaminoglycan (GAG) (11, 12), consisting of an ordered arrangement of domains rich in N- and O-sulfate groups, in which the basic disaccharide repeat unit is GlcA/IdoA-GlcNSO₃, spaced apart by regions of low sulfation in which N-acetylated disaccharides (GlcA-GlcNAc) predominate (13-15). Since bFGF is a heparin-binding growth factor the sulfated domains which contain some "heparin-like" regions are the most likely location of the binding site. However, the size of these domains, their sulfation pattern, and their IdoA content are highly variable (13-18), and the strong interaction with bFGF may require a strictly defined sequence of sulfated monosaccharide isomers in a manner similar to the specific pentasaccharide in heparin which has high affinity for antithrombin III (19). Endothelial cell-derived HS has been demonstrated by affinity chromatography to bind strongly to bFGF (4), and a weaker interaction with HS from the Engelbreth-Holm-Swarm (EHS) tumor has also been reported (20), but the structural requirements for the interaction were not investigated. In the present study we report the isolation and characterization of a distinct oligosaccharide structure from the N-sulfated domains of human skin fibroblast HS which exhibits strong affinity for bFGF.

**EXPERIMENTAL PROCEDURES**

Materials—Human recombinant bFGF was prepared in a manner similar to that described previously for acidic FGF (21). Briefly, the recombinant bFGF was purified by heparin-Sepharose chromatography and reverse phase or cation-exchange HPLC from lysates of bacterial cells, harboring a PKK 233-2-bFGF construct (22) encoding

$\Delta$GlcA₁₋₄GlcNSO₃₋₅(1₋₄)IdoA(2-OSO₃)₆, 4GlcNSO₃

**REFERENCES**

1. The abbreviations used are: bFGF, basic fibroblast growth factor; GAG, glycosaminoglycan; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; dp, degree of polymerization (i.e. for disaccharide, $dp = 2$ etc); GlcA, glucuronic acid; IdoA, iduronic acid; IdoA(2-OSO₃), iduronic acid 2-sulfate; GlcNAc, N-acetyl glucosamine; GlcNSO₃, N-sulfated glucosamine; GlcNSO₃(6-OSO₃), N-sulfated glucosamine 6-sulfate; GlcA(2-OSO₃), glucuronic acid 2-sulfate; $\Delta$HexA, unsaturated uronic acid residue; $\Delta$GlcA, unsaturated hexuronic acid residue defined as in original polymer on the basis of the known specificity of heparitinase scission (14, 27); aMan₉₋₁₆, 2,5-anhydro-b-mannitol formed by reduction of terminal 2,5-anhydro-mannose residues with NaBH₄; HPLC, high performance liquid chromatography.

2. Y. Ke, M. C. Wilkinson, D. G. Fernig, J. A. Smith, P. S. Rudland, and R. Barraclough, manuscript in preparation.
amino acids 1-155 of human bFGF (23), to yield a single compound of M, 17,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The amino acid sequence was consistent with that of human bFGF, and the recombinant protein possessed full biological activity. Affi-Gel 10 activated affinity gel was from Bio-Rad. Heparinase and heparitinases I and II and HS unsaturated disaccharide standards were from Seikagaku Kogyo Co., Tokyo, Japan.

General Methods—HSPG and HS chains biosynthetically radiolabeled with [3H]glucosamine were prepared from confluent cultures of adult human skin fibroblasts as described by Tumbull and Gallagher (14). Depolymerization of HS chains with heparitinases or heparinase and RP-HPLC chromatography of oligosaccharides on Bio-Gel P6 or P2 were also as previously described (14, 15). Chemical N-desulfation-N-acetylation was carried out as described by Inoue and Nagasawa (24). Deaminative scission with low pH nitrous acid was carried out by the method of Shirley and Conrad as described previously (14). Briefly, equal volumes of 3.5 M HNO3 and 0.5 M Ba(NO3)2 (precoced on ice) were mixed and the barium sulfate precipitate pelleted by centrifugation. Samples dried by centrifugal evaporation were reconstituted in 100 µl of the supernatant (1 M HNO3 solution) and incubated for 15 min at 20 °C. The reaction was stopped by addition of 20 µl of 1 M Na2CO3.

Preparation of bFGF-Affi-Gel 10 Affinity Matrix—Affi-Gel 10 (1 ml of packed gel) was washed four times with five volumes of double distilled water by centrifugation at 800 × g for 1 min. Heparin (500 µg) was added to bFGF (500 µg in 3 ml of 0.6 M NaCl, 25 mM Na2HPO4, pH 6.6) and mixed with 1 ml of washed and packed Affi-Gel 10 overnight at 4 °C. 2 ml of 4 M Tris-HCl, pH 8.0, was added to block unreacted groups on the gel. Heparin (5 mg) was added to stabilize bound bFGF and 4.5 µl of 20% (w/v) NaN3 as preservative. The gel was washed with 10 volumes of 2 M NaCl in 10 mM Tris-HCl, pH 6.5. No bFGF was detected in the wash by reverse-phase HPLC, indicating a high coupling efficiency.

Affinity Chromatography—Approximately 1 ml of bFGF-Affi-Gel 10 affinity matrix was packed into a glass column (bed dimensions 6 × 35 mm). Samples were loaded onto the column in 10 mM Tris-HCl, pH 6.5, at a flow rate of 0.25 ml/min. Unbound material was eluted by collecting five 1-ml fractions. Bound material was eluted with a step gradient of sodium chloride (0-2 M NaCl in column buffer in 0.25 M steps) at a flow rate of 0.6 ml/min. Five 1-ml fractions were collected at each concentration. The column was stored at 4 °C in running buffer containing 10 µg/ml heparin (Sigma). 0.01% (w/v) sodium azide, and 0.2 M NaCl.

Strong-anion Exchange HPLC of Disaccharides—Disaccharide composition was analyzed by complete depolymerization of HS oligosaccharides with a mixture of heparitinase, heparitinase II, and heparinase. Disaccharides were recovered by Bio-Gel P2 chromatography and separated by HPLC on a ProPac PA1 analytical column (4 × 250 mm; Dionex, UK). After equilibration in mobile phase (double-distilled water adjusted to pH 3.5 with HCl) at 1 ml/min, the eluted disaccharides were collected in 1-ml fractions eluted with a linear gradient of sodium chloride (0-1 M over 45 min) in the same mobile phase. The eluant was monitored in-line for UV absorbance (A232 for unlabeled disaccharides and A260 for labeled disaccharides) and radioactivity (Radiomatic Flo-one/Beta Analyzer).

RESULTS

Fractionation of Native and Partially Depolymerized HS on a bFGF Affinity Column—HSPG, metabolically labeled with [3H]glucosamine, was purified from the medium of cultures of human skin fibroblasts. HS chains were prepared by Pronase treatment of the HSPG and applied to an affinity column prepared with human recombinant bFGF (see "Experimental Procedures"). Bound material was eluted stepwise with NaCl concentrations ranging from 0.25 to 2.0 M in 0.25 M steps. The majority of the HS bound strongly to bFGF, the major peak eluting at 1.25 M NaCl (Fig. 1A, solid line). A similar elution profile was obtained for the intact HSPG (Fig. 1A, dashed line), indicating that the HS chains are the principal determinant of proteoglycan binding to bFGF. Hyaluronic acid did not bind to the bFGF column and fibroblast-derived condroitin and dermatan sulfate displayed only weak affinity (26, 38, and 36% of material eluting at 0, 0.25, and 0.5 M NaCl, respectively). In contrast, commercial heparin eluted mainly at 1.25, 1.5, and 1.75 M NaCl (20, 41, and 10% of label, respectively). These results indicate a specific interaction of bFGF with N-sulfated polysaccharides. The importance of N-sulfate groups was confirmed by findings that either deaminative scission with low pH nitrous acid, or N-desulfation/re-N-acetylation of HS, abolished the high affinity interaction (Fig. 1B, solid and dashed lines, respectively).

The problem of identifying the bFGF binding domains in HS was addressed using the enzymes heparinase and heparitinase which cleave the polysaccharide in different structural
domains. Heparinase acts in the N-sulfated regions and specifically cleaves disaccharides that contain 2-O-sulfated iduronate, i.e. GlcNSO_3(±6-OSO_3)α1,4IdoA(2-OSO_3) (27); the major products are oligosaccharides of 9–10 kDa (14). By contrast, heparitinase cleaves GlcA-containing disaccharides (27), principally GlcNAcα1,4GlcA, which are present in regions of low sulfation; this enzyme does not attack contiguous N-sulfated sequences of type [GlcNSO_3(±6-OSO_3)α1,4IdoA(±2-OSO_3)] (13, 14).

Heparinase scission of HS resulted in products with significantly reduced affinities for bFGF, elution occurring in the range 0.25–0.75 M NaCl (Fig. 1C). The effects of heparitinase digestion were even more marked with the majority of the material either failing to bind to the column or eluting at 0.25–0.75 M NaCl (Fig. 1D). However, a minor population of oligosaccharides in the heparitinase digest displayed an affinity for bFGF that was comparable to the intact HS (eluting in the range 1.0–1.5 M NaCl). Gel chromatography on Bio-Gel P-6 showed that these high affinity products comprised two oligosaccharide fractions predominantly dp12 and dp14 in size (Fig. 1E), equivalent to six and seven disaccharide units. These are the largest fragments present in significant quantities in heparitinase digests of human skin fibroblast HS (13, 14). The foregoing data suggest that extended N-sulfated sequences in HS contain the highest affinity binding site for bFGF, and that IdoA(2-OSO_3) residues make an important contribution to the interaction.

Specificity of Binding of N-sulfated Oligosaccharides—In view of the above findings, we decided to study the specificity of oligosaccharide interaction in more detail by examining whether all extended N-sulfated sequences in HS bind strongly to bFGF or alternatively, whether a particular O-sulfation pattern is required. To address this issue, large heparitinase-resistant oligosaccharides were prepared from heparitinase digests of HS using Bio Gel P-6 chromatography (see Ref. 14). Components of size dp12 and dp14 (12.5% of total products) were pooled and fractionated by bFGF affinity chromatography. Three major fractions were identified which eluted at 0.75, 1.0, and ≥1.25 M NaCl and were designated oligo-L (low), oligo-M (medium), and oligo-H (high) affinity oligosaccharides. Re-application of the fractions to the column confirmed their different affinities for bFGF (Fig. 2).

Oligosaccharides of size dp14 were mainly present in the oligo-H fraction, whereas the oligo-M and oligo-L fractions were predominantly dp12 (Fig. 3).

Heparinase treatment caused a marked reduction in binding of these oligosaccharides to bFGF (Fig. 2), and the extent of depolymerization (Fig. 3) correlated closely with loss of affinity. The presence of major products dp4 and dp6 in size (Fig. 3) was indicative of cleavage of internal linkages.

Disaccharide Composition of Oligosaccharides—The disaccharide composition of the H, M, and L oligosaccharides was assessed by polysaccharide lyase depolymerization and strong anion exchange HPLC as described under “Experimental Procedures” (Table I). The most striking aspect of the analyses was the high content of disulfated disaccharides of the type ΔHexA(2-OSO_3)α1,4GlcNSO_3, particularly in oligo-H and oligo-M (approximately 74% and 60%, respectively, of disaccharide units). The heparinase sensitivity of these fractions (Figs. 2 and 3) suggests that the majority of the 2-sulfated HexA residues were originally IdoA(2-OSO_3). Since the content of IdoA(2-OSO_3)-disaccharides in the native HS was approximately 10–12% (13, 14), the results indicate an enrichment of approximately 7-fold in oligo-H and 6-fold in oligo-M. Overall the concentration of ΔHexA(2-OSO_3)α1,4GlcNSO_3 was strongly correlated with the differing bFGF affinities of the H, M and L oligosaccharides (Table I and Fig. 2). In contrast there was a marked inverse correlation of binding strength with the content of the 6-O-sulfated derivatives [GlcA α1,4GlcNAc(6-OSO_3)] and ΔHexA α1,4GlcNSO_3(6-OSO_3). These accounted for 26% of disaccharides in oligo-L but were minor components in oligo-H (Table I).

The amount of the N-acetylated disaccharide ΔHexA α1,4GlcNAc was similar in each of the oligosaccharides and corresponded to approximately one per fragment (Table I).

Deaminitive scission with low pH nitrous acid resulted almost exclusively in disaccharide products with oligo-H and oligo-M (Fig. 4, A and B; 99 and 95%, respectively), whereas both disaccharides (76%) and tetrasaccharides (24%) were major products from oligo-L (Fig. 4C). Thus, virtually all the internal hexosaminidic linkages within the oligosaccharides in the H and M fractions involved GlcNSO_3 residues, and the N-acetylated unit will be at the reducing end of the fragment (see below). The disaccharides released from the H, M, and L fractions by nitrous acid were examined by strong anion exchange as described under “Experimental Procedures” in order to establish the identity of the constituent uronic acid residues. Oligo-H yielded 71% of label eluting in the position of the standard IdoA(2-OSO_3)-aMan_8; the remaining label eluted as an unsulfated peak, corresponding to ΔGlcA-aMan_8 and IdoA-GlcNAc (results not shown). Oligo-M and oligo-L yielded 61 and 37%, respectively, as IdoA(2-OSO_3)-aMan_8; thus, the content of the disaccharide IdoA(2-OSO_3)-aMan_8 in each of the fractions correlated well with that of ΔHexA(2-OSO_3)α1,4GlcNSO_3.
Specific bFGF-binding Sequence in Heparan Sulfate

**TABLE I**

Disaccharide composition of HS oligosaccharides with differing affinities for bFGF

| Disaccharide | Oligo-L | Oligo-M | Oligo-H |
|-------------|---------|---------|---------|
| ΔHexA-GlcNAc | 13.6    | 16.0    | 11.3    |
| ΔHexA-GlcNAc(6-OSO₃) | 12.4    | 2.6     | 0.9     |
| ΔHexA-GlcNSO₃ | 27.3    | 11.3    | 7.5     |
| ΔHexA-GlcNSO₃(6-OSO₃) | 14.0    | 4.5     | 1.4     |
| ΔHexA(2-OSO₃)-GlcNSO₃ | 31.0    | 59.8    | 74.2    |
| ΔHexA(2-OSO₃)-GlcNSO₃(6-OSO₃) | 0.6     | 2.2     | 1.0     |
| Disaccharide yield (%) | 98.9    | 96.4    | 96.3    |

Minor variations in structure would be caused by the occasional presence of 6-0-sulfate groups on any of the amino sugars (e.g. 1% of disaccharides contain GlcNAc(6-OSO₃); Table I). Polysaccharide lyase depolymerization of this sequence would produce a disaccharide composition for oligo-H which closely matches that shown in Table I. The GlcNAc residue has been placed at the reducing end of the sequence (position 14) because essentially all the internal linkages were sensitive to deaminative scission (Fig. 4A), indicating the presence of a single contiguous sequence of N-sulfated disaccharides. If the GlcNAc was located elsewhere in the sequence, even at position 2, deaminative scission would also produce a significant fraction of tetrasaccharide products in addition to disaccharides (e.g. as observed with oligo-L, Fig. 4C).

To our knowledge this is the first time an extended sequence of IdoA(2-OSO₃)Δ1,4GlcNSO₃ units has been identified in HS. The surprisingly low content of 6-0-sulfate groups clearly distinguishes this oligosaccharide from typical N-sulfated sequences in heparin in which the GlcNSO₃ residues are frequently sulfated at C-6, i.e. [IdoA(2-OSO₃)Δ1,4GlcNSO₃]ₙ. Although the oligo-H sequence identified here may not represent the minimal sequence for optimal binding to bFGF it is of some interest that full activation of bFGF (measured by its ability to bind to the flg receptor) requires heparin fragments of about the same size as oligo-H, i.e. dp14–dp16 (28). The related cytokine acidic FGF is also strongly

FIG. 3. Bio-Gel P6 chromatography of HS oligosaccharides with differing affinities for bFGF. HS oligosaccharides (dp12–14) with low (oligo-L), medium (oligo-M), and high (oligo-H) affinity for bFGF were prepared as described in Fig. 2, and their size distribution established by Bio-Gel P6 chromatography either intact (solid line) or after heparinase treatment (dashed line).

FIG. 4. Bio-Gel P6 chromatography of bFGF-binding HS oligosaccharides subjected to deaminative scission. HS oligosaccharides (dp 12–14) with low (oligo-L), medium (oligo-M), and high (oligo-H) affinity for bFGF were prepared as described in Fig. 2, treated with nitrous acid, and fractionated by Bio-Gel P6 chromatography. The disaccharides (dp2) were partially resolved into monosulfated (main peak) and non-sulfated species.
activated by heparin oligosaccharides in this size range (29, 30).

An indication of the structural requirements for high affinity interactions with bFGF can be obtained by comparing the composition of oligo-H with the oligosaccharides of medium and low affinity for bFGF, which, as oligosaccharides resistant to heparitinase digestion, contain the same basic disaccharide repeat of IdoA-GlcN(3S). Oligo-H and oligo-M have similar degrees of sulfation (1.6 and 1.5 sulfates/disaccharide, respectively) but oligo-M contains 60% of disaccharides in the form of IdoA(2-OSO3)~1,3GalNAc(4-OSO3) compared to 74% in oligo-H. About 10% of amino sugars in oligo-M are 6-O-sulfated (Table I), which in terms of overall sulfation largely offsets the lower concentrations of IdoA(2-OSO3). The only other detectable difference between the two fractions is in size, oligo-M containing predominantly six disaccharides compared with seven in oligo-H (Fig. 3). Therefore, it seems likely that the combined effects of fragment size and enrichment of IdoA(2-OSO3) are the key properties that facilitate a stronger interaction of oligo-H with bFGF. The importance of IdoA(2-OSO3) is emphasized by the analytical data on the low affinity fragment oligo-L (size dp12) in which only 31% of disaccharides contain this component. However, oligo-L is still quite highly sulfated (1.3 sulfates/disaccharide), because of the higher content of GlcN(3S) and GlcNAc(6-OSO3) (Table I). It is of interest that HS from the EHS tumor displays weak affinity for bFGF (20), and this may well be due to its very low level of O-sulfation (31).

Our data provide some revealing insights into the structural heterogeneity of HS. The differential O-sulfation of the large N-sulfated oligosaccharides (Table I) probably reflects a complex mechanism of HS biosynthesis in which the 2- and 6-sulfotransferases may be regulated independently. A specific sequence consisting of GlcN(3S) and IdoA(2-OSO3) appears to be designed for strong binding to bFGF. Perhaps sequences with different sulfation patterns, especially those with mixed 2- and 6-sulfated isomers exemplified by oligo-L, will interact with other HS-binding proteins, for example fibronectin and laminin. It is also possible that other members of the FGF family will bind preferentially with different HS sequences to those recognized by bFGF.

The identification of specific protein binding sequences in GAGs is central to our understanding of their biological functions. The sequencing of the antithrombin-III binding region in heparin was a major advance in this field (19). The interaction is specific, requiring a distinct sugar sequence and sulfation pattern, rather than being determined mainly by relatively unspecific electrostatic forces. Antithrombin-III is activated by heparin in a manner analogous to the HS/heparin activation of bFGF. Thus, specific interactions with GAGs convert proteins from latent to active forms. We are currently investigating the relationship between oligosaccharide affinity and activation of bFGF using the structures described in Table I. Other GAGs such as keratan sulfate and dermatan sulfate are complex structures and may also contain specialized domains for protein recognition. Indeed a heparan sulfate comprising of the rare disaccharide IdoA(2-OSO3)~1,3GalNAc(4-OSO3) has been implicated in the binding of dermatan sulfate to heparin co-factor II (32). When a protein ligand for HS is known, the strategy adopted here, combining specific polysaccharide scission and affinity chromatography may prove useful for isolating and characterizing the protein-binding domains.

Acknowledgments—We thank J. Wassell and H. Bourne for technical assistance, Dr. K. Yoshida for heparitinase and unsaturated disaccharides, Prof. Ulf Lindahl, Prof. Ed Conrad, and Prof. John Hopwood for providing 3H-labeled disaccharides, Dr. M. Lyon for N-desulfated/re-N-acetylated HS, and P. Jones for secretarial help. J. E. T. and J. T. G. thank Prof. D. Crowther, Dr. A. Howell, and Dr. E. Anderson for their support.

REFERENCES
1. Klagsbrun, M. (1989) Prog. Growth Factor Res. 1, 207–235
2. Faasen, P. B. & Singer, S. J. (1989) Proc. Natl. Acad. Sci. U. S. A., 86, 5459–5463
3. Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M., Ingber, D. & Vlodavsky, I. (1988) An. J. Pathol. 130, 395–400
4. Saksela O., Moscatelli, D., Sommer, A. & Rifkin, D. B. (1988) J. Cell Biol. 107, 743–751
5. Presta, M., Maier, J. A. M., Ruusuvuori, M. & Ragnotti, G. (1989) J. Cell. Physiol. 140, 67–74
6. Rifkin, D. B. & Moscatelli, D. (1989) J. Cell Biol. 109, 1–6
7. Sommer, A. & Rifkin, D. B. (1989) J. Cell. Physiol. 138, 215–220
8. Yayon, A., Klagsbrun, M., Eako, J. D., Leder, P. & Ornitz, D. M. (1991) Cell 64, 841–846
9. Rapraeger, A. C., Krufka, A. & Owlin, B. B. (1991) Science 252, 1705–1708
10. Klagsbrun, M. & Baird, A. (1991) Cell 67, 229–231
11. Gallagher, J. T., Lyon, M. & Steward, W. F. (1986) Biochem. J. 236, 313–325
12. Gallagher, J. T. (1989) Curr. Opin. Cell Biol. 1, 1201–1218
13. Turnbull, J. E. & Gallagher, J. T. (1990) Biochem. J. 265, 715–724
14. Turnbull, J. E. & Gallagher, J. T. (1991a) Biochem. J. 273, 553–559
15. Turnbull, J. E. & Gallagher, J. T. (1991b) Biochem. J. 277, 297–303
16. Gallagher, J. T. & Walker, A. (1985) Biochem. J. 230, 665–674
17. Gallagher, J. T. & Lyon, M. (1989) in Heparin (Lane, D. & Lindahl, U., eds) pp. 135–168. Edward Arnold, London
18. Gallagher, J. T., Turnbull, J. E. & Lyon, M. (1990) Biochem. Soc. Trans. 18, 207–209
19. Lindahl, U., Thumberg, L., Backstrom, G., Rosenfeld, J., Nordling, K. & Bjork, I. (1984) J. Biol. Chem. 259, 12368–12376
20. Vigny, M., Ollier-Hartmann, M. P., Lavigne, M., Faystein, N., Jeann, J. C., Laurent, M. & Courtois, Y. (1988) J. Cell Physiol. 137, 321–329
21. Ke, Y., Fennig, D. G., Smith, J. A., Wilkinson, M. C., Rudland, P. S. & Barraclough, R. (1989) Biochem. Biophys. Res. Commun. 171, 963–971
22. Amann, E. & Bronius, J. (1986) Gene (Amst.) 40, 183–190
23. Abraham, J. A., Wbang, J. L., Tunolo, A., Mergia, A., Friedman, J., Gospodarowics, D. & Fiddes, J. C. (1986) EMBO J. 5, 2523–2528
24. Inoue, Y. & Nagase, K. (1976) Carbohydr. Res. 46, 87–95
25. Feijer, G. & David, G. (1987) Biochem. J. 248, 69–77
26. Birkenesovski, M. J. & Connell, H. E. (1980) J. Biol. Chem. 260, 356–365
27. Linhardt, R. J., Turnbull, J. E., Wang, H. M., Loganathan, D. & Gallagher, J. T. (1990) Biochemistry 29, 2611–2617
28. Ornitz, D. M., Yayon, A., Flanagan, J. G., Svahn, C. M., Levi, K. & Leder, P. (1992) Mol. Cell. Biol. 12, 240–247
29. Barna, T., Lormeuse, J.-C., Petitos, M., Michelon, S. & Chaoy, J. (1989) J. Cell. Physiol. 140, 538–548
30. Southern, J., Folkman, J., Svahn, C. M., Berland, K. & Damore, P. A. (1989) J. Biol. Chem. 264, 6892–6897
31. Trescony, P. V., Ogema, T. R., Farnam, B. J. & Deloria, L. B. (1991) Connect. Tissue Res. 19, 219–245
32. Mainone, M. M. & Tollesfolen, D. M. (1990) J. Biol. Chem. 265, 1823–1827