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Hyaluronan Research in Uppsala*

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Abstract

Studies of the polysaccharide hyaluronan (hyaluronic acid) started more than a century ago in Uppsala. This article describes the general development of hyaluronan research from an Uppsala point of view and is thus strongly biased. The readers are referred to other reviews for a more objective description of the history.

Introduction

Hyaluronan (previously hyaluronic acid) is a polysaccharide distributed ubiquitously in tissues of vertebrates. It belongs to the family of polymers termed glycosaminoglycans (previously mucopolysaccharides). Hyaluronan has for a century interested scientists in Uppsala. It is also in Uppsala that it was first developed on industrial scale to an important product for clinical use. This article is intended to give a brief history of hyaluronan research and specifically point out contributions originated in Uppsala. For less biased reviews see e.g. (1–4) and for the most recent developments (5,6).

In previous reviews (5,7) I have compared the development of hyaluronan research to a tree (Figure 1). It has its roots in connective tissue biology, carbohydrate chemistry and polymer science. New branches are growing out from the stem when enough basic knowledge has accumulated for them to be viable. I will use this metaphor also in this article.

How it started

Hyaluronan was discovered by Karl Meyer in 1934 (8). He prepared from bovine vitreous humour a non-sulfated polysaccharide, which contained equal amounts of uronic acid and hexosamine. He gave it the name hyaluronic acid after hyalos (=glassy) and uronic acid. However, Carl Thore Mörner in Uppsala isolated a "mucin" from the vitreous already in 1894 (9). It presumably consisted of hyaluronan contaminated with proteins.

Meyer and collaborators determined the structure of hyaluronan in a series of papers during the 1950ies (for references see e.g. Brimacombe and Webber (10)). It is a linear polymer with alternating D-glucuronic acid and D-N-acetyl glucosamine units linked by €– (1–3) and €– (1–4) linkages (Figure 2).

The present author was introduced to hyaluronan in 1949 when serving as a jun-

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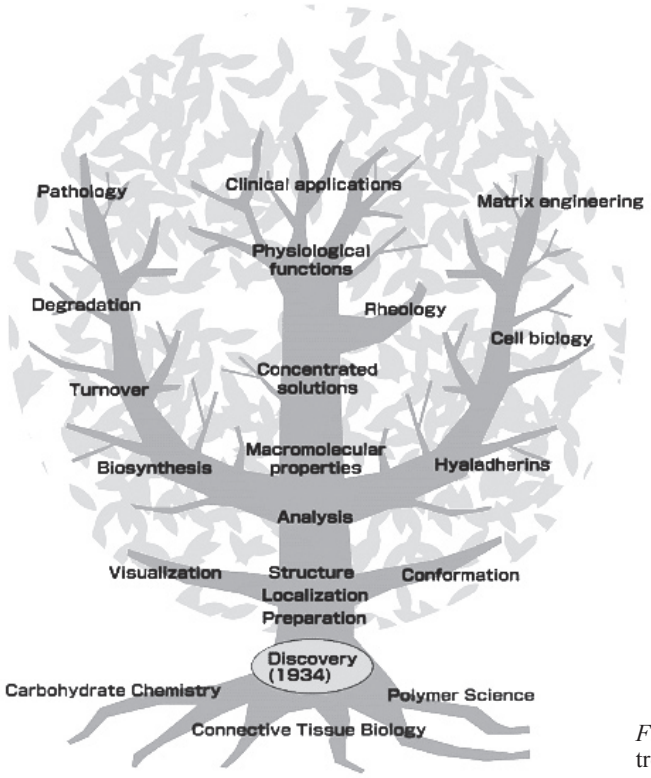

Figure 1. The hyaluronan research tree. (From reference 7).

ior instructor in histology at the Karolinska Institute in Stockholm. My mentor was a Hungarian visiting scientist, Endre A. Balazs, who studied the biological effect of hyaluronan on fibroblasts in tissue culture. During the preparation of hyaluronan from umbilical cords we discovered the polyelectrolyte viscosity of hyaluronan (11) and that it was degraded by ultraviolet light (now known to be free radical degradation) (12). At this time the structures of hyaluronan and heparin had not yet been determined but it was known that both polymers contained uronic acid and glucosamine and that heparin was sulfated. We could show that sulfated hyaluronan was not identical to heparin (13).

In 1951 Balazs moved to an eye research laboratory in Boston, MA and I continued with physical-chemical studies on hyaluronan in the Chemistry Department under supervision of Bertil Jacobson.

The macromolecular properties of hyaluronan

The first larger branch on the hyaluronan tree started with the physico-chemical characterization of the molecule.

The high visosity of hyaluronan solutions was early reported by many authors. The first attempt to investigate the macromolecular properties by other techniques was carried out in Uppsala by Gunnar Blix and Olle Snellman in 1945. They used

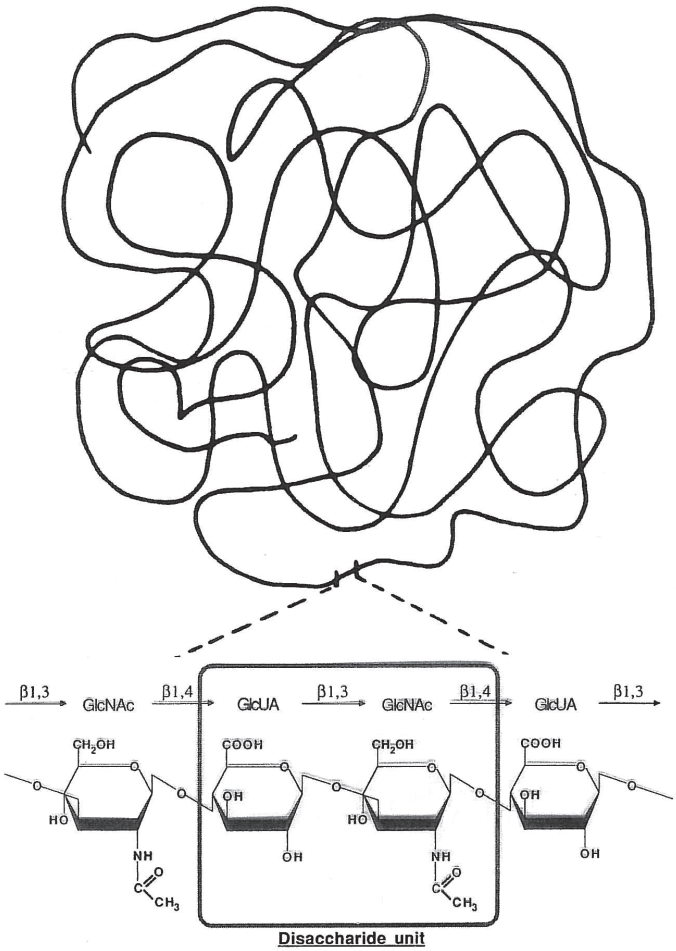

Figure 2. The chemical and macromolecular structure of hyaluronan.

streaming birefringence measurements and concluded that hyaluronan was a rod-like molecule with a length of 4.800–10.000 Å (14). Blix also described the electrophoretic mobility of hyaluronan (15).

In a series of papers on a hyaluronan-protein complex isolated from synovial fluid, Ogston and Stanier in Oxford studied its hydrodynamic properties and described the complex as a large hydrated sphere based on a random coil configuration (see e.g. ref. 16). At the same time the present author using light-scattering technique described a purified hyaluronan from umbilical cord as an extended random coil with molecular weight in the millions (17) (Figure 2). The work was carried out in Balazs' laboratory in Boston and extended in Stockholm where it formed the basis for a doctoral thesis (18). This also included studies on the hydration of the molecule and it was concluded that the solvent was mechanically immobilized within the coil rather than forming an organized hydration shell around the chain.

In 1959 the author returned to Boston for an extended postdoc period. We had previously observed that hyaluronan from bovine vitreous humour must be poly-

disperse and an attempt was made to fractionate it according to molecular weight by detergent precipitation (19) which previously had been used to fractionate differently charged polysaccharides as described by John Scott. The attempt was successful and the mechanism could later be explained theoretically (20).

The characterization of hyaluronan has continued in various laboratories. Fessler and Fessler (21) showed by electron microscopy that the hyaluronan molecule is a long unbranched chain molecule (an example of visualization). Robert L. Cleland, an experienced polymer chemist, worked for long periods in Uppsala between 1968 and 1993 on the macromolecular properties of hyaluronan. He refined the techniques for fractionation, he characterized the random coil and he analyzed the polyelectrolyte behavior (see e.g. 22–24). Cleland's experimental and theoretical studies confirmed the random coil structure of hyaluronan and its worm-like behavior. A doctoral thesis on the physical chemistry of hyaluronan was also presented by Ove Wik (25).

The conformation of hyaluronan

Atkins, and Sheehan showed in 1972 that hyaluronan in fibrous form has a helical structure (26). Later John Scott analyzed the conformation in solution using periodate oxidation and NMR and found that it was a helical structure stabilized by hydrogen bonds between the sugar residues in the chain (for references see (27)). The helix also displays hydrophobic patches which possibly could lead to chain-chain associations in the form of double helices. The helix formation explains the stiffness of the chain, which leads to the extended dimensions of the random coil.

Hyaluronan networks

In the above mentioned work on the fractionation of hyaluronan from the vitreous humour (19) the molecular weights were determined in the ultracentrifuge. There was a striking concentration dependence of the sedimentation rates. At high dilution the sedimentation rates mirrored the molecular weights while at increasing concentrations the difference decreased. Already at 2 mg/ml all the fractions sedimented at the same rate and very slowly. The conclusion was that the molecules had entangled and formed a three-dimensional chain network that resisted water flow and retarded the sedimentation.

When I moved to Uppsala in 1961 it was my intention to study the properties of this network and the possible physiological implications (Table 1). Uppsala turned out to be an ideal place for such research. The polysaccharide dextran had been developed into a plasma substitute by Ingelman and Grönwall at the Department of Biochemistry in the 1940ies and Pharmacia, which manufactured dextran, had moved to Uppsala. There was intensive experimental and clinical research on dextran and one could find interesting parallels between dextran and hyaluronan.

Rheology. The rheological characteristics of hyaluronan solutions have been investigated in many laboratories e.g. by Ogston (16) and Balazs (28). Concentrated

Table 1. Physiological functions of hyaluronan networks

Physico-chemical property	Function
Viscosity	Lubrication in joints and muscles
Osmotic pressure	Water homeostasis between tissue compartments
Flow resistance	Formation of flow barriers
Sieve effect	Formation of diffusion barriers. Protection.
Excluded volume	Partition of proteins between compartments. Pathological depositions.

solutions of hyaluronan are viscoelastic due to the entanglement of the chains and the viscosity is markedly shear dependent due to stretching of the chains along the streamlines. The shear dependence was studied in detail already in a thesis by Lars Sundblad in Uppsala in 1953 (29). A more recent Uppsala contribution is the thesis by Hege Bothner Wik in 1991 (30). The rheological properties of hyaluronan have been related to its function as a lubricant in joints and other tissues. The shear-dependence gives a low viscosity of the lubricant during rapid movements and a high viscosity during slow precision movements, which is functional. Due to the elasticity kinetic energy is stored in the network and used in the return of joints to the rest positions.

Osmotic pressure. Under ideal conditions the osmotic pressure of macromolecular solutions is a linear function of the concentration. Hyaluronan solutions are, however, markedly non-ideal and the osmotic pressure rises rapidly with concentration (see e.g. ref.31). For this reason, hyaluronan acts as an osmotic buffer in the tissues. A small drop in concentration of hyaluronan due to influx of water from the circulation to the matrix leads to a marked drop in osmotic pressure and the water returns to the blood stream.

Flow resistance. As shown by the sedimentation experiments cited above (19) the hyaluronan networks exert a high resistance to solvent flow. Thus the network prevents rapid flow through the tissue matrices and bulk flow is taking place mainly within blood and lymph vessels. The first one to demonstrate the high flow resistance was Day (32) in a classic experiment in which he filled a capillary with hyaluronan and measured the flow of solvent through the capillary before and after hyaluronidase treatment.

Sieve effect. The network also acts as a filter for other molecules; retarding large ones and letting small molecules diffuse freely. This effect could be demonstrated both in sedimentation (33) and diffusion (34) experiments. From experimental data an empirical relationship between retardation, size of the moving molecule and concentration of hyaluronan was deduced (34). Ogston then showed that this relationship described the statistical probability that there would be a space in the network next to a spherical molecule into which it could move (35). The network does not, as expected, have any effect on the rotation of a spherical molecule (36). It is possible to hypothesize that the sieve effect protects cells from other cells and

microorganisms as well as large proteins, such as antibodies, while letting nutrients and waste products freely diffuse to and from the cell surface.

Exclusion effect. While the sieve effect describes the retardation of transport through the network the exclusion describes the volume no longer available for other molecules in the network. Exclusion was first described by Ogston and Phelps (37) and then put on a quantitative basis by equilibrium dialysis and gel chromatography on cross-linked hyaluronan (38). For large particles like viruses the exclusion is quite dramatic. The exclusion is important in the tissue since it keeps plasma proteins in the blood stream as there is limited space for them in the extravascular space.

The addition of a polysaccharide to a solution of another macromolecule removes space from the latter, which leads to an increase in its concentration or alternatively expressed an increase in its chemical activity. There are interesting consequences e.g. the macromolecule may precipitate if its solubility limit is reached (39). It is notable that many physiological or pathological precipitations occur in polysaccharide containing compartments e.g. collagen fiber formations, immune precipitations (40), formation of lipid plaques and amyloid deposition. Also the equilibrium in chemical reactions between macromolecules may be affected (41).

The clinical use of concentrated hyaluronan (hyaluronan networks)

The first clinical use of hyaluronan is founded on our knowledge of its behaviour in concentrated solutions.

Endre Balazs was at an early stage looking for clinical use of hyaluronan especially in replacement of the vitreous body. A Swedish orthopaedic surgeon, Nils Rydell, joined his laboratory. He had experience of treating horses with arthrotic joints by cortison injections. However, the cortison is insoluble in water and precipitated on and destroyed the cartilage. To avoid the precipitate formation he and Balazs dispersed the cortison in a hyaluronan solution before injection. It turned out that hyaluronan alone had a beneficial effect on the joints (42). Balazs contacted the present author and asked for advice which pharmaceutical company would be interested in developing hyaluronan for treatment of human joints. Through my mediation Pharmacia in Uppsala was engaged in the project in 1972. Hyaluronan was produced from rooster combs according to a procedure described by Balazs. However, when clinical trials were started on patients with arthrosis there was a very high placebo effect.

At this point a completely new aspect arose. The eye surgeon David Miller in Boston was performing lens extraction on rabbits and replacing them with plastic lenses. However, the plastic injured the corneal endothelium. When he dipped the artificial lens in a solution of concentrated hyaluronan that he had received from Balazs the cornea was protected. A South African ophthalmic surgeon, Robert Stegmann, made a clinical trial and showed that if he filled the anterior chamber of the eye with hyaluronan during cataract surgery and intraocular lens implantations he had much better operating conditions and protected the sensitive eye tissues

Table 2. Practical applications of visco-elastic solutions of hyaluronan or hyaluronan gels.(From Balazs, E.A., Chapter 20 in reference 4)

Viscosurgery Eye surgery, e.g. cataract surgery. Arthroscopic surgery.
Viscoaugmentation Filling facial wrinkles. Treatment of glottal insufficiency Treatment of urinary incontinence.
Viscoseparation To prevent adhesions after surgery.
Viscosupplementation Injections in joints to relieve pain
Viscoprotection Eye drops to treat dry eyes. Protection of wounds.
Drug delivery
Matrix and tissue engineering

(43). Hyaluronan, under the name of Healon, immediately became a commercial product for eye surgery and a special division of Pharmacia, Pharmacia Ophthalmics, was formed.

Concentrated hyaluronan has since then been tried for many medical purposes such as other surgical applications, in tissue implants, in antiadhesion and wound-healing, as a moisturizer, as drug carrier etc. (Table 2). In Uppsala Bengt Ågerup, who had experience of hyaluronan from Pharmacia, started to use cross-linked hyaluronan for tissue implants. He has been highly successful and his company, Q-Med, is now a leading biotech firm in Uppsala. Among recent reports on the use of hyaluronan can be mentioned hyaluronan implants in the treatment of vocal cord insufficiency (44). Linking insulin covalently to hyaluronan protects it during oral administration (45).

Analytical techniques

Development of new analytical techniques has been part of the main stem of the "tree" ever since the discovery of hyaluronan.

In early years the concentration of hyaluronan was determined by color reactions specific for uronic acid or hexosamine. The analyses required pure polysaccharides and could not be applied to body fluids. The sensitivity was in the order of 0.01–1 mg/ml. In the end of the sixties we developed an isotope dilution technique that could measure the hyaluronan directly in aqueous humour (46) It had a sensitivity of 1Γg/ml.

The real breakthrough came when the first hyaluronan binding proteins were discovered. Hardingham and Muir found in 1972 that hyaluronan aggregates cartilage proteoglycans (46) and Heinegård and Hascall subsequently showed that hyaluronan binding proteins could be isolated by trypsin digestion of these aggregates (48). Using these affinity proteins Anders Tengblad developed a radio assay for

Table 3. Hyaluronan concentrations in some human body fluids. For references see (3)

Fluid	Concentration (mg/l)
Joint fluid	1,400–3,600
Thoracic lymph	8.5–18
Amniotic fluid (week 16)	21.4
(at term)	1.1
Skin blisters	0.8–5.6
Cerebrospinal fluid	0.2–0.5
Aqueous humour	0.3–2.2
Urine	0.1–0.3
Blood serum	0.01–0.1

hyaluronan, which could determine nanogram quantities of the polysaccharide (49) and could be used on biological material (50). This technique and various modifications have been extensively used to determine the hyaluronan content in organs and tissue fluids. Of special interest have been analyses on blood (51,52). Table 3 lists the concentration of hyaluronan in some tissue fluids.

The distribution of hyaluronan in various organs was studied in the rat (53). An animal weighing 200 g contained about 60 mg hyaluronan. About 56% were found in the skin, 8% in muscles, 27% in skeleton and supporting tissues, 1% in intestines and stomach and 9% in remaining internal organs and blood. Hyaluronan has not been found in animals below vertebrates (Laurent, T.C. and Lilja, K. unpublished).

The availability of hyaluronan binding proteins did also open up the possibility of staining hyaluronan specifically in histological sections (54).

Turnover of hyaluronan

A new main branch of the hyaluronan tree started to grow out from the stem a quarter of a century ago. In 1980 the present author met Robert Fraser in Australia. Fraser had grown synovial cells in the presence of tritium-labeled acetate and isolated labeled hyaluronan. We decided to use this material for studies on the turnover of hyaluronan in the living organism.

When injected in the circulation of a rabbit it disappeared with a half-life of a few minutes and after 20 minutes tritiated water appeared in the blood. The main part of the radioactivity was taken up in the liver in its non-parenchymal cell fraction (55). The uptake could be prevented by preinjection of excess unlabelled hyaluronan and was therefore due to a receptor mediated mechanism. When the labeled hyaluronan was injected in humans a similar half-life in the circulation was recorded (56). The central role of the liver in the uptake of hyaluronan was confirmed in whole-body autoradiographs of mice injected with C-14 labelled hyaluronan (57).

Bård Smedsröd identified the cells, which endocytosed the hyaluronan, as sinusoidal liver endothelial cells (58). This came as a surprise; we had expected the

known scavengers – the Kupffer cells. The endothelial cells were found to carry an endocytose receptor which now has been identified and which is named stabilin-2 (59,60). The scavenger functions of the liver endothelial cells have been reviewed (61).

It was early recognized that the endogenous hyaluronan in blood must have been carried from the tissues by the lymph (62,63). Subsequent studies on the turnover of radioactive hyaluronan injected in various tissue compartments such as joints, skin, muscles, amniotic fluid and the eye compartments have shown a surprisingly rapid turnover in the tissues (half-lives in the order of hours up to a few days). Rolf Reed from Bergen, Norway, has taken a very active part in this work; The turnover in the tissues has been reviewed (64,65). Part of the hyaluronan is degraded locally. A major part is carried by the lymph to the lymph nodes where in the order of 90% is endocytosed and degraded (66). Only a minor part is thus entering the general circulation.

Studies on pathological hyaluronan turnover

The new knowledge of the turnover of hyaluronan and the availability of a technique to measure hyaluronan in body fluids started an intensive research activity in the clinical field. A number of conditions with pathologically high serum levels were discovered. There are a few rare genetic diseases such as progeria and Werner's syndrome and one, recently discovered by Robert Fraser, named cutaneous hyaluronanosis (67). There are also some rare tumors such as Wilm's tumor and mesothelioma, which produce large amounts of hyaluronan.

However, the most common conditions are inflammatory diseases, e.g. rheumatoid arthritis, in which there is overproduction of hyaluronan. It is especially worth mentioning that in rheumatoid arthritis the plasma level often shows a daily variation with a high peak in the morning (68). When this was discovered an explanation could for the first time be given to the symptom "morning stiffness". Hyaluronan is accumulated during the night in the inflamed joints, which become swollen and stiff. When the physical activity starts in the morning the excess hyaluronan is pumped via the lymph into the blood stream.

In severe liver conditions (liver cirrhosis) the uptake of hyaluronan is impaired and one usually finds very high levels of serum hyaluronan.

There is now a very large literature on serum hyaluronan as a clinical marker and the reader is referred to a review which contains 150 references (69). The author wants especially to acknowledge the great contribution to this work carried out in Uppsala or in collaboration with research groups in Uppsala by former graduate students Anna Engström-Laurent (70), Lena Lebel (71), Henning Onarheim (72), Ulla Lindqvist (73), James Alston-Smith (74) and Sören Berg (75).

Except blood serum a number of other fluids and organs have been studied under normal and pathological conditions. Ulla Laurent investigated the concentration and turn-over in the aqueous humour and vitreous body (76). Claude Laurent analyzed the compound in ear tissues and investigated the use of exogenous hyaluro-

nan in otosurgery (77). Lauritz Dahl followed the hyaluronan level in amniotic fluid during the foetal development of sheep and could define the sources of the compound and the routes of its degradation (78). Greta Edelstam studied hyaluronan in peritoneal fluid and the female reproductive tract during inflammatory conditions (79). An extensive research on pulmonary hyaluronan has been carried out by Roger Hällgren and his associates. They have followed the compound in broncho-alveolar lavage fluid in various pathological conditions and shown increase during both experimentally induced (80) and other inflammatory conditions such as adult respiratory distress syndrome (81), sarcoidosis (82) and farmer's lung (83). Hans Johnsson (84) has been especially interested in lung hyaluronan during the perinatal period.

Synthesis of hyaluronan

Hyaluronan is synthesized by enzymes, hyaluronan synthases (Has), situated in the cell membrane. Glucuronic acid and N-acetyl glucosamine are transferred from their respective UDP-derivatives to the growing chain on the inside of the membrane and the chain is simultaneously translocated to the pericellular space. For reviews on the biosynthesis see e.g. an article by Weigel in ref. (4) and (85,86). Peter Prehm was the first one to describe the mechanism (87,88) and according to him sugars are added to the reducing end of the growing chain in eucaryotic cells. A similar result was obtained by Heldin and collaborators (89) while a report from Lindahl's laboratory describes the elongation as taking place in the non-reducing end (90). Further analyses are required.

On the outside of the cell the hyaluronan forms a thick coat which was ingeniously visualized by Clarris and Fraser (91). If particles, e.g. erythrocytes, were added to a culture of synovial fibroblasts the particles were completely excluded from the pericellular layer. Heldin and Pertoft (92) described similar coats around mesothelial cells while the malignant counterpart, mesothelioma cells, were without them. When studied in culture, cells can be seen to shed the coats and there are apparently mechanisms at the cell surface that can release the hyaluronan chains from the synthase.

We now know that there are three mammalian synthases (Has 1, Has 2 and Has 3) and that they are expressed in different tissues, during different periods of the life cycle, are synthesizing chains of different molecular weights and are regulated by different mechanisms (4). Has 2 is preferentially expressed during the embryonal stage and makes very high molecular weight hyaluronan.

Studies in Uppsala on synthesis of hyaluronan started with an important observation that growth factors can induce fibroblasts to make the polymer (93). The largest effect was recorded with platelet-derived-growth-factor (PDGF-BB) but lower effects were also obtained with EGF, bFGF and TGF-€ The hyaluronan synthesis did not correlate with the mitogenic properties of the factors. Since then a number of other growth factors, cytokines and other compounds have been shown to have a regulatory function.

The discovery that hyaluronan synthesis is regulated by external factors has led to an extensive work in the research group of Paraskevi Heldin on the biosynthesis of hyaluronan and its biological implications. Further analyses of the stimulatory activity of PDGF-BB, TGF-€and TPA (a tumor promoter) showed that it was mediated by protein kinase C (89, 95,96). The effect of the two growth factors was also dependent on an active protein synthesis, while TPA seemed to directly activate PKC.

To investigate the role of the three synthases (Has 1, Has 2 and Has 3) they were expressed in recombinant form in CHO-cells (97) alternatively that the expression of mRNA for the three isoforms was studied after external stimulation (98). Stimulation of mesothelial cells with PDGF-BB upregulated mRNA for Has 2 whereas the genes for Has 1 and Has 3 were only slightly induced. A similar upregulation of Has 2 was found in dermal fibroblasts (98) together with some increase in Has 1. With inhibitors to the Erk MAP kinase and P13 kinase signaling pathways both the expression of Has 2 mRNA and hyaluronan synthesis could be completely suppressed while inhibitors for other pathways had less effect. The signal transduction pathway for PDGF-induced hyaluronan synthesis thus seems to be clarified. Studies by several research groups have revealed that different types of cells react differently to various activators of the synthesis and in these cases alternative signaling pathways may exist (85).

Much of the above work has been described in doctoral theses by Asplund (99), Teder (100), Brinck (101), Rahmanian (102), Jacobson (103) and Li (104).

Hyaluronidases

The degradation of hyaluronan into fragments by hyaluronidases is essentially taking place intracellularly. The fragments are then degraded by exoglycosidases.

The discovery of hyaluronidase was made before the discovery of hyaluronan. Duran-Reynals (105) found that extracts from certain tissues promoted the spreading of vaccinia virus in skin and he designated the activity the "spreading factor". Hyaluronidases are wide-spread and found e.g. in certain microrganisms, bee venom, leeches and in higher organisms. For a review see (106). The mammalian hyaluronidases are endo-€N-acetyl hexosaminidases. They split hyaluronan into fragments of various sizes from tetrasaccharides and upwards. We know of six hyaluronidase genes in the genom. One is a pseudogene. Four code for hyaluronidases 1–4 (Hyal 1–4), which are distributed in the tissues. Hyal 1 is found at a high concentration in liver, Hyal 2 in spleen and Hyal 3 in bone marrow. The last hyaluronidase, PH20, is found in testis and sperm and takes part in the fertilization process by helping the sperm to penetrate the zona pellucida.

Hyaladherins

As already mentioned Hardingham and Muir discovered in 1972 (47) the binding between cartilage proteoglycan (aggrecan) and hyaluronan. That was the first ex-

ample of a hyaluronan-binding protein (hyaladherin). Since then a number of proteins that recognize hyaluronan have been discovered. For details of each of them see (4). The hyaladherins have different functions. Some are matrix components, which bind to hyaluronan to form organized structures extracellularly. To these belong aggrecan, versican, neurocan, brevican, link protein and TSG-6.

A second group of hyaladherins are cell surface receptors. To this group belong CD44, RHAMM, toll-like receptor 4, laylin and PH20 (mentioned above as a hyaluronidase). The hyaluronan-endocytosing receptors in liver (stabilin-2) (59,60) and lymph tissues (LYVE) do also belong to this group.

There is also one example of a protein, which binds covalently to hyaluronan, i.e. serum-derived-hyaluronan-binding-protein (SHAP). SHAP is identical to the heavy chain of the serum protein inter-α -trypsin inhibitor and it is enzymatically transferred in the tissues to hyaluronan with which it forms an esterbond.

Hyaladherins have been studied in Uppsala in various connections. Interestingly the first observation of hyaluronan-binding proteins on cells was made by Wasteson et al. (107). Stefan Gustafson later studied the binding of hyaluronan to a number of cell types; some of the work has been presented in the thesis of Nina Forsberg (108). She characterized hyaladherins on macrophages and corneal endothelium and also followed brevican and neurocan in the developing brain The work on stabilin-2 has already been mentioned (59,69). Erik Fries and collaborators studied the inter-α -inhibitor and found that the transfer of SHAP was important for the formation of stable hyaluronan coats around cells in culture (109).

Cell biological functions of hyaluronan

It has for a long time been known that hyaluronan plays a role in a number of biological processes (Table 4) but until about twenty-five years ago it was believed that the characteristic physical-chemical properties of the polymer were the basis for its biological functions. With the new knowledge that cell surface receptors recognize hyaluronan and our deeper understanding of hyaluronan synthesis and degradation, the interest has instead been focused on the specific interactions between cells and hyaluronan. The growing interest is mirrored in general reviews (1–4) and especially in the proceedings from the latest international conferences on the polymer (5,6).

The first report that hyaluronan influenced a cellular activity was probably the observation that it promoted cell growth in fibroblast cultures (13). Although most cell biological studies since then have been performed elsewhere some interesting observations have been made in Uppsala in recent years.

As mentioned earlier there is an increased production of hyaluronan in inflammatory conditions. Efforts have been made to understand the mechanism of this increase. In one experimental system bleomycin was instilled in the lungs of rats causing an inflammation and this increased hyaluronan in brocnhoalveolar lavage fluid (110). It could be shown that stimulatory activity for hyaluronan biosynthesis increased in the lavage fluid and that a major part of this activity was produced by

Table 4. Some cell biological activities in which hyaluronan takes part

Embryonal development
Cell differentiation
Cartilage formation
Cell motility
Nerve cell generation
Tumor growth
Wound healing
Inflammation
Angiogenesis

the alveolar macrophages. On the same time the binding and uptake of hyaluronan in the macrophages decreased. Also irradiation-injury causes similar increase in lung hyaluronan. When irradiated rat lungs were analyzed the expression of Has 2 increased and that of Hyal 2 decreased which explains the accumulation of hyaluronan (111). Especially interesting was the observation that fragments of hyaluronan induced Type I and Type III collagen synthesis, which can explain lung fibrosis after irradiation.

Another function of hyaluronan, which has attracted attention, is the role it plays in cell migration, especially of malignant cells. Many times the transformation of normal cells to malignant cells leads to a change in hyaluronan synthesis or expression of hyaluronan receptors. Examples of cell lines which carry receptors have been derived from lymphoma (107), colon carcinomas (112); squamous lung cancer (100) and mesothelioma (113). In certain cases malignant cells can induce normal cells to produce hyaluronan e.g. mesothelioma cells produce factors that increase hyaluronan synthesis in mesothelial cells and fibroblasts (113). There are also examples of cancer cells that produce large amounts of hyaluronan, e.g. a malignant breast cancer (114). Not surprising, binding of hyaluronan to cell surface receptors has been associated with migration of malignant cells and metastasis. Evidence was recently found in the breast cancer cell line (114). It expressed preferentially Has 2 mRNA for production of hyaluronan. When this was silenced by RNA interference the tumor became less aggressive and exhibited less cell migration. This could partly be reversed by addition of exogenous hyaluronan.

A recent observation may have interesting implications for cell growth. In dermal fibroblasts PDGF-BB had only mitogenic effect if hyaluronan was bound to CD44 on the cell surface implying that the PDGF-receptor and CD44 co-operate in the plasma membrane to induce mitosis (115).

In 1985 the interesting observation was made by West et al. (116) that fragments of hyaluronan had angiogenic activity while high molecular hyaluronan was antiangiogenic. Since then a number of examples of biological activities of hyaluronan fragments have been described (for a review see (117)).

Rahmanian et al. (118) have confirmed the angiogenic activity. They studied a brain endothelial cell line growing in a collagen gel. When they added hyaluronan

fragments to the cells these organized into tubes ("capillaries"). In an extension of this work Takahashi et al. (119) compared the angiogenic activity of hyaluronan dodecasaccharides and fibroblast growth factor 2 (a known angiogenic factor). Both induced endothelial cell differentiation and by micro array technique it could be shown that there was common gene activation. The hyaluronan fragment also induced the formation of a chemokine.

The discovery that hyaluronan fragments have various biological activities may have important future medical implications. One of the most astounding discoveries was recently reported by Asari and coworkers (120). They showed that hyaluronan tetrasaccharides could protect cells from apoptosis by inducing the synthesis of the chaperon Hsp 72 in the cells. This prevents proteins from denaturation and could very well be a natural mechanism for protecting cells in inflammatory locuses. In rat experiments Asari has also shown that the tetrasaccharides protect neural tissues from destruction after mechanical injuries (personal communication).

In retrospect

When the author became engaged in work on hyaluronan 58 years ago only a few research groups in the world were interested in the compound and only a very limited number of publications had appeared. A search on PubMed today gives more than 10,000 references. Now we also have an International Society for Hyaluronan Sciences (ISHAS) that organizes international congresses every third year. At the last conference there were about 400 participants and 130 scientific contributions.

Looking back it is striking how little of the development in the hyaluronan field could have been predicted. During the first 35 years after the discovery of hyaluronan the research was purely preclinical. After 1980 it has become of major clinical interest. Of the 29 graduate students working on hyaluronan projects and cited in this review fourteen came from various clinical departments and graduated after 1980. Three students came from industry.

When the University of Uppsala celebrated its 500th anniversary in 1977 the author gave the traditional lecture at the jubilee graduation ceremony. It had the title "Modern medicine is founded on basic research" (121). I described a number of discoveries in Uppsala that unexpectedly have been of practical importance in clinical medicine. New examples have since then appeared in my own filed. No one could predict that hyaluronan would revolutionize cataract surgery; that it would be used to treat speech problems due to flaccid vocal cords; that it would explain morning stiffness in rheumatoid arthritis; that it would lead to the discovery of the liver endothelial cells as scavengers; that it could serve as a serum marker for liver cirrhosis; that it would explain why polymers can enhance immune reactions; or that hyaluronan oligosaccharides perhaps will be used to prevent tumor metastasis or to minimize neural damage. The largest use of hyaluronan today is probably as moisturizer in beauty creams. The commercial success of hyaluronan has certainly contributed to the scientific and clinical interest and I am convinced that we can expect many new practical applications of the compound in the future. I would

like to cite Stern et al (117) who suggest that the change of the name "hyaluronic acid" to "hyaluronan" has to be reconsidered. The name ought to be "highly ironic acid".

As stated above the author has used this opportunity to present hyaluronan research carried out in Uppsala which of course is a small fraction of all research carried out in the field. Contributions from outside Uppsala have only been included to give a general background. But even so there has only been space to cite a fraction of all publications that have originated in Uppsala. I apologize to all those who have not been cited.

Finally, I would like to express my gratitude to all collaborators I have had during the years and for the enjoyment to climb the "hyaluronan tree" together with them.

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