DISSERTATIONS IN
HEALTH
SCIENCES

Tiina Jokela

Regulation of Hyaluronan Synthesis by UDP-sugars

Publications of the University of Eastern Finland
Dissertations in Health Sciences

UNIVERSITY OF
EASTERN FINLAND

TIINA JOKELA

Regulation of hyaluronan synthesis by UDP-sugars

To be presented by permission of the Faculty of Health Sciences,
University of Eastern Finland for public examination in Auditorium L21,
Kuopio, on Saturday, December 10th 2011, at 12 noon

Publications of the University of Eastern Finland
Dissertations in Health Sciences
Number 89

Department of Biomedicine, School of Medicine, Faculty of Health Sciences,
University of Eastern Finland
Kuopio
2011

Kopijyvä oy
Kuopio, 2011

Series Editors:
Professor Veli-Matti Kosma, M.D., Ph.D.
Institute of Clinical Medicine, Pathology
Faculty of Health Sciences

Professor Hannele Turunen, Ph.D.
Department of Nursing Science
Faculty of Health Sciences

Professor Olli Gröhn, Ph.D.
A.I. Virtanen Institute for Molecular Sciences
Faculty of Health Sciences

Distributor:
University of Eastern Finland
Kuopio Campus Library
P.O.Box 1627
FI-70211 Kuopio, Finland
http://www.uef.fi/kirjasto

ISBN (print): 978-952-61-0625-0
ISBN (pdf): 978-952-61-0626-7
ISSN (print): 1798-5706
ISSN (pdf): 1798-5714
ISSN-L: 1798-5706

Author's address: Department of Biomedicine / School of Medicine / Anatomy
University of Eastern Finland
KUOPIO
FINLAND

Supervisors: Professor Markku Tammi, M.D., Ph.D.
Department of Biomedicine / School of Medicine/Anatomy
University of Eastern Finland
KUOPIO
FINLAND

Professor Raija Tammi, M.D., Ph.D.
Department of Biomedicine / School of Medicine/Anatomy
University of Eastern Finland
KUOPIO
FINLAND

Reviewers: Professor Jukka Finne, M.D., Ph.D.
Department of Biosciences
University of Helsinki
HELSINKI
FINLAND

Docent Sakari Kellokumpu, Ph.D.
Department of Biochemistry
University of Oulu
OULU
FINLAND

Opponent: Professor Jens Fischer, Ph.D.
Hautklinik
Universitätsklinikum Düsseldorf
DÜSSELDORF
GERMANY

Jokela, Tiina. Regulation of hyaluronan synthesis by UDP-sugars
University of Eastern Finland, Faculty of Health Sciences, 2011
Publications of the University of Eastern Finland. Dissertations in Health Sciences
Number 89. 2011. 78p.

ISBN (print): 978-952-61-0625-0
ISBN (pdf): 978-952-61-0626-7
ISSN (print): 1798-5706
ISSN (pdf): 1798-5714
ISSN-L: 1798-5706

ABSTRACT

Hyaluronan is a large glycosaminoglycan consisting of alternating N-acetylglucosamine (GlcNAc) and glucuronic acid (GlcUA) units. It is synthesized by hyaluronan synthase enzymes (HAS1,2,3). In many tissues hyaluronan is a major component of the extracellular matrix. It enhances cell proliferation, migration, and controls differentiation. High hyaluronan levels are associated with cancer progression and inflammation. In this study a new inhibitor of the hyaluronan synthesis, mannose, was discovered and it was demonstrated that it depletes content of UDP-GlcNAc. The three HAS enzymes showed different sensitivities to the cellular content of the UDP-GlcNAc. HAS3 had the highest affinity to the precursors and HAS1 the lowest, suggesting that the HAS-isoenzyme distribution in a particular cell type determines the sensitivity of its hyaluronan synthesis to UDP-sugar supply. Interestingly, a feedback mechanism from UDP-sugar content to *HAS2* expression was found since fluctuations in UDP-GlcNAc content caused reciprocal changes in *HAS2* transcription. This regulation is likely mediated by O-GlcNAc modifications of transcription factors YY1 and SP1. This study also showed that the hyaluronan-dependent binding of leukocytes can be induced by the inflammatory mediators and cell stress, and inhibited with mannose. In an *in vivo* wound model mannose reduced the hyaluronan level, granulation tissue growth and accumulation of leukocytes. Altogether, this work shows that cellular UDP-sugar content regulates hyaluronan synthesis and hyaluronan-mediated functions, such as cell migration, proliferation, and leukocyte adhesion. Therefore, inhibition of hyaluronan synthesis by reduction of UDP-GlcNAc using mannose or similar effectors may provide novel ways to treat pathological processes that involve excessive hyaluronan production, e.g. in inflammation and cancer.

National Library of Medical Classification:
Medical Subject Headings: Hyaluronan, UDP-sugars, inflammation

Jokela, Tiina. UDP-sokerit säätelevät hyaluronaani synteesiä
University of Eastern Finland, Faculty of Health Sciences, 2011
Publications of the University of Eastern Finland. Dissertations in Health Sciences
Numero 89. 2011. 78 s.

ISBN (print): 978-952-61-0625-0
ISBN (pdf): 978-952-61-0626-7
ISSN (print): 1798-5706
ISSN (pdf): 1798-5714
ISSN-L: 1798-5706

TIIVISTELMÄ

Hyaluronaani on suuri glykosaminoglykaani joka koostuu vuorottelevista N-asetyyliglukosamiinin (GlcNAc) ja glukuronihapon (GlcUA) muodostamista sokeriyksiköistä. Hyaluronaanisyntaasien (HAS1,2,3) tuottama hyaluronaani on monissa kudoksissa soluvälitilan tärkeimpiä rakennusaineita. Hyaluronaani edistää solujen jakaantumista ja liikkumista, sekä säätelee erilaistumista. Liiallista hyaluronaanin tuotantoa esiintyy monissa syövissä ja tulehdussairauksissa. Tässä tutkimuksessa kuvataan uusi hyaluronaanisynteesin inhibiittori – mannoosi – jonka vaikutus perustuu sen kykyyn vähentää solunsisäistä UDP-GlcNAc-pitoisuutta. Työ osoitti myös että HAS isoentsyymit tarvitsivat erilaiset UDP-sokeripitoisuudet tuottaakseen maksimaalisesti hyaluronaania, HAS3 pystyi tähän pienimmällä pitoisuudella ja HAS1 tarvitsi suurimman pitoisuuden UDP-sokereita. Yksi mielenkintoisimmista löydöksistä oli takaisinsäätelymekanismi, jossa UDP-GlcNAc:n pitoisuuden muutos aiheutti päinvastaisen muutoksen *HAS2*-geenin ilmenemisessä. Tulokset viittaavat siihen että tässä säätelymekanismissa on mukana transkriptiotekijät YY1 ja SP1, sekä niihin sitoutuva O-GlcNAc. Tässä työssä osoitettiin myös että tulehduksen välittäjäaineet ja solustressi lisäsivät hyaluronaanista riippuvaa monosyyttien sitoutumista keratinosyytteihin, ja että tämä sitoutuminen estyi mannoosilla. Myös *in vivo* haavamallissa mannoosi-injektiot vähensivät hyaluronaanin määrää, estivät granulaatiokudoksen muodostumista ja rajoittivat leukosyyttien määrää haava-alueella. Tässä työssä osoitettiin että UDP-sokerisubstraattien konsentraatiota kontrolloimalla voidaan säädellä hyaluronaanin synteesiä ja hyaluronaanin välittämiä solun toimintoja, kuten kasvua, liikkumista ja valkosolujen sitoutumista. Mannoosi, uusi hyaluronaanisynteesin inhibiittori, tai vastaava UDP-GlcNAc:in konsentraatiota vähentävä tekijä voi löytää käyttökohteita tulehduksen ja syövän hoidossa.

Acknowledgements

This work was carried out in the School of Medicine, Institute of Biomedicine at the University of Eastern Finland (formerly University of Kuopio, Department of Anatomy), during 2005-2011.

First of all, I would like to thank my supervisors, Professors Markku and Raija Tammi for introducing me to the world of research. The expedition to the field of hyaluronan has been a great adventure and you certainly have shown me the best of views.

Tammi's research group has always had top-class facilities and an inspiring athmosphere, I am proud of being a member of it. I am also very grateful for the possibility to visit and work at the Cleveland Clinic Lerner Research Institute. The hospitality of Dr. Vincent C. Hascall and Dr. Edward Maytin during my stay in Cleveland is greatly appreciated.

I sincerely thank Professor Jukka Finne and Docent Sakari Kellokumpu, the official reviwers of my thesis, for their careful review and constructive criticism.

My warm thanks go to Professor Heikki Helminen for his genuine interest in my work and for his encouraging attitude. I also wish to thank Dr. Rita Sorvari for giving me an incentive to teaching. Teaching in the Department of Anatomy has been both challenging and enjoyable, and my great colleagues there have made it even more so.

I also want to thank my co-authors Marjo Jauhiainen, M.Sc., Professor Seppo Auriola, Miia Kauhanen, M.Sc., Antti Lindgren, M.D., Jukka Kuokkanen M.D., Katri Makkonen, Ph.D., Sanna Oikari Ph.D., Juha Hyttinen, Ph.D., Elina Koli M.Sc., Professor Gerald Hart, Professor Carsten Carlberg and Professor Matti Laato for their efforts and assistance with publications.

I would like to extend my warm thanks to all the present and former members of the hyaluronan-group, especially to Anne Kultti, Sanna Pasonen-Seppänen, Kirsi Rilla and Kari Törrönen for inspiring collaboration in science and also for the moments outside the working hours, e.g. memorable conference trips.

My deepest thanks go to Riikka Kärnä, who has done a major portion of the lab work; her top class expertise and motivated attitude have made our collaboration productive and enjoyable. I also would like to thank Sari Maljanen, Tuula Venäläinen, Arja Venäläinen, Kari Kotikumpu, Eija Rahunen, Eija Kettunen, Eija Vartiainen and Arja Winberg for their contribution and help. Big thanks also goes to our "gym-team", Hertta Pulkkinen, Piia Takabe, Hanna Siiskonen and Virpi Tiitu, I have really enjoyed our discussions and also got exercise on the side.

I am grateful for the financial support from the Glycoscience Graduate School, North-Savo Cancer Foundation, Emil Aaltonen Foundation and Aarne and Aili Turunen Foundation.

My loving thanks to my parents Pirkko and Jarmo for continuous support in my studies and in all my life. I also would like to thank my aunt Minna for her genuine interest in my studies and encouraging attitude.

Finally I am grateful for having a family that has balanced my life between science and home. I want to express my most loving thanks to Sami, thank you for always helping me with the graphics and layouts, without you my thesis and publications would look less impressive. I am also very grateful for your support and encouragement during this thesis project.

Kuopio, November 2011

Tiina Jokela

List of the original publications:

This dissertation is based on the following original publications:

I. Jokela T, Jauhiainen M, Auriola S, Kauhanen M, Tiihonen R, Tammi M, Tammi R.
Mannose inhibits hyaluronan synthesis by down-regulation of the cellular pool of UDP-N-acetylhexosamines
J Biol Chem, 283(12): 7666-73, 2008

II. Jokela T*, Makkonen K*, Oikari S, Kärnä R, Koli E, Hart G, Tammi R, Carlberg C, Tammi M.
Cellular content of UDP-*N*-acetylhexosamines controls hyaluronan synthase 2 expression and correlates with O-linked *N*-acetylglucosamine modification of transcription factors YY1 and SP1
J Biol Chem, 286(38): 33632-40, 2011

III. Rilla K*, Oikari S*, Jokela T*, Hyttinen J, Kärnä R, Tammi R, Tammi M.
Hyaluronan synthase 1 (HAS1) requires higher UDP-GlcNAc concentration than HAS2 and HAS3, and its expression correlates with the cellular UDP-sugar pool size
Submitted 2011

IV. Jokela T, Lindgren A, Rilla K, Maytin E, Hascall V, Tammi R, Tammi M.
Induction of hyaluronan cables and monocyte adherence in epidermal keratinocytes
Connect Tissue Res, 49(3):115-119, 2008

V. Jokela T, Kuokkanen J, Lindgren A, Kärnä R, Hyttinen M, Kössi J, Peltonen J, Laato M, Tammi R, Tammi M.
Mannose reduces hyaluronan and leukocytes in granulation tissue and inhibits hyaluronan-dependent monocyte binding
Manuscript

* Equal contribution.
The Publications were adapted with the permission of the copyright owners.
This thesis also contains unpublished data.

Contents

Abbreviations

A2ar	alpha-2 adrenergic receptors
ARPO	acidic riboprotein P0
bHABC	biotinylated HABC
CBP	CREB-binding protein
Ccl	chemokine (C-C motif) ligand
CD44	cluster of differentiation 44 / hyaluronan receptor
ChIP	chromatin immunoprecipitation
Cox-2	prostaglandin-endoperoxide synthase 2
CREB	cAMP response element binding protein
CRG-2	cytokine responsive gene-2
CSF	colony-stimulating factor
Cxcl	chemokine (C-X-C motif) ligand
EGF	epidermal growth factor
ERK	extracellular signal-regulated kinase
ERM	ezrin, radixin, moesin; actin-binding proteins
GalNAc	N-acetylgalactosamine
GFAT	glutamine fructose-6-phosphate amidotransferase
Glc	glucose
GlcN	glucosamine
GlcNAc	N-acetylglucosamine
GlcUA	glucuronic acid
GLUT	glucose transporter
GM-CSF	granulocyte-macrophage CSF
GPI	glucosamine-6-phosphate isomerase
HA	hyaluronan

HABC	hyaluronan binding complex of the cartilage aggrecan G1 domain and link protein
HaCaT	human keratinocyte cell line
HAPLN	hyaluronan and proteoglycan link proteins
HARE	hyaluronan receptor for endocytosis
HAS	hyaluronan synthase protein
HAS/*Has*	hyaluronan synthase gene, human/animal
HC	heavy chain
HexNAc	N-acetylhexosamine
HMW	high molecular weight
HPLC	high pressure liquid chromatography
HYAL	hyaluronidase
Hyalp1	hyalurono-glucosaminidase pseudogene 1
IαI	inter-alpha-inhibitor
IFN	interferon
IGF	insulin-like growth factor
IL	interleukin
IP-10	interferon gamma-induced protein-10
KC	keratinocyte-derived chemokine
KGF	keratinocyte growth factor
LMW	low molecular weight
LYVE-1	lymph vessel endothelial hyaluronan receptor 1
MCP	monocyte chemotactic protein
M-CSF	macrophage-CSF
MIG	monokine induced by gamma interferon
MIP	macrophage inflammatory protein
MME	membrane metallo-endopeptidase

MMP	matrix metalloproteinase
MyD88	myeloid differentiation primary response gene 88
4-MU	4-methylumbelliferone
NF-κB	nuclear factor κB
NCoR	nuclear receptor corepressor
O-GlcNAc	O-linked-N-acetylglucosamine
OGT	O-GlcNAc transferase
PAI-1	plasminogen activator inhibitor-1
PBS	phosphate buffered saline
PCAF	P300/CBP-associated factor
PDGF	platelet-derived growth factor
PGE	prostaglandin E
PI3K	phosphatidylinositol-3-kinase
Poly I:C	polyinosinic: polycytidylic acid
p-selectin	cell adhesion molecule
RAR	retinoid acid receptor
RE	response element
REK	rat epidermal keratinocyte
RHAMM	receptor for hyaluronan mediated motility
SCF	serum stem cell factor
siRNA	short interfering RNA
SMC	smooth muscle cell
SP1	specificity protein 1
Spam1	sperm adhesion molecule 1
Socs	suppressor of cytokine signaling protein
STAT	signal transducer and activator of transcription
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinases
TLR	toll-like receptor

TNF	tumor necrosis factor
TRAF	TNF receptor associated factor
TSG-6	tumor necrosis factor alpha simulated gene-6
U937	human monocyte cell line
UDP	uridine diphosphate
UGDH	UDP-glucose dehydrogenase
uPA	urokinase-type plasminogen activator
VEGF	vascular endothelial growth factor
YY1	ying-yang 1

1 Introduction

Hyaluronan is a large glycosaminoglycan localized mainly in the extracellular space. Due to its anionic nature hyaluronan is strongly hydrophilic and acts as a space filler in tissues. Interestingly, hyaluronan also has special properties: it can regulate cell proliferation, migration, invasion and differentiation just by expanding the space inside the tissue or activating intracellular signaling by binding to receptors (Jiang 2011). During embryonic development the synthesis of hyaluronan is essential (Camenisch 2000, Tien 2005) and constantly renewable tissues such as skin epidermis produce high levels of hyaluronan (Tammi 1994). On the other hand, hyaluronan levels increase in many cancers and inflammatory diseases (Jiang 2011, Tammi 2008). Elevated hyaluronan levels indicate poor prognosis in many cancer types (Tammi 2008), while in chronic inflammatory diseases like rheumatoid arthritis, hyaluronan causes joint stiffness, pain and probably maintains inflammation (Naor 2003). Thus, hyaluronan is beneficial in promoting normal tissue regeneration, but in pathological cases it would be useful to control excessive hyaluronan synthesis.

The regulation of hyaluronan synthases (HAS) has been studied widely at transcriptional level, and lately also post-translational modifications have been revealed. *HAS* transcription is controlled by transcription factors such as retinoid acid receptor (RAR), nuclear factor κB (NFκB), cAMP response element binding protein 1 (CREB1), signal transducer and activator of transcription (STAT), specificity proteins 1 (SP1) and 3 (SP3), and often associates with growth factor and cytokine triggered signaling pathways (Jiang 2011, Pasonen-Seppänen 2003, Karvinen 2003, Saavalainen 2005). HAS enzymes are normally active only when localized in plasma membrane (Rilla 2005) and post-translational modifications such as glycosylation, phosphorylation and ubiqitination control enzyme activation (Vigetti 2011, Karousou 2010, Vigetti 2009).

The precursor sugars for hyaluronan synthesis - UDP-GlcNAc and UDP-GlcUA - have received less attention as factors that could determine the rate of hyaluronan production. Hyaluronan synthesis depends on the concentration of intracellular UDP-GlcUA and is inhibited when the UDP-GlcUA level is decreased by siRNA specific for UDP-glucose-6-dehydrogenase (UGDH), or 4-MU (Vigetti 2006, Kultti 2009). Conversely, an elevated UDP-GlcUA level caused, by overexpression of UGDH, increases hyaluronan synthesis (Vigetti 2006). The present study focused on the other precursor – UDP-GlcNAc – and shows that both UDP-sugars are equally

important to hyaluronan production, and that a lack of UDP-GlcNAc inhibits, whereas an overdose stimulates hyaluronan production. Interestingly, UDP-GlcNAc also dampens its own stimulation of hyaluronan synthesis by downregulating *HAS2* mRNA.

Hyaluronan has multiple roles in inflammation. A special form of hyaluronan coat, called cables, binds leukocytes and platelets onto cells of inflamed tissues (De la Motte 2003, De La Motte 1999). In inflammatory sites hyaluronan is digested to short fragments by platelet derived HYAL2 and reactive oxygen species, for example (Al-Assaf 2006, De la Motte 2009). The short hyaluronan fragments activate and maintain inflammation by inducing cytokine secretion and regulating leukocyte functions (Jiang 2011). Hyaluronan cables and their leukocyte binding have been studied in many cell types including fibroblasts, smooth muscle cells, mesangial cells, kidney epithelial cells and endothelial cells (De La Motte 1999, Evanko 2009, Vigetti 2010, Wang 2004, Selbi 2006) following their induction by proinflammatory cytokines, viral infection, ER-stress and hyperglycemic conditions (De La Motte 1999, Wang 2004, Majors 2003, Shi 2006). This study shows that epidermal keratinocytes also produce hyaluronan cables and bind monocytes when treated with cytokines, hyperglycemia, and tunicamycin. The results also show that hyaluronan-mediated inflammatory responses, such as the cable-dependent monocyte binding, can be inhibited by depletion of intracellular UDP-GlcNAc using mannose, an inhibitor of hyaluronan synthesis discovered in this study.

2 Review of the literature

2.1 THE HYALURONAN MOLECULE

Hyaluronan is a large glycosaminoglycan, which consists of repeating disaccharide units of N-acetyl glucosamine (GlcNAc) and glucuronic acid (GlcUA) (figure 1). Hyaluronan synthesis takes place in the plasma membrane and the growing chain is simultaneously extruded into the extracellular space, allowing the synthesis of very long polymers, typically in the range of 10^4 disaccharides (~3.7 x 10^6 Da). At physiological pH the carboxyl groups on the GlcUA residues are negatively charged, making hyaluronan highly hydrophilic. The anionic nature, spatial restrictions around the glycosidic bond, and the weak and transient intramolecular hydrogen bonds determine the physico-chemical properties of hyaluronan. Unlike other glycosaminoglycans, hyaluronan is not built on a core protein and does not contain sulfate groups (Jiang 2011, Toole 2004, Hascall 2000).

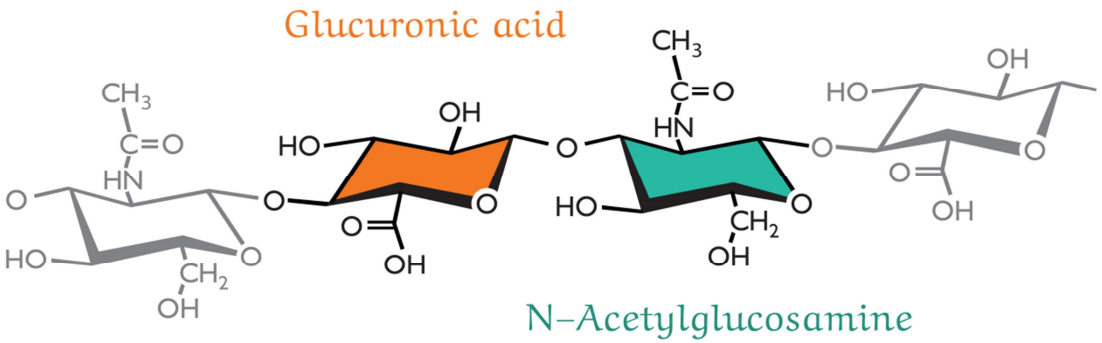

Figure 1. *Chemical structure of the hyaluronan chain with its N-Acetylglucosamine and Glucuronic acid repeating units, linked via alternating β1,4 and β1,3 glycosidic bonds.*

2.2 UDP-SUGARS

2.2.1 Synthesis

Hyaluronan synthesis requires activated precursors in the form of the nucleotide sugars, UDP-GlcUA and UDP-GlcNAc. UDP-glucose-6-

dehydrogenase (UGDH) synthesizes UDP-GlcUA directly from UDP-Glc. UDP-Glc is produced when glucose is first converted to glucose-6-P by hexokinase and then converted into glucose-1-P by phosphoglucomutase. Finally, the reaction of glucose-1-P with UTP forms UDP-Glc (figure 2). UDP-GlcNAc is the end product of the hexosamine biosynthesis pathway. Since it is estimated that about 2% of the total intracellular glucose goes to hexosamine biosynthesis, this pathway can act as a cellular glucose sensor and control cellular energy metabolism. The pathway begins with the formation of glucosamine-6-P from fructose-6-P by glutamine fructose-6-phosphate amidotransferase (GFAT). This is considered to be the rate-limiting step of HBP (Marshall 1991). Glucosamine-6-P is then N-acetylated via an acetyl-CoA-mediated reaction and isomerizes to N-acetylglucosamine-1-P. Finally, a reaction with UTP forms UDP-GlcNAc (figure 2). With a high supply of NH_3, glucosamine-6-phosphate deaminase (GPI) may also convert fructose-6-P into glucosamine-6-P although this enzyme is believed to mainly function in the reverse direction (Varki 2008).
Both GlcUA and GlcNAc can also be salvaged from glycoconjugates degraded by cells. Specific N-acetylhexosamine, sialic acid and glucuronic acid carriers export salvaged sugars from lysosomes for reuse. As much as 80 % of N-acetylglucosamine on glycoproteins can be converted into UDP-GlcNAc after degradation in liver lysosomes (Aronson 1983). UDP-GlcNAc can be converted into UDP-GalNAc by UDP-galactose 4-epimerase, resulting in an equilibrium between UDP-GlcNAc/UDP-GalNAc in a ~3:1 ratio. The common pool of UDP-GlcNAc and UDP-GalNAc is called UDP-HexNAc (Varki 2008).

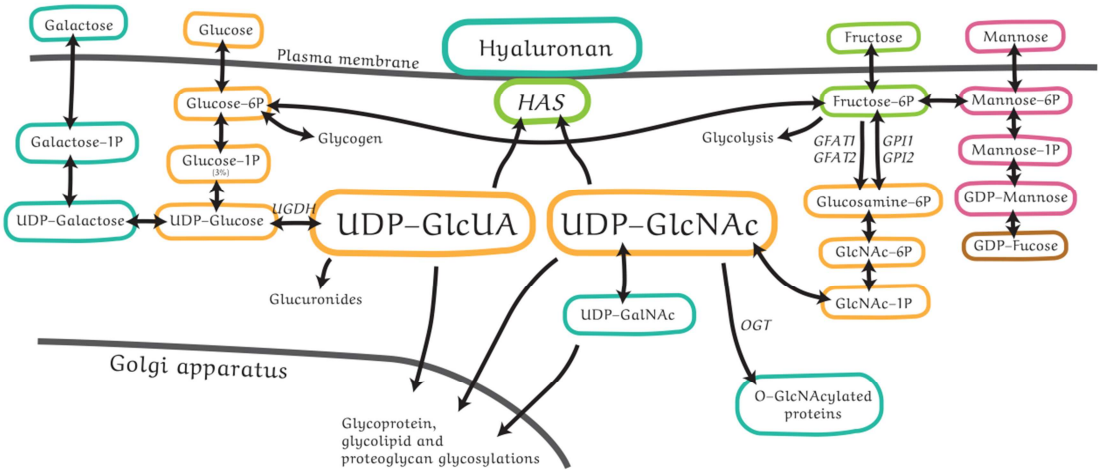

Figure 2. *Main metabolic pathways related to the UDP-sugar precursors of hyaluronan*.

2.2.2 Functions

UDP-GlcUA and UDP-GlcNAc are transported to the Golgi where they are used as building blocks for glycoproteins, proteoglycans and glycolipids. UDP-sugar concentrations are ~20-fold higher in the ER/Golgi than in the cytosol (Hirschberg 1998). Golgi transporters have high affinity to UDP-sugars (Km 1-10 mM) and it has been shown that overexpression of UDP-GlcNAc transporter increases cellular release of UDP-GlcNAc, probably in vesicles together with secreted glycoconjugates, reflecting their content in the Golgi (Sesma 2009).
UDP-GlcUA can also be used in the glucuronidation reactions of xenobiotic metabolism. For instance, glucuronic acid coupling to bile acids and xenobiotic compounds in the liver increases their solubility, and facilitates secretion (Varki 2008).
UDP-GlcNAc is involved in intracellular signaling as a substrate for O-GlcNAc transferase (OGT). OGT catalyzes the addition of O-GlcNAc to serine or threonine residues of many nucleocytoplasmic proteins. Dynamic O-GlcNAc modification regulates protein activation, degradation and localization. The main function of O-GlcNAcylation appears to be the modulation of cellular processes in response to nutrients and stress. Crosstalk between O-GlcNAcylation and phosphorylation is extensive, and many proteins are reciprocally modified under different conditions at the same site by either O-GlcNAc or phosphate. O-GlcNAcylation and phosphorylation also dynamically modify the enzymes controlling each other's cycling. Phosphatases are associated with the OGT, indicating that the same protein complexes can both remove phosphate and add an O-GlcNAc residue to some proteins (Hart 2011). O-GlcNAcylation is highly abundant on chromatin-associated proteins, e.g. histones (Hart 2011) and transcription factors such as SP1 and YY1 (Özcan 2010).

2.2.3 Regulation of UDP-sugar pools

The content of UDP-sugars is likely to be very tightly controlled. UDP-HexNAc concentration varies between tissues and changes during aging (Fulop 2008). UDP-sugar pools have been manipulated by regulating the key enzymes of their synthesis or by controlling the availability of the synthesis precursors. The content of UDP-GlcUA has been decreased by using siRNA to block UGDH activity and increased by overexpression of UGDH. UDP-GlcUA concentration can also be decreased by 4-MU, an excellent substrate of glucuronidation which consumes large amounts of UDP-GlcUA (Vigetti 2009, Kultti 2009, Vigetti 2006). UDP-GlcNAc content can be upregulated by overexpression of GFAT (Schleicher 2000), or treatment with glucosamine (Marshall 2005) or ammonium chloride (Ryll 1994).

2.3 HYALURONAN SYNTHASES

2.3.1 HAS enzymes

The mammalian *HAS* genes (*HAS 1-3*) are highly homologous. They are located in chromosomes 19, 8 and 16. HAS is a multispan transmembrane protein, including at least 6 transmembrane domains and 1-2 membrane-associated domains. Hyaluronan synthesis occurs on the inner surface of the plasma membrane and the growing chain is extruded through the membrane into the extracellular space (Prehm 1984). HAS enzymes have dual catalytic activities, i.e. for the transfer of GlcNAc and GlcUA units from the corresponding nucleotide sugars. *In vitro*, in membrane preparations containing HAS enzymes need the precursor sugars and Mg^{2+} or Mn^{2+} to synthesize hyaluronan (Weigel 2007). The monosaccharides are added to the reducing end of the hyaluronan chain (Weigel 2007). HAS enzymes seem to have 3-14 times higher affinity to UDP-GlcUA (K_m: 190 μM) than to UDP-GlcNAc (K_m: 400 μM) (Pummill 2002), whereas UDP-GlcNAc has 2-17 times higher cellular concentration than UDP-GlcUA.

2.3.2 Transcriptional regulation of *HAS*

The three *HAS* genes with divergent loci in the genome provide versatile transcriptional regulation. All *HAS* genes have distinct promoter regions and each isoenzyme can be differentially controlled by specific cellular signaling cascades. *HAS1* seems to have the highest respective promoter activity and *HAS2* the lowest, making *HAS2* a more potent target for regulation than the other *HAS* isoforms (Monslow 2003). The expression of *HAS* genes is typically regulated in parallel (Kultti 2009, Vigetti 2009b, Pasonen-Seppänen 2003, Karvinen 2003), but in some cases divergent regulation has also been reported. For example, progesterone reduces *Has1* and *Has2* expression in mouse uterine fibroblasts whereas *Has3* expression is induced (Uchiyama 2005). Also, TGF-β treatment upregulates *HAS1* expression in human synoviocytes but inhibits *HAS3* expression (Stuhlmeier 2004).

Several transcription factors have been reported to regulate *HAS* gene expression. The most studied is the promoter of *HAS2*, which has been shown to contain functional response elements for different transcription factors including retinoid acid receptor (RAR), nuclear factor κB (NFκB), cAMP response element binding protein 1 (CREB1), signal transducer and activator of transcription (STAT), SP1 and SP3, and several cofactors that take part in regulation (Saavalainen 2005, Saavalainen 2007, Makkonen 2009, Monslow 2006). In epidermal keratinocytes, epidermal growth factor (EGF) and retinoic acid induce *HAS2* transcription: EGF receptor activation by EGF elicites binding of phosphorylated STAT3 to the *HAS2* promoter, whereas retinoic

acid induces binding of RAR, retinoid X receptor (RXR), mediator protein (MED), CREB binding protein (CBP), SP1 and RNA polymerase II in to the *HAS2* promoter (Saavalainen 2005).

HAS genes also have alternative splicing which may influence enzyme activation or localization. A *HAS1* splice variant has been detected in bladder cancer (Golshani 2007), multiple myeloma (Adamia 2003) and Waldenström's macroglobulinemia (Adamia 2005). *HAS3* has 3 splice variants, variant 1 and 3 encode an identical protein and variant 2 has a different C-terminus (Gene-database, NCBI).

2.3.3 HAS and embryonic development

During mouse development, HAS2 is expressed throughout all embryonic stages while HAS1 expression disappears after embryonic day 8.5, and HAS3 is expressed later, particularly during the development of sensory organs. HAS2 is the only isoform highly expressed between embryonic days 8.5 and 9.5, a time period of heart valve development. This is why HAS2 knockout mice die from several cardiovascular defects at embryonic day 9.5-10, while HAS1 and HAS3 knockouts show no obvious phenotypes (Camenisch 2000, Tien 2005).

2.3.4 Comparison of HAS isoenzymes

Even though the HAS isoenzymes are highly homologous there are some known differences between their properties. The affinity of HAS1 to its substrates is lower than that of HAS2, and HAS3 has the highest affinity. It has been reported that HAS1 produces a significantly smaller pericellular hyaluronan coat than HAS2 and HAS3 (Itano 1999). Different HAS isoforms have also been reported to produce hyaluronan of different size, but there is some ambiguity in this property. Plasma membrane preparations of COS-1 cells transfected with HAS3 produced smaller hyaluronan ($1x10^5$-$1x10^6$ Da) than corresponding HAS1 and HAS2 ($2x10^5$-$2x10^6$ Da) (Itano 1999). On the other hand, Brinck et al. (1999) found that CHO cell membrane preparations with transfected HAS2 produced large hyaluronan ($3.9x10^6$ Da), while HAS3 and HAS1 synthesized relatively shorter molecules ($0.12\text{-}1.0x10^6$ Da and $0.12x10^6$ Da, respectively). In live CHO cells all isoforms produced hyaluronan larger than 3.9×10^6 Da (Itano 1999, Brinck 1999), and in live aortic smooth muscle cells HAS1 and HAS2 produced high molecular weight hyaluronan ($2\text{-}10x10^6$ Da), while HAS3 produced much lower molecular weight ($\sim 2x10^6$ Da) (Wilkinson 2006). These partly inconsistent results suggest that the size of hyaluronan is not dependent only on HAS isoform. Obviously some post-translational and cell type specific modifications of HAS protein or

its environment also regulate the chain length of newly synthesized hyaluronan.

2.3.5 Regulation of HAS enzymatic activity

The enzymatic activity of HAS has been suggested to require plasma membrane localization, and be regulated by post-translational modifications and interactions with other membrane components. Inhibition of hyaluronan synthesis by 4-MU is associated with the disappearance of HAS from the plasma membrane (Rilla 2005). Intracellular domains of HASs contain potential phosphorylation sites and phosphorylation induces HAS activity (Vigetti 2011, Bourguignon 2007, Anggiansah 2003, Goentzel 2006, Ohno 2001). It has also been shown that HAS2 is ubiquitinated on Lys190, and mutation of this residue leads to inactivation of the enzyme (Karousou 2010). The microenvironment is important to HAS activity. In bacterial cells HAS activity depends on cardiolipin (Weigel 2006). Mammalian cells do not contain cardiolipin in plasma membrane, but cholesterol might have a role in the regulation of HAS activity (Sakr 2008).

A recent report also shows that HAS2 can form homodimers, and heterodimers with HAS3 (Karousou 2010). This offers even more ways to regulate HAS activity and hyaluronan synthesis.

2.4 HYALURONIDASES

In most tissues hyaluronan turnover is very rapid. The half-life of hyaluronan in blood is just a few minutes, in the dermal connective tissue about 2-3 days and in the epidermis 12 hours, while in the vitreous body and cartilage the half-life can be several weeks (Tammi 1991, Morales 1988, Fraser 1997). Enzymes called hyaluronidases on cell surface and lysosomes cooperate with exoglycosidases in the degradation of hyaluronan chains. Human cells express hyaluronidase (*Hyal*) genes, which include two sets of three contiguous genes located on two chromosomes. The cluster on chromosome 3p21.3, - containing *Hyal 1-3* - are the most important in hyaluronan catabolism. The genes in chromosome 7q31.3 - *Hyal4, Hyalp1 and Spam1* - make no major contribution to hyaluronan degradation in somatic tissues (Jiang 2011, Varki 2008).

According to our current understanding, hyaluronan catabolism involves hyaluronan binding to a specific cell-surface receptor such as CD44, LYVE-1 and HARE, which then internalize hyaluronan for lysosomal catabolism. Uptake may be facilitated by fragmentation of high molecular weight hyaluronan by the membrane-associated, glycosylphosphatidylinisotol-anchored Hyal2 hyaluronidase. The fragments are taken into vesicles and

eventually enter lysosomes for complete degradation to monosaccharides, probably by Hyal1 and two exoglycosidases, β-glucuronidase and β-N-acetylglucosaminidase (Stern 2003). Reactive oxygen species or free radicals can also cleave hyaluronan, especially during inflammation. Free radicals have been reported to enhance hyaluronan turnover (Al-Assaf 2006, Ågren 1997).

2.5 HYALADHERINS

2.5.1 Properties and classification of hyaladherins

Proteins able to interact with hyaluronan are called hyaladherins or hyaluronan binding proteins. Hyaladherins containing one or two hyaluronan binding domains (so called, link modules) belong to the hyaluronan and proteoglycan link protein gene family (HAPLN) (Day 2002). HAPLN can be divided into two subgroups, cell-surface receptors CD44, LYVE-1, HARE, STABILIN-1, and secreted HAPLNs such as aggrecan, versican, brevican, neurocan, and TSG-6. Hyaladherins without link modules, e.g. RHAMM, interact with hyaluronan via a specific, spatially ordered set of clustered basic amino acids (Day 2002).

2.5.2 Cell-surface receptors

The human CD44 gene, located in chromosome 11, produces in most cell types a transcript that creates the standard isoform, comprising exons 1-5 and 16-20. Epithelial and endothelial cells, activated lymphocytes, and some malignant cells also express splice variants containing variable sets of additional exons between exons 5 and 16. The outermost N-terminal extracellular domain in exons 1-5 includes one hyaluronan binding link module. The variant exons bring in different extensions between the hyaluronan binding region and the standard C-terminus with plasma membrane and cytoplasmic domains (exons 16-20) (Isacke 2002). Additional variation occurs in the length of the cytoplasmic domain (Knudson 2002), while the transmembrane domain is highly conserved. CD44 is heavily modified post-translationally by N- and O-glycosylation, xylose-linked GAGs, sulphation, phosphorylation and acylation. A part of CD44 associates with cholesterol-rich microdomains called lipid rafts, and cholesterol depletion enhances CD44 shedding and inhibits its activation (Murai 2010). CD44 anchors hyaluronan on the plasma membrane (Tammi 1998), which can lead to hyaluronan internalization (Knudson 2002). Phosphorylation of CD44 following hyaluronan binding activates intracellular signaling molecules and regulates CD44 interaction with the actin cytoskeleton through ERM (Ezrin,

Radixin and Moesin) (Legg 2002). CD44 forms complexes with matrix metalloproteinases (MMP) and with the ErbB group of growth factor receptors (Kim 2008). It can modulate several receptor tyrosine kinase activities and anchor an active form of MMP to cell surfaces (Yu 1999). Hyaluronan binding can induce CD44 clustering and thus activate CD44-associated signals (Liu 1998). Through all these mechanisms CD44-hyaluronan interaction can induce leukocyte rolling on endothelium (DeGrendele 1996), cell migration (Bourguignon 2001, Turley 2002), invasion (Bourguignon 2010, Zhang 2002), proliferation (Bourguignon 2001, Meran 2011), differentiation (Bourguignon 2006) and chemotaxis (McKee 1996).
The LYVE-1 hyaluronan receptor was found to be highly homologous with CD44, and the hyaluronan binding link module of LYVE-1 especially is almost identical with that of CD44. While CD44 is ubiquitously expressed in hematopoietic cells, fibroblasts and epithelial cells, LYVE-1 expression is quite unusual, mostly limited to lymphatic endothelium. LYVE-1 binds hyaluronan, but its role in hyaluronan endocytosis is still unclear (Jackson 2004).
The hyaluronan receptor HARE, also known as Stabilin-2, in expressed in liver, spleen and lymph nodes. It has an important role in systemic hyaluronan clearance. Two active isoforms have been found in tissues (Zhou 2003). Cells stably transfected with full-length HARE create the other isoform when a minor subset of the receptor is proteolytically cleaved (Harris 2007).

2.5.3 Aggregating hyaladherins

Aggrecan, versican, neurocan and brevican are members of the hyalectin gene family. They have specific tissue distribution patterns, aggrecan being most prominent in cartilage, neurocan and brevican in the central nervous tissue and versican in soft connective tissues. Chondroitin sulphates of variable size, number and structure are attached to these core proteins, the N-terminals of which bind to a hyaluronan chain via two link modules. The binding to hyaluronan is stabilized by a link protein. Large aggregates are formed in ECM when multiple hyalectins are bound to a hyaluronan chain (Buckwalter 1982, Sommarin 1983, Kiani 2002).

2.5.4 TSG-6 and IαI

TSG-6 is encoded by the TNFAIP6-gene in chromosome 2. Its size is 35 kDa and it contains one hyaluronan binding link module. TSG-6 is not constitutively present in healthy adult tissues, but its expression is induced during inflammation and ovulation. IαI is primarily a serum macromolecule, synthesized by hepatocytes in the liver. It consists of three polypeptides: a 16

kDa bikunin and two HCs ~83 kDa each. TSG-6 secreted into ECM can catalyze the transfer of HC from IαI into a covalent complex with hyaluronan. HC binding changes the mechanical and cell-binding properties of hyaluronan. TSG-6 - IαI complex can also modulate the protease network and thus inhibit inflammation (Milner 2003). Furthermore, it was shown recently that binding to hyaluronan changes the conformation of TSG-6, leading to its dimerization, and that these dimers can form a non-covalent crosslink between hyaluronan chains (Baranova 2011).

2.5.5 RHAMM

The human RHAMM gene located in chromosome 5 produces alternatively spliced proteins. RHAMM localizes to cell surfaces, cytosol and the nucleus in most tissue types. It binds to hyaluronan via special types of binding motifs, which differ from HAPLNs (Yang 1994). RHAMM interaction with hyaluronan initiates several signaling cascades (Turley 2002). RHAMM appears to be involved in cellular motility and migration due its interactions with the cytoskeleton (Hall 1995). RHAMM-type hyaluronan binding motifs have also been found in a special matrix protein called Spacrcan, which binds hyaluronan in the interphotoreceptor matrix of the eye (Chen 2004).

2.5.6 Non-classified hyaladherins

Layilin with no link module or sequence homology to previously known hyaluronan receptors has been reported to bind hyaluronan (Bono 2001). Layilin has a role in cell migration and is found in microvilli, filopodia, lamellipodia and membrane ruffles (Bono 2001). It has also been reported that short hyaluronan fragments can interact with TLR 4, and induce secretion of inflammatory cytokines (Campo 2010).

2.6 BIOLOGICAL FUNCTIONS OF HYALURONAN

For a long time hyaluronan was thought to be just a space filler in tissues but several more specific roles have been discovered. The hydrophilic hyaluronan together with hyaladherins such as aggrecan, versican, TSG-6 and IαI can form extracellular ”goo” (Toole 2000), which, for example in cartilage, makes a critical contribution to the maintenance of ECM viscoelasticy and osmotic pressure (Varki 2008). Hyaluronan is also an ideal lubricant in the joint. It can act as a scavenger of free radicals. Hyaluronan coat around the cell clears way for cell migration and proliferation. A special shape of hyaluronan coat (’cables’) can immobilize leukocytes in the tissue. Hyaluronan interactions with cell surface receptors such as CD44 and

RHAMM are known to be important activators for many cellular functions. Thus hyaluronan has multiple roles in early development, tissue organization, cell proliferation and differentiation.

It can be said that hyaluronan is the initiator of new life. Hyaluronan-based matrix induces extrusion of the oocyte at ovulation and regulates the penetration of spermatozoa into an oocyte (Varki 2008). During embryonic development hyaluronan is abundant in tissues and especially prominent in sites of cell migration, such as the pathways for cell migration from neural crest, and in the developing cardiovascular system. In *HAS2* knockout mice the absence of hyaluronan inhibits migration from heart tube endothelium to underlying mesenchyme, resulting in defective heart valves and embryonic lethality (Camenisch 2000).

Skin epidermis is a good example of a tissue where hyaluronan may have a marked effect on homeostasis. In the human epidermis hyaluronan is the main component of ECM, which is found between all vital cell layers, but not in the terminally differentiated keratinocytes of the stratum corneum (Tammi 1994). In basal and spinous layers of epidermis hyaluronan may assist keratinocyte proliferation and migration and therefore increase stratification. Hyaluronan in the stratum granulosum and stratum corneum may prevent the normally tight cell-cell contacts, and the organization of intercellular lipids, compromising the permeability barrier (Tammi 1998). Hyaluronan synthesis in the upper layers of the epidermis, induced by retinoic acid, EGF or KGF, weakens the adhesion between keratinocytes and leads to incomplete differentiation (Tammi 1988, Pasonen-Seppänen 2003, Karvinen 2003, Pasonen-Seppänen 2008). On the other hand, removal of hyaluronan from epidermis enhances the expression of differentiation-related proteins (Maytin 2004, Passi 2004).

2.7 HYALURONAN METABOLISM DURING INFLAMMATION

Hyaluronan has multiple roles in inflammation. In acute inflammation several cytokines stimulate hyaluronan synthesis and induce hyaluronan coat transformation into cables (Table 1). Elevated hyaluronan levels and cables attract and bind platelets and leukocytes into the area of inflammation (De la Motte 2003, De la Motte 2009, Vigetti 2010). Hyal2 and reactive oxygen species mediate hyaluronan fragmentation into short oligosaccharides (figure 3) (De la Motte 2009, Al-Assaf 2006, Soltes 2006), which then interact with TLR2, TLR4 and CD44, and induce cytokine expression (Table 2) (figure 3). In the later phase of inflammation hyaluronan assists in tissue remodeling by inducing cell migration and proliferation. Short hyaluronan fragments enhance angiogenesis by stimulating MMP activation and endothelial cell

migration (Isacke 2002, Deed 1997, Yu 2000) (figure 3). Elevated hyaluronan synthesis is often seen in chronic inflammation, and a high serum level of hyaluronan in patients with septic conditions is a sign of poor prognosis (Laurent 1996). In the chronic inflammation of rheumatoid arthritis, accumulation of free hyaluronan around synovial joints causes swelling, immobility and stiffness (Naor 2003).

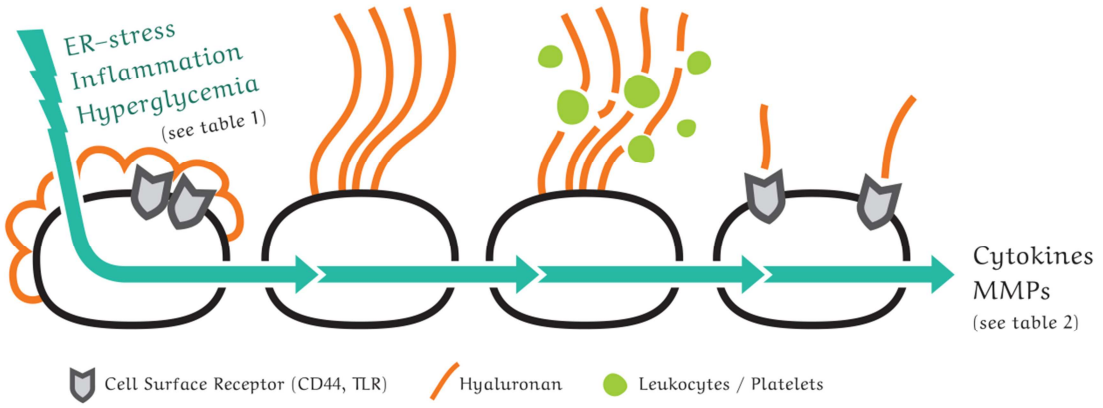

Figure 3. *Hyaluronan metabolism in inflammation.* Inflammation, viral infection, ER-stress and hyperglycemic conditions stimulate recasting of cell surface hyaluronan into cable-like structures. Hyaluronan cables attract and bind leukocytes and platelets. Platelet-derived Hyal2 or reactive oxygen species from leukocytes digest hyaluronan into shorter fragments. Hyaluronan fragments stimulate cell surface receptors such as CD44 and TLR4 and induce inflammatory reactions in host cells (De la Motte 2009)

2.8 HYALURONAN INTERACTION WITH LEUKOCYTES

Hyaluronan interacts in multiple ways with different leukocytes. Intraepithelial γδT-lymphocytes respond quickly to self-antigens released from damaged cells, and after activation they increase their own hyaluronan synthesis and stimulate that of the neighboring epithelial cells. Elevated hyaluronan levels attract macrophages into the injury site (Jameson 2005). Activated circulating T-lymphocytes gain the capacity to bind hyaluronan by expressing an active splice variant of CD44 with V6 and V9 exons (Bollyky 2007). Hyaluronan interaction with CD44 enhances the transepithelial migration of leukocytes (Khan 2004), activates T- and B-lymphocytes (Bollyky 2007, DeGrendele 1996, DeGrendele 1997, Lefebvre 2010, Maeshima 2011, Rafi 1997), prolong neutrophil survival (Esnault 2003), and induces eosinophil

cytokine secretion (Ohkawara 2000). The interaction of hyaluronan with leukocytes is size dependent. HMW hyaluronan suppresses whereas LMW activates T-cell actions (Bollyky 2007). Only LMW hyaluronan causes prolonged neutrophil survival, and no effects are seen with HMW hyaluronan (Ohkawara 2000).

Table 1: Inflammatory agents which regulate hyaluronan synthesis. (↑) indicates upregulation and (↓) downregulation of hyaluronan synthesis. The form of cell surface hyaluronan is indicated as follows: HA-cables = cable-type hyaluronan coat, (-) no hyaluronan cables, n.s. = not studied.

Agent	*Cell/ Tissue*	*HA*	*Form of hyaluronan*	*Reference*
EGF	*keratinocyte, primary skin fibroblast, oral mucosa fibroblast*	↑	*-*	*(Pasonen-Seppänen 2003, Yamada 2004)*
IFN-γ	*keratinocyte, lung fibroblast*	↑	*-*	*(Sampson 1992, Sayo 2002)*
IGF	*fibroblast, mesothelial cell*	↑	*n.s.*	*(Honda 1991, Kuroda 2001)*
IL-1β	*skin fibroblast, oral mucosa fibroblast, synoviocyte from rheumatoid arthritis, fetal skin fibroblast, vascular endothelial cell*	↑	*HA-cables*	*(Yamada 2004, Vigetti 2010, Oguchi 2004, Kennedy 2000)*
IL-4	*synovial membrane*	↑	*n.s.*	*(Hyc 2009)*
IL-6	*skin fibroblast*	↑	*n.s.*	*(Duncan 1991)*
IL-15	*vascular endothelial cell*	↑	*n.s.*	*(Estess 1999)*
KGF	*keratinocyte*	↑	*n.s.*	*(Karvinen 2003)*
PDGF	*fibroblast, mesothelial cell, vascular endothelial cell, vascular SMC*	↑	*n.s.*	*(Heldin 1992, Heldin 1989, Evanko 2001, Jacobson 2000, Suzuki 2003)*
PolyI: C	*primary mucosa SMC, lung fibroblast*	↑ -	*HA-cables*	*(Evanko 2009, de la Motte 2003)*
TGF-β	*keratinocyte, synoviocyte*	↓	*n.s.*	*(Pasonen-Seppänen 2003, Sayo 2002, Kawakami 1998)*
TGF-β	*synoviocyte from rheumatoid arthritis, fibroblast, keratinocyte, vascular endothelial cell*	↑	*n.s.*	*(Oguchi 2004, Heldin 1989, Suzuki 2003, Sugiyama 1998)*
TNF-α	*fetal skin fibroblast, lung fibroblast, vascular endothelial cell, synovial membrane*	↑	*HA-cables*	*(Sampson 1992, Vigetti 2010, Kennedy 2000, Hyc 2009)*
TNF-β	*vascular endothelial cell*	↑	*HA-cables*	*(Vigetti 2010)*

Table 2: Inflammatory agents upregulated by hyaluronan. HMW > 1000 kDa, LMW < 1000 kDa, Mix= hyaluronan size not defined.

Inflammatory agent	*Cell/ Tissue*	*Form of HA*	*Receptor*	*Reference*
Ccl2/ MCP-1	*renal tubular epithelial cell, peripheral blood mononuclear cell, macrophage*	*LMW*	*TLR4, CD44*	*(Yamawaki 2009, McKee 1996, Beck-Schimmer 1998)*
Ccl3/ MIP-1α	*monocyte*	*Mix*		*(Wallet 2010)*
Ccl3/ MIP-1α	*macrophage*	*LMW*	*CD44*	*(McKee 1996)*
Ccl3/ MIP-1α	*macrophage*	*LMW*	*TLR2, TLR4*	*(Scheibner 2006)*
Ccl4/ MIP-1β	*macrophage, cumulus cell*	*LMW*	*TLR2, TLR4*	*(Jiang 2005, Shimada 2008)*
Ccl4/ MIP-1β	*macrophage*	*LMW*	*CD44*	*(McKee 1996)*
Ccl4/ MIP-1β	*monocyte*	*Mix*		*(Wallet 2010)*
Ccl5 / Rantes	*macrophage, cumulus cell*	*LMW*	*CD44, TLR4, TLR2*	*(McKee 1996, Shimada 2008)*
CSF1/ M-CSF	*dermal epithelial cell*	*LMW*		*(Taylor 2004)*
CSF2/ GM-CSF	*monocyte, eosinophil*	*Mix, LMW*		*(Wallet 2010, Esnault 2003)*
Cxcl1/KC	*endothelial cell, macrophage*	*LMW*	*CD44*	*(McKee 1996, Takahashi 2005)*
Cxcl1/KC	*bronchoalveolar lavage fluid*	*LMW*	*Non-TLR4*	*(Zhao 2010)*
Cxcl2/ MIP-2α	*macrophage*	*LMW*		*(Bai 2005)*
Cxcl9/MIG	*macrophage*	*LMW*		*(Horton 1998)*
Cxcl10/ crg-2/IP-10	*macrophage, monocyte*	*LMW*	*CD44*	*(McKee 1996, Wallet 2010, Horton 1998)*
I-CAM	*epithelial cell, eosinophil*	*LMW*		*(Taylor 2004, Ohkawara 2000)*
IFN-γ	*T-lymphocyte*	*LMW*		*(Blass 2001)*
IGF-1	*macrophage*	*LMW*	*CD44*	*(Noble 1993)*
IL-1α	*monocyte*	*Mix*		*(Wallet 2010)*
IL-1β	*monocyte*	*Mix*		*(Wallet 2010)*
IL-1β	*bronchoalveolar lavage fluid*	*LMW*	*Non-TLR4*	*(Zhao 2010)*
IL-1β	*dendritic cell, chondrocyte*	*LMW*	*TLR4, CD44*	*(Campo 2010b, Termeer 2000)*
IL-6	*bronchoalveolar lavage fluid*	*LMW*	*Non-TLR4*	*(Zhao 2010)*

IL-6	*chondrocyte, peripheral blood mononuclear cell, monocyte, B-lymphocyte, cumulus cell*	*LMW, MIX*	*TLR4, TLR2, CD44*	*(Yamawaki 2009, Wallet 2010, Shimada 2008, Campo 2010b, Iwata 2009)*
IL-8	*melanoma cell, dermal endothelial cell, macrophage, monocyte*	*LMW*	*TLR4, CD44, MD-2*	*(McKee 1996, Taylor 2004, Taylor 2007, Voelcker 2008)*
IL-10	*fibroblast-like synoviocyte, monocyte, serum, T-lymphocyte, B-lymphocyte*	*HMW, MIX*		*(Wallet 2010, Iwata 2009, Huang 2011, Asari 2010, Bollyky 2007)*
IL-12	*macrophage, dendritic cell*	*LMW*	*CD44*	*(Termeer 2000, Hodge-Dufour 1997)*
IL-12p40	*monocyte*	*Mix*		*(Wallet 2010)*
MIP-2	*bronchoalveolar lavage fluid*	*LMW*	*Non-TLR4*	*(Zhao 2010)*
MIP-2	*macrophage, dermal endothelial cell, monocyte*	*LMW*	*TLR4, CD44, MD-2*	*(Taylor 2004, Taylor 2007, Zheng 2009)*
MME	*macrophage*	*LMW*		*(Horton 1999)*
MMP-2	*melanoma cell*	*LMW*	*TLR4*	*(Voelcker 2008)*
MMP-3	*macrophage*	*Mix*		*(Taylor 2007)*
MMP-9	*dendritic cell*	*LMW*	*Non-CD44 non-RHAMM, non-TLR4*	*(Fieber 2004)*
MMP-10	*dermal microvascular endothelial cell*	*LMW*		*(Taylor 2004)*
MMP-12	*macrophage*	*LMW*		*(Horton 1999)*
MMP-13	*dendritic cell*	*LMW*	*Non-CD44 non-RHAMM, non-TLR4*	*(Fieber 2004)*
MyD88	*chondrocyte*	*LMW*	*TLR4*	*(Campo 2010a)*
PAI-1	*macrophage*	*LMW*		*(Horton 2000)*
Socs3	*macrophage*	*Mix*		*(Taylor 2007)*
TGF-β	*eosinophil, B-lymphocyte*	*LMW, MIX*	*CD44, TLR4*	*(Ohkawara 2000, Iwata 2009)*
TGF-β2	*monocyte*	*Mix*	*CD44, MD-2, TLR4*	*(Taylor 2007)*
TIMP-1	*fibroblast-like synoviocyte*	*HMW*		*(Huang 2011)*
TIMP-2	*fibroblast-like synoviocyte*	*HMW*		*(Huang 2011)*

TNF	*monocyte*	*Mix*		*(Wallet 2010)*
TNF-α	*bronchoalveolar lavage fluid*	*LMW*	*Non-TLR4*	*(Zhao 2010)*
TNF-α	*dendritic cell, chondrocyte, macrophage, B-lymphocyte*	*LMW, Mix*	*TLR4, CD44*	*(Campo 2010b, Iwata 2009, Zheng 2009, Termeer 2002)*
TRAF-6	*chondrocyte*	*LMW*	*TLR4*	*(Campo 2010a)*

Table 3: Inflammatory agents downregulated by hyaluronan. HMW > 1000 kDa, LMW < 1000 kDa, Mix= size not defined.

Inflammatory agent	*Cell/ Tissue*	*Form of HA*	*Receptor*	*Reference*
A2ar	*macrophage*	*LMW*	*CD44*	*(Collins 2011)*
Cox-2	*synovial fibroblast*	*HMW*	*CD44*	*(Mitsui 2008)*
IL-1β	*fibroblast-like synoviocyte*	*HMW*	*CD44*	*(Mitsui 2008, Huang 2011)*
IL-6	*corneal epithelial cell, synovial fibroblast*	*HMW*	*CD44*	*(Mitsui 2008, Pauloin 2009)*
IL-8	*corneal epithelial cell, fibroblast-like synoviocyte*	*HMW*	*CD44*	*(Pauloin 2009, Wang 2006)*
MCP-5	*serum*	*HMW*		*(Asari 2010)*
MIP-2	*serum*	*HMW*		*(Asari 2010)*
MMP-1	*chondrocyte*	*HMW*	*CD44*	*(Hashizume 2010)*
MMP-1	*synovial fibroblast*	*LMW, HMW*	*CD44*	*(Shimizu 2003)*
MMP-3	*chondrocyte, fibroblast-like synoviocyte*	*HMW*	*CD44*	*(Huang 2011, Hashizume 2010)*
MMP-13	*chondrocyte*	*HMW*	*CD44*	*(Hashizume 2010)*
PGE(2)	*macrophage*	*HMW*	*CD44*	*(Yasuda 2010)*
p-selectin	*serum*	*HMW*		*(Asari 2010)*
Rantes	*serum*	*HMW*		*(Asari 2010)*
SCF	*serum*	*HMW*		*(Asari 2010)*
Socs7	*macrophage*	*Mix*		*(Taylor 2007)*
TNF-α	*fibroblast-like synoviocyte*	*HMW*	*CD44*	*(Mitsui 2008, Huang 2011)*
uPA	*macrophage*	*LMW*		*(Horton 2000)*
VEGF	*serum*	*HMW*		*(Asari 2010)*

2.9 HYALURONAN CABLES

A special form of hyaluronan coat, called hyaluronan cables, appears in inflammation, ER-stress, hyperglycemic conditions, α1-adrenergic receptor stimulation, viral infection or mimic of viral infection by double-stranded RNA (polyinosinic:polycytidylic acid=Poly I:C) (De La Motte 1999, Majors 2003, Shi 2006, Wang 2004). Cells respond to these stress factors by reshaping their hyaluronan coat, and long hyaluronan cables appear in the extracellular space. The cables attract and bind leukocytes and platelets (De La Motte 1999, De la Motte 2009). *In vitro*, IL-1β– and TNFα-treatments induce hyaluronan cables (Vigetti 2010). TNFα induces cable formation in endothelial and epithelial cell cultures but not in smooth muscle cells (SMC). IFNα,β and γ are incapable of stimulating hyaluronan-dependent monocyte binding in SMC cultures (De La Motte 1999, Vigetti 2010). *In vivo*, similar hyaluronan structures are present in the intestine of patients with inflammatory bowel disease (Kessler 2008). *In vitro*, hyaluronan cables have also been found in fibroblasts and mesangial cells (De La Motte 1999, Wang 2004, Vigetti 2010, Evanko 2009, Selbi 2006a).

Hyaluronan cables span long distances, combine hyaluronan produced by several cells and anchor to the cells by HAS2 and/or CD44 (Vigetti 2010). The formation of cables does not require intact cytoskeleton or membrane protrusions (Evanko 2009). It has been suggested that some kind of hyaluronan chain cross-linking exists in the cables. However, there seems to be a lot of variation in the structure and composition of hyaluronan cables present in different cell types. In tissue sections from inflammatory bowel disease hyaluronan colocalizes with IαI and versican (De la Motte 2003). Also in cultured human primary colon mucosal SMC and immortalized human proximal tubular epithelial cells hyaluronan cables contain IαI and versican, which are hyaladherins that may cross-link hyaluronan chains (De la Motte 2003, Selbi 2006b). TSG-6 remains the only documented enzyme capable of the covalent transfer of IαI-derived HCs to hyaluronan, but hyaluronan cable formation can take place without TSG-6 (Selbi 2006b). In murine and human airway SMC cultures, no IαI and versican is detectable in hyaluronan cables (Lauer 2009). The formation of the hyaluronan cables does not require *de novo* protein synthesis and they can be built up in a serum-free medium (De la Motte 2003). Poly I:C stimulates hyaluronan cable formation in a STAT1- and PI3K/Akt-dependent, but TLR3-independent manner (Bandyopadhyay 2010). In summary, the molecular structure and origin of hyaluronan cables are still unclear.

Monocytes bind to hyaluronan cables via their CD44 receptors (De La Motte 1999). Platelets also bind to cables, and the membrane bound Hyal2 on the surface of platelets can mediate hyaluronan fragmentation (figure 3) (De

la Motte 2009). Hyaluronan cables obscure cell surface adhesion molecules, such as intracellular adhesion molecule (ICAM), and therefore inhibit signaling through this receptor (Zhang 2005).

2.10 HYALURONAN FRAGMENTS

Hyaluronan plays different structural and signaling roles depending on its molecular size. Newly synthesized hyaluronan generally exists as a HMW polymer (>1000 kDa), but in many pathological conditions hyaluronan becomes more polydisperse and LMW fragments have been reported. HMW hyaluronan is thought to be anti-inflammatory whereas its fragments stimulate inflammation by inducing the expression of inflammatory cytokines (Table 2) and by regulating leukocyte functions (Jiang 2011). Hyaluronan fragmentation might be due to dysregulation of HAS and Hyal enzymes. Also reactive oxygen species released during tissue injury can cause fragmentation (Al-Assaf 2006, Soltes 2006). In lung injury, extracellular superoxide dismutase can inhibit inflammation by preventing oxidative fragmentation of hyaluronan (Gao 2008). Hyaluronan fragments can induce CD44 cleavage, resulting in signals different from those of HMW hyaluronan (Sugahara 2003) (Table 2 and 3).

2.11 TISSUE REMODELING

Because hyaluronan can induce cell migration and proliferation, it is important in tissue repair. Hyaluronan accumulates in tissues that undergo rapid remodeling due to injury, cancer or inflammation (Tammi 2009, Toole 2002). High hyaluronan content in the matrix opens the way for migrating cells, induces detachment of the cells, and amplifies growth factor signals (Jiang 2011). After skin wounding hyaluronan accumulates transiently in the granulation tissue and epidermis (Tammi 2005). In epidermis hyaluronan enhances proliferation and retards differentiation in a pool of migration-competent keratinocytes, which rapidly cover the wound area and re-establish the permeability barrier (Tammi 2009). Hyaluronan also upregulate dermal fibroblast proliferation (Röck 2010) and in granulation tissue hyaluronan stimulates the expression of many cytokines (Table 3), and short hyaluronan fragments induce angiogenesis by stimulating endothelial cell proliferation through the hyaluronan receptors CD44 and RHAMM (Jiang 2011). CD44 also provides a cell surface docking receptor for MMP9, the proteolytic activity of which induces angiogenesis and tissue remodeling (Yu 1999).

3 Aims of the study

The aim in this thesis was to study the mechanisms and signals that regulate hyaluronan synthesis, including the expression and activity of HAS enzymes, and the organization of the hyaluronan coat on cell surfaces. The main focus was in the hyaluronan precursor sugars - UDP-GlcUA and UDP-GlcNAc - because the role of UDP-GlcNAc in particular had received little attention previously.

Considering the surge of hyaluronan metabolism in wound healing, inflammation, and cancer, the work was also motivated by opportunities to find new therapeutic targets in the processes that control hyaluronan synthesis. In particular, two issues were considered important:

1. Analyzing cellular UDP-GlcNAc content as a regulator of hyaluronan synthesis.

2. Studying the formation of cell surface hyaluronan cables and their influence on monocyte binding.

4 Materials and Methods

4.1 MATERIALS

The cells used in this project are shown in Table 4. When studying UDP-sugars and hyaluronan synthesis, cells were incubated for 2 hours to 5 days with 1-20 mM mannose, fructose, and galactose, 0-25 mM glucose, 0.25-6 mM glucosamine, and 0.2 mM 4-MU (all from Sigma). Hyaluronan cable expression was induced by adding recombinant IL-1β and TNF-α (Biosource, Camarillo, CA, USA) at 10 ng/ml, and tunicamycin (Streptomyces, from Sigma) at 2.5 μg/ml.

Table 4: Cell lines used in the project. Culture conditions for each are presented in the original publications.

Origin of cells	*Name of cells*	*Original publication*	*Reference*
Newborn rat skin keratinocytes	*REK*	*I, IV and V*	(Baden 1983)
Human epidermal keratinocytes	*HaCaT*	*II*	(Boukamp 1988)
African green monkey kidney	*COS-1*	*III*	(Gluzman 1981)
Monocytes from a human histiocytic lymphoma	*U937*	*IV and V*	*Dr. Carol de la Motte, Lerner Res. Inst., Cleveland, OH, USA*
Human mesothelium	*LP-9,*	*III*	*Dept. Pathol., Kuopio Univ.*
Human cutaneus fibroblasts	*Skin fibroblast*	*III*	*Dr. Reidar Grenman, Turku Univ.*
Human fibroblast from cutaneus tumor stroma	*Tumor fibroblast*	*III*	*Dr. Reidar Grenman, Turku Univ.*
Human diploid lung fibroblast	*WI-38*	*III*	(Hayflick 1961)
Human chondrosarcoma	*HCS*	*III*	(Takigawa 1989)
Human breast adenocarcinoma	*MCF-7*	*III*	(Soule 1973)

4.2 METHODS

The methods used in this thesis are listed in Tables 5-8. Specific details of every method are presented in the original publications (I-V). Table 5 itemizes the methods used to study the content of hyaluronan. The UDP-sugar analysis methods are presented in Table 6. Table 7 shows the methods used to study the expression and function of genes and proteins. Table 8 shows the methods used to study cell and tissue functions.

Table 5: Methods used to study hyaluronan. Specific details are presented in the original publications.

Purpose	*Method*	*Original publication*	*Reference*
Content of hyaluronan	*Enzyme –linked sorbent assay for hyaluronan*	*I,II, III, IV and V*	(Hiltunen 2002)
Quantity of newly synthesized glycosaminoglycans	*Metabolic labeling with $[^{35}S]SO_4$ and $[^{3}H]$glucosamine*	*I*	(Tammi 2000)
Demonstration of hyaluronan in fixed cell cultures	*Staining with biotinylated Hyaluronan Binding Complex (bHABC) for light- and fluorescence microscopy*	*I, III, IV and V*	(Tammi 1998)
Demonstration of hyaluronan coat on live cells	*Incubation with fluorescently labeled HABC for confocal microscopy*	*III and V*	(Rilla 2008)
Quantitation of hyaluronan in granulation tissue	*Stainings tissue sections with bHABC and densitometry with Microtek® scanner*	*V*	*Method optimised by Jukka Kuokkanen and Mika Hyttinen, Univ. Kuopio*

Table 6: Methods used to quantify UDP-sugars. Specific details are presented in original publications I-III.

Purpose	*Method*	*Original publication*	*Reference*
Content of UDP-sugars	*ion pairing HPLC-MS/MS*	*I*	*Method optimised by Marjo Jauhiainen and Dr. Seppo Auriola, Univ. Kuopio*
Content of UDP-sugars	*anion-exchange HPLC*	*II, III*	*Method optimized from:* (Tomiya 2001)

Table 7: Methods used to study genes and proteins. Specific details are presented in the original publications.

Purpose	*Method*	*Original publication*	*Reference*
Gene transcription (HAS1,2,3; CD44; YY1; SP1; GFAT1,2; UGDH)	*Quantitative PCR*	*II, III, IV*	
YY1- and SP1-gene silencing	*Transient siRNA transfection*	*II*	
HAS-overexpression	*Transient HAS-pDendra2-C transfection*	*III*	*HAS- pDendra2-C-constructs made by Dr. Juha Hyttinen, Univ. Kuopio*
Protein biosynthesis	*Metabolic labeling with L-[^{35}S]methionine*	*I*	
YY1 and SP1 protein expression	*Western blot*	*II*	
YY1 and SP1 substitution with O-GlcNAc	*Immunoprecipitation and western blot*	*II*	
Transcription factor binding sites in HAS2 promoter	*In silico promoter analysis*	*II*	*(http://mordor.cgb.ki.se/cgi-bin/CONSITE/consite/)*
Transcription factor binding to HAS2 promoter (YY1, SP1, Ncor, CBP and PCAF)	*Chromatin immunoprecipitation*	*II*	

Table 8: Methods used to study cell and tissue functions. Specific details are presented in the original publications.

Purpose	*Method*	*Original publication*	*Reference*
Keratinocyte migration	*Scratch wound-assay in monocyte cultures*	*I*	(Pienimäki 2001)
Keratinocyte proliferation	*Cell counting with a hemocytometer*	*I*	(Rilla 2004)
Hyaluronan dependent monocyte binding to keratinocytes	*Counting of monocytes attached to keratinocyte cultures before and after hyaluronidase treatment*	*IV, V*	*Optimised from* (De la Motte 1999)
Formation of granulation tissue	*Rat wound healing model with subcutaneous sponge implant*	*V*	(Kössi 1999)
Number of leukocytes in granulation tissue	*Rat wound healing model with subcutaneous sponge implant*	*V*	(Kössi 1999)

5 Results

5.1 REGULATION OF HYALURONAN SYNTHESIS BY UDP-SUGAR SUPPLY

5.1.1 Regulation of intracellular UDP-sugar pools

The intracellular pool of UDP-HexNAc was increased by glucosamine, whereas mannose and GFAT1-siRNA treatments reduced it (I, II, III). To reduce the pool of UDP-GlcUA, 4-MU treatment was used (I). The contents of UDP-sugars show up to 50-fold differences between cell types (III Fig. 8). Cells of mesenchymal origin generally contained higher amounts of UDP-HexNAc and UDP-GlcUA, compared with epithelial cells (III Fig. 8). The magnitude of the UDP-GlcNAc increase following glucosamine treatment also varied between rat keratinocytes (REK), human keratinocytes (HaCat) and monkey kidney cells (COS-1) (I, II, III).

5.1.1.1 Glucosamine

A dose-dependent increase in UDP-HexNAc content was detected following the addition of glucosamine at 0.1-6.0 mM final concentrations in the growth medium, although close to maximum effect was reached already with 1 mM concentration (II Fig. 2B). In REK and COS-1 cultures 2 mM glucosamine caused a more than 11-fold increase in the UDP-HexNAc pool (I, Fig. 4A and III, Fig. 2A) and in the HaCaT culture 6 mM glucosamine increased it x 9-fold (II, Fig. 2B). Analysis of different time points after the introduction of glucosamine (6 mM) indicated that already at 2 hours the size of the UDP-HexNAc pool had increase 2.5-fold, and remained elevated at 24 hours. Glucosamine also caused a 6-fold increase in UDP-GlcUA, but this response emerged later, and was detectable only 6 hours after the introduction of glucosamine (I Fig. 4A, II Fig. 1). This study shows that both UDP-HexNAc and UDP-GlcUA pools can be elevated by glucosamine (I, II, III).

5.1.1.2 Mannose

Mannose decreased the UDP-HexNAc pool size in a dose-dependent way. A significant, 20% reduction in the intracellular UDP-HexNAc content was seen already with 5 mM mannose, while 20 mM mannose caused the maximal, ~50% downregulation in the pool size (I, Fig. 3A, II, Fig. 2A). The effect of

mannose was rapid and a reduction of UDP-HexNAc was seen already after 30 minutes (I, Fig. 3B), and the low levels were still detected after 24 hours (I, Fig. 4A), but in HaCaT cells the UDP-HexNAc content was normalized to control level after 24 h incubation with mannose (II, Fig. 1A). Mannose was found to increase the intracellular UDP-GlcUA content. In REK cells, 2 h incubation with mannose caused a 25% increase in UDP-GlcUA content (I, Fig. 3), whereas in HaCaT cells the upregulation was over 50% (II, Fig. 1B). When REKs were treated simultaneously with mannose and glucosamine, glucosamine dominated the response and the UDP-HexNAc pool was increased 8.5-fold (I, Fig. 4A). In summary, mannose depleted the level of UDP-HexNAc and elevated the level of UDP-GlcUA (I, II).

5.1.1.3 4-MU

4-MU decreased both UDP-HexNAc and UDP-GlcUA (I). The response was most potent after 2 h incubation, but was still evident after 12 h. With 0.2 mM 4-MU a 60% decrease in the UDP-GlcUA pool and a 20% decrease in the UDP-HexNAc pool were noted, while with a 1 mM dose the UDP-GlcUA content was reduced by 85% and UDP-HexNAc content by 40%. Combined with mannose (10 mM), 0.25 mM 4-MU caused about the same response in UDP-sugar contents as mannose or 4-MU alone, UDP-HexNAc levels being reduced by 35% and UDP-GlcUA content by 50% (I Fig. 5). This study demostrated that the depletions of the UDP-sugar pools by 4-MU and mannose are not additive (I).

5.1.1.4 Gfat1-siRNA

GFAT1 is one of the key enzymes of the hexosamine biosynthesis pathway, which ends in UDP-GlcNAc (figure 2). By siRNA-silencing of *GFAT1* for 48h, UDP-HexNAc levels were reduced by ~30% (II, Fig. 4A). The result confirmed that GFAT1 makes a significant contribution to the maintenance of the cellular UDP-HexNAc level (II).

5.1.2 Changes in the UDP-sugar pool sizes are reflected in hyaluronan synthesis

Cellular concentrations of UDP-HexNAc and UDP-GlcUA were closely correlated with hyaluronan synthesis (figure 4A) (III). Changes in UDP-sugar contents were rapidly reflected on hyaluronan synthesis (figure 4B) (I, II). In addition, comparison of different cell lines showed that their basal hyaluronan synthesis was highly correlated with the precursor sugar contents. Glucosamine, mannose, *GFAT1*-siRNA and 4-MU which all caused changes in UDP-HexNAc and UDP-GlcUA contents, also regulated hyaluronan synthesis (figure 4B and I, II, III, V).

The hyaluronan synthesis rate and the content of UDP-sugars were studied in seven cell lines (III). Cells were cultured close to confluence in standard culture conditions to reach a steady state, past the most active phase of proliferation. Up to 50-fold differences were found in the content of UDP-GlcNAc and UDP-GlcUA between the cell lines, and, interestingly, hyaluronan synthesis was found to correlate with the content of precursor sugars. The content of the two UDP-sugars also correlated with each other.

The stimulatory effect of glucosamine on hyaluronan synthesis was significant already with 0.25 mM glucosamine, and in HaCaT cells the maximum (1.5-fold) effect was obtained with 1 mM concentration (II, Fig. 2D). In REK cultures a somewhat higher, 2.5-fold increase in hyaluronan synthesis was seen with 2 mM glucosamine (I, Fig. 4B). In contrast to glucosamine, mannose dose-dependently inhibited hyaluronan synthesis. With 5 mM mannose a ~25% inhibition was noted, and with 20 mM concentration hyaluronan synthesis was decreased by ~55% (I, Fig. 1B, II, Fig. 2C). The inhibition induced by mannose was rapid; a 2h incubation with 20 mM mannose decreased hyaluronan levels by 50% (I Fig. 2A), and inhibition was still significant (~55%) after 24 h (I, Fig. 1B, II, Fig. 2C). When cells were treated simultaneously with mannose and glucosamine, hyaluronan synthesis increased 2.5-fold, as much as with glucosamine alone (I, Fig. 4B). Treating cells with 20 mM glucose, galactose and fructose did not markedly influence hyaluronan synthesis (I, Fig. 1A). The synthesis rates of sulfated GAGs, chondroitin and heparan sulphates, analyzed in REK cultures using metabolic labeling, were less sensitive to mannose than hyaluronan. A decrease in chondroitin sulphates was not seen until 10 h after adding 20 mM mannose (I, Fig. 2B).

4-MU (0.2 mM) significantly inhibited hyaluronan synthesis (-40%) after 6 h incubation in REK cells (I, Fig. 5). A combined treatment with mannose and 4-MU caused as strong inhibition as mannose or 4-MU alone, 4-MU being slightly a more potent inhibitor at the 6 h time point whereas mannose was slightly more efficient at 24 h, then resulting in 35% inhibition (I, Fig. 5E,F).

To summarise these data, glucosamine increases the intracellular pool of UDP-HexNAc and stimulates hyaluronan synthesis, whereas mannose reduces the UDP-HexNAc level and 4-MU UDP-GlcUA level, and both inhibit hyaluronan synthesis (I, II, III, V).

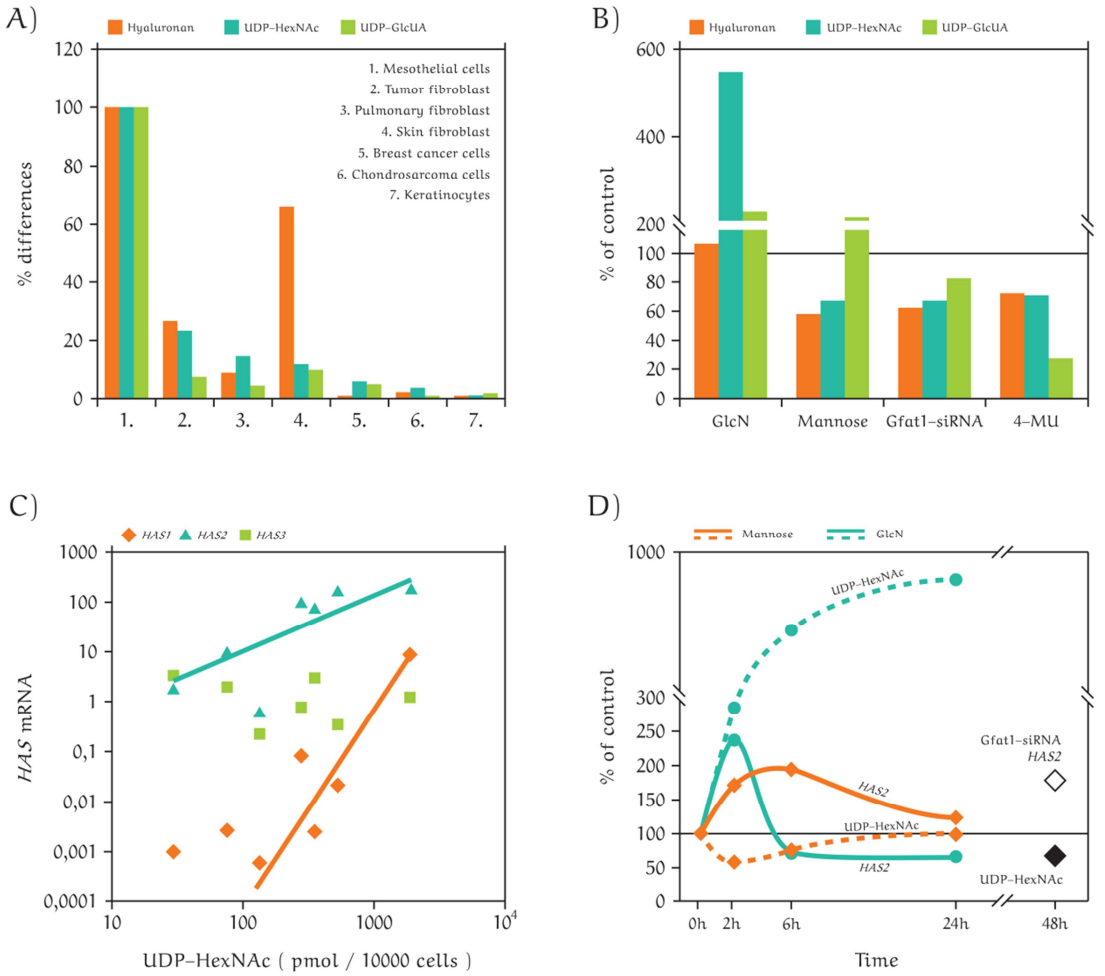

Figure 4. *Interrelationship between hyaluronan synthesis and cellular UDP-sugar contents.* (A) Hyaluronan synthesis, UDP-HexNAc and UDP-GlcUA contents in different cell lines are presented as % of those in mesothelial (LP-9) cells (100 %). (B) Hyaluronan synthesis and UDP-sugar levels after glucosamine, mannose, GFAT1-siRNA and 4-MU treatments. REK-cultures were incubated for 6 h with 0.25 mM 4-MU. HaCaT cells were incubated for 6 h with 6 mM glucosamine, 20 mM mannose, or transfected with GFAT1-siRNA (48 h after transfection). The results are presented as % of non-treated cultures. (C) *HAS1-3* expressions correlated with UDP-HexNAc. mRNA expressions were studied in the cell lines named in panel A and correlated with the content of intracellular UDP-HexNAc. (D) *HAS2* mRNA and UDP-HexNAc contents after glucosamine, mannose and GFAT1-siRNA treatments. mRNA and UDP-HexNAc levels were analysed in HaCaTs incubated for 0-24 h with mannose (20 mM) and glucosamine (6 mM), or for 48h with GFAT1-siRNA (white and black diamonds). All *HAS* mRNA levels were related to that of the ARPO gene.

5.2 UDP-HEXNAC CONTROL OF *HAS* EXPRESSION

The expression of *HAS* genes was also studied when cells were treated with mannose and glucosamine. Interestingly, *HAS2* expression was induced when the UDP-HexNAc pool was reduced with mannose or *GFAT1*-siRNA while the glucosamine-induced increase of the UDP-HexNAc pool was associated with decreased *HAS2* expression (II). All three *HAS* isoforms were regulated by glucosamine and mannose (figure 5A,B). Human keratinocytes (HaCaT) and rat keratinocytes (REK) responded in a slightly different time scale, in REKs glucosamine treatment caused *Has2* downregulation already after 2h incubation (figure 5B) whereas in HaCaTs glucosamine first upregulated *HAS2* expression and the downregulation was not seen until after 6h incubation (figure 4D and II, Fig. 3). *Has1* and *Has3* mRNA levels were also studied in REK cells. Glucosamine after 2 and 6h incubations did not remarkably change *Has1* expression but 24h incubation caused about 50% inhibition (figure 5B). Conversely, mannose upregulated *Has1* at all three time points (2h, 6h, and 24h), with the most potent effect occurring at 6h, when the increase was almost 2.5-fold (figure 5A). Two-hour and 6h incubations with glucosamine inhibited *Has3* expression more than 50%, but, unexpectedly, 24h incubation caused a 3-fold upregulation (figure 5B). A 24h incubation with mannose upregulated *Has3* expression 2-fold, whereas no effect was found in shorter time points (figure 5A).

5.2.1 Regulation of *HAS2* expression

Because *HAS2* is the dominant isoform in HaCaT cells, its regulation was studied in more detail (II). In HaCaTs, glucosamine regulated *HAS2* expression in a time-dependent manner. A 2h incubation with glucosamine caused a 2.4-fold induction of *HAS2* expression, while after 6 h and 24 h incubations its expression was significantly downregulated (-50%). Mannose slightly induced *HAS2* expression already after 2 h incubation; a 2-fold upregulation was seen after 6 h and inhibition continued at 24 h (II fig. 3). Like mannose, *GFAT1*-siRNA caused a significant upregulation (~1.5-fold) of *HAS2* expression (II fig. 4B). Glucosamine reduced *HAS2* expression in a dose-dependent manner, 2 mM glucosamine caused a ~30% decrease while 20 mM inhibited *HAS2* expression by 70% (figure 5C). With the 6 mM dose of glucosamine the amount of HAS2 enzyme probably became the limiting factor of hyaluronan synthesis, explaining why 2 mM glucosamine stimulated hyaluronan synthesis more effectively than 6 mM (II, Fig. 2D).

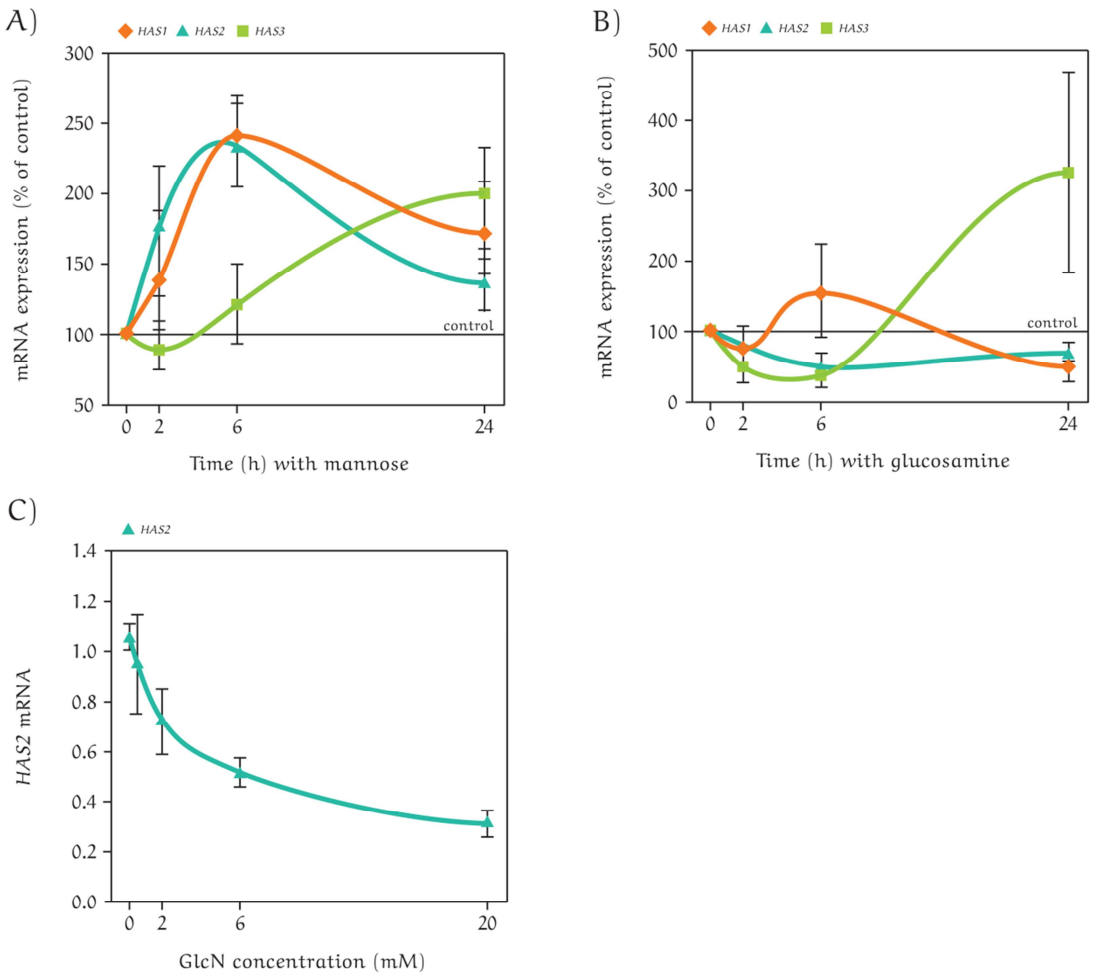

Figure 5. *Time-dependent regulation of Has expression by mannose and glucosamine.* REKs were incubated with 20 mM mannose (A) and 2 mM glucosamine (B), and mRNA levels of the *Has* isoforms were analyzed after 2, 6 and 24 h. (C) Concentration dependence of *Has2* expression in REKs incubated for 6 h with 0-20 mM glucosamine. All *Has* mRNA levels were related to that of ARPO.

5.2.2 Effects of YY1 and SP1 on *HAS2* expression

The above results reveal a negative correlation between UDP-HexNAc content and *HAS2* expression. To study the mechanism of this regulation in more detail, the binding of transcription factors to the *HAS2* promoter was studied (II). The binding of CREB1, NF-κB and STAT3, transcription factors that have been previously verified to be functional in *HAS2* promoter (Saavalainen 2005, Saavalainen 2007, Makkonen 2009, Monslow 2006), were

not influenced by mannose or glucosamine. In contrast, the transcription factors YY1 and SP1 showed altered binding to the *HAS2* promoter after mannose and glucosamine treatments. The binding of cofactors that can enhance and suppress transcription, such as CBP, NcoR1 and PCAF, were also studied. YY1 binding to the *HAS2* promoter increased significantly after glucosamine treatment, and mannose decreased SP1 binding in region 8 and YY1 in region 1, suggesting that they are important for *HAS2* expression (II, Fig. 6A, B). Coactivator-CBP binding to the *HAS2*-promoter was reduced, whereas corepressor-NcoR1 binding was increased, after glucosamine treatment. Binding of the coactivator PCAF to the promoter was increased after mannose treatment (II, Fig. 6A,B). A schematic view of the recruitment of YY1, SP1, CBP, PCAF and NcoR1 after glucosamine and mannose treatments is shown in figure 6. The importance of YY1 and SP1 was further studied by using specific siRNAs, YY1 and SP1 knockdown caused 2.4–fold and 1.5-fold upregulation of *HAS2* expression, respectively (II, Fig. 7). So, glucosamine increased their binding to the promoter, leading to transcriptional downregulation, and when their binding was inhibited by mannose, transcription was upregulated.

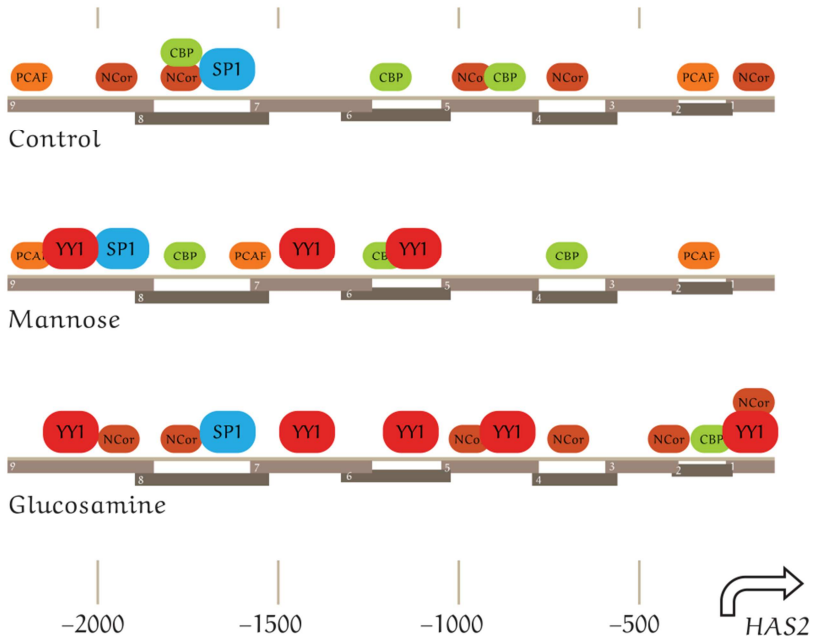

Figure 6. *Recruitment of SP1 and YY1 and transcription co-factors after mannose and glucosamine treatments.* HaCaTs were incubated for 6 h with 20 mM mannose and 6 mM glucosamine. Transcription factors bound to the proximal *HAS2* promoter were analyzed with ChIP.

5.2.3 O-GlcNAc modification in YY1 and SP1

YY1 and SP1 can both be modified by O-GlcNAc glycosylation (Özcan 2010). We studied whether glucosamine and mannose change the level of O-GlcNAc modification of these transcription factors (II, Fig. 8). Glucosamine significantly increased O-GlcNAc modification of YY1 1.6-fold and SP1 2.7-fold, whereas mannose slightly decreased both (II, Fig. 8). The results suggest that O-GlcNAc modification can regulate the binding of these transcription factors to the *HAS2* promoter, and in that way regulate *HAS2* expression, as a response to the changed intracellular content of UDP-HexNAc.

5.3 UDP-SUGAR REQUIREMENTS OF DIFFERENT HAS ISOFORMS

5.3.1 Substrate sensitivity of HAS1-3

To study the differences in the hyaluronan synthetic capacity between the three HAS isoenzymes (III), COS-1 cells were transiently transfected with Dendra2-*HAS1-3* constructs. COS-1 cells expressed low levels of endogenous HASs and synthesized very little hyaluronan (III, Fig. 1B). Interestingly, hyaluronan synthesis remained at a low level after HAS1 overexpression, whereas HAS2 and HAS3 overexpression significantly increased hyaluronan synthesis (III, Fig. 1B). Because HAS1 is known to have a lower *in vitro* affinity for precursor sugars than HAS2 and HAS3 (Itano 1999), the effect of increased UDP-sugar levels was studied by adding 2 mM glucosamine to the culture medium. The content of UDP-HeXNAc increased more than 10-fold and that of UDP-GlcUA ~3-fold (III, Fig. 2). The elevated UDP-sugar content caused by glucosamine was associated with a dose-dependent, up to 4-fold increase in the amount of hyaluronan synthesized in HAS1-transfected cells. In contrast, HAS2-transfected cells showed only a 1.6-fold increase, and no change in the hyaluronan synthesis rate was found in HAS3- transfected cells (III, Fig. 3). This suggests that the UDP-sugar concentrations needed by HAS1 are markedly higher than those needed by HAS2 or HAS3.

To confirm the differences in the hyaluronan synthesis rate between the HAS-isoenzymes, the cell surface coat formed by hyaluronan was analyzed in HAS-overexpressing cells. During the on-going synthesis the growing hyaluronan chain is still attached to the HAS enzyme, forming a hyaluronan coat, which can be visualized with fluorescently labeled HABC in live cells. This microscopic examination showed that HAS2- and HAS3-transfected cells had prominent hyaluronan coats (III, Fig. 4A). The coat present in HAS2–transfected cells appeared larger and more diffuse than that on HAS3-transfected cells (III, Fig. 4A). Glucosamine treatment did not change the size

of HAS2- and HAS3-induced coats. In contrast, cells transfected with HAS1 and maintained in the basal medium showed no cell surface hyaluronan, while supplementation of the medium with 1 mM glucosamine resulted in the formation of intensely stained, large hyaluronan coats also on HAS1-transfected cells (III, Fig. 4).

The consumption of UDP-sugars, studied in HAS-transfected cells, caused a clear depletion of both UDP-GlcNAc and UDP-GlcUA, even when the medium was supplemented with 1 mM glucosamine. Again, the largest depletion was found in cells transfected by HAS3, a smaller decrease was noted with HAS2, but no significant changes were observed with HAS1 (III, Fig. 7).

5.3.2 Effect of glucose on hyaluronan synthesis by HAS1-3

As both hyaluronan precursor sugars are downstream metabolites of glucose, the effect of glucose concentration on hyaluronan synthesis was studied in HAS-transfected cultures (III). COS-1 cells are normally cultured with a medium, containings 25 mM glucose. When cells were transferred to a glucose-free medium, hyaluronan synthesis was significantly inhibited, even in HAS3-transfected cultures (III, Fig. 5). Raising glucose from 0 to 5 mM (physiological level), caused an increase in hyaluronan synthesis. However, increasing glucose concentration from 5 to 25 mM showed a less marked elevation in hyaluronan production with all HAS isoforms (III, Fig. 5D,E,F). Hyaluronan coats formed in the total absence of glucose (0 mM) were markedly smaller than those formed in the control medium, even in HAS2- and HAS3 -transfected cells. Their coat sizes were not changed when the glucose concentration was raised from 5 mM to 25 mM (III, Fig. 6). HAS1-transfected cells did not produce hyaluronan coats at any glucose concentration tested (III, Fig. 6).

5.3.3 Correlation of UDP-sugar contents with hyaluronan synthesis and different *HAS* isoforms

The contents of UDP-GlcNAc and UDP-GlcUA, synthesis of hyaluronan, and expression of *HAS*-genes were studied in 7 different human cell lines (III). The UDP-sugars and the synthesis of hyaluronan were higher in the cells of mesodermal origin, i.e. in fibroblasts and mesothelial cells, while epithelial cells, such as keratinocytes and breast cancer cells contained generally lower UDP-precursor sugar concentrations and synthesized less hyaluronan (figure 4A, III, Fig. 8).

When the expression of *HAS* isoenzymes was studied in these 7 cell types, a strong, positive correlation was found between *HAS1* expression and the level of both UDP-precursor sugars (R=0.978 for UDP-GlcNAc and R=0.997

for UDP-GlcUA). *HAS2* expression also correlated with UDP-GlcNAc and UDP-GlcUA (R=0.892 and R=0.815), whereas no correlation between *HAS3* and UDP-sugars was found (figure 4C, III, Fig. 9). The mRNA levels of *GFAT1*, *GFAT2*, *UGDH* were measured on different cell lines to check whether a correlation exists between the expression of UDP-sugar synthesizing enzymes, UDP-sugar contents and hyaluronan synthesis. No correlations were found between the expression levels of *GFAT1* or *UGDH* and UDP-sugars, but there was a tendency to a positive correlation between the expression of *GFAT2* and UDP-GlcNAc (R=0.883) and UDP-GlcUA (R=0.883). The expression of *UGDH*, producing the UDP-GlcUA, was found to be high in cancer cell lines compared with non-malignant cells, but it did not correlate with the hyaluronan synthesis rate in these cell lines (III, Fig. 10F).

5.4 HYALURONAN IN INFLAMMATION

Hyaluronan has been shown to have an important role in skin inflammation (Tammi 2009, Tammi 2005). In this study a new hyaluronan-mediated inflammatory change was revealed in cultured keratinocytes. Microscopic analysis showed that epidermal keratinocytes could form hyaluronan cables (figure 7) that bind monocytes in a hyaluronan-dependent manner (IV, V). Mannose, the novel inhibitor of hyaluronan synthesis inhibited this hyaluronan-dependent monocyte binding (V). In an *in vivo* wound model mannose also reduced hyaluronan, the formation of granulation tissue, and the number of leukocytes accumulated in the granulation tissue (V) (Kössi 1999). Mannose also inhibited keratinocyte migration, and slightly slowed down cell proliferation (I).

5.4.1 Hyaluronan cables in keratinocytes

Previous studies have described a special form of hyaluronan coat called cables in many cell types (De La Motte 1999, Evanko 2009, Vigetti 2010, Wang 2004, Selbi 2006). This study (IV) showed that epidermal keratinocytes can also form these cable-like structures when subjected to inflammatory stimuli such as IL-1β and TNFα, or to tunicamycin causing ER-stress, or to hyperglycemic conditions (20 mM Glucose) (IV, Fig.1). The formation of hyaluronan cables did not require increased hyaluronan synthesis. Tunicamycin, IL-1β and TNFα actually decreased the content of hyaluronan in medium (IV, Fig. 3), tunicamycin and IL-1β also inhibited the expression of *Has* genes (IV, Fig.4). The microscopic structure of the cables was highly sensitive to the fixation. In live cells stained with fluorescently labeled HABC, the cables rose up above the cell layer as hair-like structures, whereas in fixed

cultures the "hair" collapsed into cables that fell down to form horizontal bundles of hyaluronan chains (figure 7, IV, Fig. 1, V, Fig. 3). The biological significance of these hyaluronan cables probably is in their capability to bind leukocytes into injured tissue. In this study we show that IL-1β and tunicamycin treated keratinocytes bind, in a hyaluronan dependent way, more monocytes than non-treated cultures (IV, Fig. 2, V, Fig. 4)

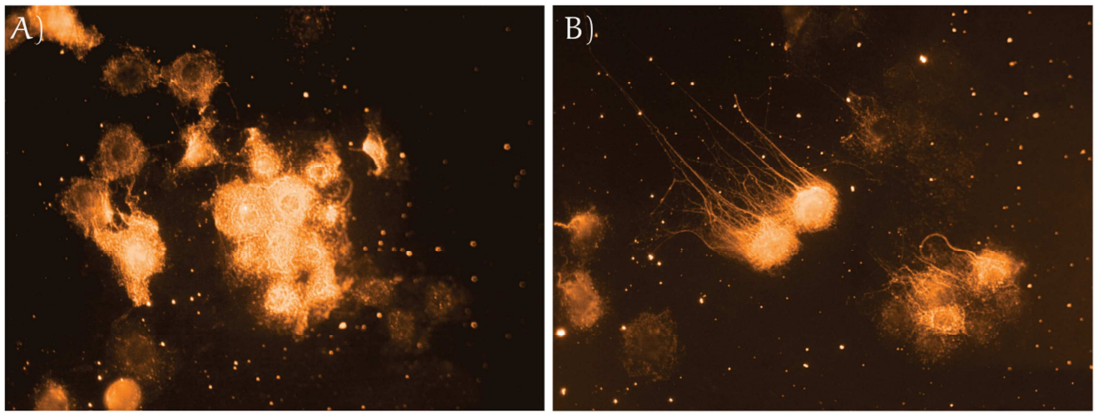

Figure 7. *Hyaluronan cables on keratinocytes*. (A) Untreated REKs. (B) REKs incubated for 3 h with tunicamycin (2.5 μg/ml). Hyaluronan on cells fixed with Histochoice MB was visualized with bHABC.

5.4.2 Inhibition of hyaluronan-dependent monocyte binding by mannose

In the first phase of the project (I) I discovered the ability of mannose to inhibit hyaluronan synthesis. The effect of mannose on the formation of hyaluronan cables and hyaluronan-dependent binding of monocytes was later studied in keratinocytes treated with tunicamycin and IL-1β to induce cable formation (V). The results show that mannose inhibited these events. IL-1β treatment doubled the number of monocytes bound to keratinocytes, and mannose significantly counteracted this effect (V, Fig. 4A). Mannose also inhibited tunicamycin-induced, hyaluronan-dependent binding of monocytes to keratinocytes (V, Fig. 4B).

5.4.3 Inhibition of hyaluronan synthesis in granulation tissue by mannose

It has been demonstrated that mannose injections inhibit the formation of granulation tissue in an *in vivo* wound-healing model (Kössi 1999). Importantly, the influx of leukocytes into the hollow sponge forming the matrix for subcutaneous granulation tissue was also significantly reduced by mannose, the numbers of neutrophils particularly being significantly

decreased (-70%) (V, Fig. 2C). The mannose isomers glucose, galactose and fructose had no such effects. Since Kössi et al. have shown that mannose reduces the amount of glycosaminoglycans in the granulation tissue, we wanted to see whether this applies to hyaluronan. For that purpose, we used paraffin sections from the experiments produced by Kössi et al., to study the amount of hyaluronan in the granulation tissue using histochemical staining with bHABC (V). Image analyses of the stainings showed that the intensity of the hyaluronan positive staining was reduced (-28%), and the depth of the hyaluronan positive area was decreased (-50%) in mannose treated sponges compared with control samples (V, Fig. 2A,B). To conclude, our data and those of Kössi et al. (1999) suggest that mannose decreased hyaluronan synthesis and leukocyte accumulation *in vivo*, leading to the granulation tissue growth block via reduced cell migration and proliferation. In line with this idea, we noticed in our experiments with keratinocyte cultures that mannose inhibited cell migration (-30%) (I, Fig. 6D), and also slightly reduced proliferation during a 5-day exposure (I, Fig. 6A,B). On the other hand, we noticed that while mannose and 4-MU inhibited hyaluronan synthesis to about the same extent (I, Fig. 5E,F), 4-MU (0.2 mM) was 4-fold more potent than mannose (10 mM) in the proliferation assay, suggesting that mannose may be less toxic to the cells.

6 Discussion

6.1 UDP-SUGAR CONTENT, HYALURONAN SYNTHESIS AND *HAS* EXPRESSION

6.1.1 Availability of precursors regulates hyaluronan synthesis

The most widely studied factors regulating hyaluronan synthesis have been the HAS enzymes, and a lot of information has been obtained about the ways *HAS* transcription is regulated, but the influence of the precursor sugars has received less attention. The present study shows that the availability of the precursor sugars UDP-GlcNAc and UDP-GlcUA regulate hyaluronan synthesis in several ways (figure 8). Whereas Kultti et al. (Kultti 2009) and Vigetti et al. (Vigetti 2009) have shown that 4-MU decreases UDP-GlcUA content, and inhibits hyaluronan synthesis, the present study shows that the contents of both precursor sugars are equally important to hyaluronan synthesis.

The metabolism of UDP-sugars is an integral part of the cellular mechanism for sensing the supply of energy, particularly that of glucose. The synthesis of UDP-sugars responds to many exogenous and endogenous stimuli (Fulop 2008, Sweeney 1993, Wice 1985, Ryll 1994), and the dynamic changes in the content of UDP-sugars by these stimuli can also regulate the synthesis of hyaluronan. This study shows that incubation with glucosamine increases the intracellular content of UDP-GlcNAc and UDP-GlcUA, and induces hyaluronan synthesis, while incubation with mannose decreases UDP-GlcNAc content and inhibits hyaluronan synthesis. The UDP-GlcUA pool size can be decreased by 4-MU and this limits hyaluronan synthesis (I, II, III).

Glucosamine is transported into the cells by glucose transporters (GLUT) simultaneously inhibiting glucose uptake (Hresko 1998). As different GLUTs have different affinities to glucosamine, the rate and kinetics of glucosamine uptake may depend on the combination and abundance of GLUTs expressed in a certain cell or tissue type (Uldry 2002). Glucosamine enters the hexosamine biosynthesis pathway in a step downstream of the rate-limiting GFAT enzymes and is converted into UDP-GlcNAc (Kim 1974, Marshall 2005), as shown also in our results (I, II, III). The three cell lines (REK, HaCat and COS) only slightly differed from each other in their response to glucosamine, i.e. 6h incubation with 1 mM glucosamine elevated UDP-HexNAc level 10-fold in HaCaT cells (II, Fig. 2B), and 9-fold in COS-1 cells

(III, Fig. 2A). At the same time, the UDP-GlcUA level was increased 2.25-fold in HaCaTs (data not shown) and 2.6-fold in COS-1 cells (III, Fig. 2B).

Mannose can also be transported into cells via GLUTs but there are also mannose-specific transporters (Panneerselvam 1996). Intracellular mannose probably modulates the activity of glucosamine-6-phosphate isomerase (GPI), which can either deplete or increase glucosamine-6P, the precursor of UDP-GlcNAc (Cayli 1999). The GPI1 and 2 and GFAT1 and 2 enzymes function in the same, rate-limiting step of hexosamine biosynthesis, and these enzymes can probably compensate for each other. This may explain why we see only a ~50% reduction in UDP-GlcNAc content after Gfat1 siRNA or mannose treatments (I, II).

4-MU has been shown to inhibit hyaluronan synthesis *in vivo* mouse model (Nagy 2010) and *in vitro* many cell types (Kultti 2009, Vigetti 2009, Kakizaki 2004). It inhibits *HAS* expression and reduces the content of UDP-GlcUA (Kultti 2009), as shown also in this study (I). 4-MU is also a potent inhibitor of cell proliferation, migration and invasion (Kultti 2009). This study shows that 4-MU markedly decreases the content of UDP-GlcUA also in keratinocytes, while its reducing effect on the UDP-HexNAc pool is smaller (I).

Other glycosaminoglycans using the same precursors as hyaluronan were less sensitive to changes in the UDP-sugar pools. For example, chondroitin sulphate synthesis stays more active after the UDP-GlcNAc depletion caused by mannose (I). Hyaluronan is probably more sensitive to changes in precursors because it is the only glycosaminoglycan synthesized on the plasma membrane and HAS uses cytosolic precursor sugars. The enzymes responsible for the synthesis of other GAGs are located in the Golgi apparatus. UDP-sugars are actively transported to the ER/Golgi, where concentrations are 20-fold higher than in cytosol (Hirschberg 1998).

6.1.2 UDP-GlcNAc content and *HAS2* expression

Interestingly, an intracellular signaling system regulated *H*AS expression when UDP-GlcNAc content was changed. Low UDP-GlcNAc content caused by mannose or *GFAT1*-siRNA induced *HAS* expression while the increased UDP-GlcNac pool induced by glucosamine was associated with downregulation of *HAS* expression (II). This signaling loop is probably meant to inhibit too radical shifts in the hyaluronan synthesis rate.

UDP-GlcNAc is also a substrate for OGT, the enzyme catalyzing the addition of O-GlcNAc to proteins. Dynamic O-GlcNAc modification controls localization, activation and degradation of signaling proteins (Love 2005). Consequently, O-GlcNAc-mediated signaling might be a direct link between UDP-GlcNAc content and the regulation of *HAS* expression. Our results showed that the transcription factors YY1 and SP1 bind to the *HAS2* promoter and that their binding is controlled by changes in the UDP-GlcNAc content

caused by mannose or glucosamine (II). YY1 and SP1 are known to regulate several genes and they can act as activators or repressors of transcription, depending on the gene and associated cofactors, such as co-repressor NcoR1 or co-activators CBP and PCAF. YY1 has a vital role in development (Gordon 2006, Shi 1997) and SP1 contributes to insulin signaling and the development of metabolic syndrome (Solomon 2008). Mannose reduced the binding of SP1 to the *HAS2* promoter, while glucosamine induced both SP1 and YY1 binding to the promoter (figure 6). SP1- and YY1-siRNA experiments showed that both of these transcription factors are repressors of *HAS2* expression. Interestingly mannose and glucosamine also modulated post-translational modifications of SP1 and YY1, mannose reducing whereas glucosamine increasing the O-GlcNAc modification in SP1 and YY1 (II). It has been shown that hypoglycosylated SP1 is subject to proteasome degradation whereas glucosamine-induced hyperglycosylation increases SP1 protein stability (Han 1997). O-GlcNAc modification also regulates the nuclear localization of SP1 since insulin-induced SP1 glycosylation causes SP1 accumulation in the nucleus (Majumdar 2006). O-GlcNAc modification enhances YY1 binding to DNA, because it disturbs the association of YY1 with retinoblastoma protein, which inhibits YY1 binding to DNA (Hiromura 2003). These results support the hypothesis that mannose, by reducing O-GlcNAc modifications in YY1 and SP1, causes reduced binding of SP1 to the *HAS* promoter, perhaps due to proteasomal degradation of SP1, and results in enhanced *HAS2* expression. In contrast, glucosamine increases O-GlcNAc modifications in YY1 and SP1, supporting their binding to the promoter and resulting in downregulation of *HAS2* expression (figure 8). This hypothesis may be simplified and the possibly independent roles of cofactors such as CBP, PCAF, and NcoR1 remain unknown. For instance, increased O-GlcNAc levels are associated with upregulated expression of corepressor genes such as NCoR1 and SMRT (Bowe 2006). Nevertheless, the transcriptional regulation of *HAS2* by the substrate of the same enzyme is interesting and probably important.

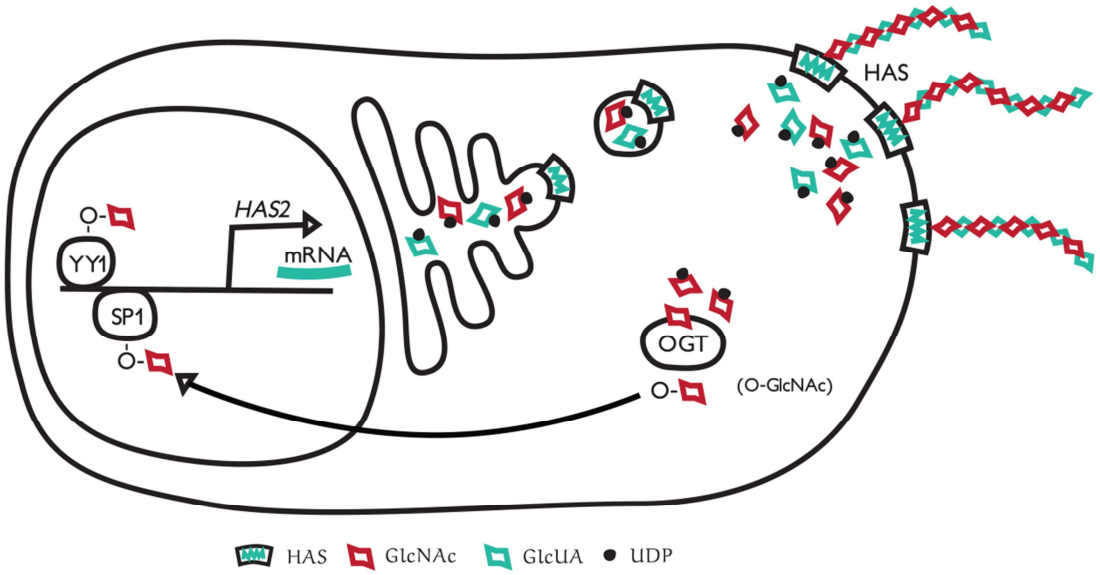

Figure 8. *Schematic model of the interrelationship between hyaluronan synthesis and UDP-precursor sugars.* The contents of the UDP-sugars, especially UDP-GlcNAc controls hyaluronan synthesis by acting as a substrate for HAS, and simultaneously regulates HAS gene expression, probably by O-GlcNAc modification of the transcription factors YY1 and SP1.

6.2 INTERRELATIONSHIPS BETWEEN UDP-SUGARS AND HAS ISOFORMS

HAS isoforms are known to have different K_m values for their precursor sugars which are probably reflected in their different affinities to these substrates. Itano et al. (Itano 1999) tested the different HAS isoenzymes with the precursor sugars using membrane fractions isolated from HAS-transfected cells and found that HAS1 has the highest and HAS3 the lowest K_m for the precursor sugars, especially UDP-GlcNAc. Our results with glucosamine-treated, HAS-transfected cells are in line with those findings. HAS1 needs high precursor concentrations to synthesize hyaluronan and to produce a notable hyaluronan coat, while HAS2 and HAS3 can effectively produce hyaluronan even when the UDP-sugar content is lower (III). These results suggest that active hyaluronan synthesis by HAS1 requires a high content of UDP-GlcNAc

Glucose is the precursor of all UDP-sugars (figure 2). It is therefore quite natural that glucose starvation inhibits hyaluronan synthesis by

downregulating UDP-GlcNAc and UDP-GlcUA contents. However, raising glucose from physiological (5 mM) to hyperglycemic (25 mM) levels caused relatively small changes in hyaluronan synthesis during a 6 h incubation in COS-1 cultures (III). Different cell types may vary in their tolerance to hyperglycemic conditions, and maintaining the cells for more extended periods in high glucose medium may be necessary to show the differences in the ability to maintain stable hyaluronan synthesis between 5 mM and 25 mM glucose concentrations. We have shown that vascular smooth muscle cells cultured in 25 mM glucose over several cell passages synthesize significantly more hyaluronan than cells cultured in 5 mM glucose (Sainio 2010). In rat keratinocyte cultures, a short incubation with 25 mM glucose caused the formation of hyaluronan cables (IV), but did not change the amount of secreted hyaluronan (data not shown).

Interestingly, the expression levels of *HAS1* and *HAS2* in different cell types correlated with precursor sugar contents. A particularly strong positive correlation occurred between the precursor sugars and *HAS1*, but the expression of *HAS2* and the total hyaluronan synthesis were also clearly related to precursor sugar contents, unlike *HAS3*, which showed no correlation (III). Apparently, in a steady state situation cells balance *HAS1* and *HAS2* expression in an economical level and express these only if there are enough precursor materials. *HAS3* sustains hyaluronan synthesis even in low UDP-sugar contents, so it may not be necessary to balance its expression with the precursor contents. Studies on human mesangial and proximal tubular cells have shown that hyperglycemic conditions induce *HAS2* expression but do not influence *HAS3* expression (Yevdokimova 2006, Jones 2001). Microarray and PCR analyses of different cell types have shown significant overexpression of *HAS2* in hyperglycemic conditions (Sainio 2010, Maier 2010, Rugheimer 2008). Evidently, the intrinsic UDP-sugar content correlates with *HAS2* expression, and probably also with *HAS1* expression. However, because of the relatively low expression level in many cell types, the analysis of *HAS1* expression is difficult and not often performed.

Expression levels of the enzymes producing hyaluronan precursor sugars were also studied in an attempt to explain the cell type-related differences in UDP-sugar levels, and correlated with hyaluronan synthesis. GFAT1 and GFAT2 are presumably the key enzymes in hexosamine biosynthesis producing UDP-GlcNAc, while UGDH is responsible for UDP-GlcUA synthesis (figure 2). A somewhat surprising finding was that there was no correlation between the mRNA levels of most of these enzymes and hyaluronan synthesis. A weak correlation was found between Gfat2 mRNA and hyaluronan (III). Gfat1 and 2 expression levels are known to be tissue specific, for instance Gfat1 is predominantly expressed in the liver and fat, whereas a high expression of Gfat2 is found in the brain (Oki 1999).

Furthermore, both Gfat1 and Gfat2 expression change during ageing (Fulop 2008). Gfat1 expression is upregulated in muscle after an isocaloric-sucrose diet (Ferrer-Martinez 2006). There are fewer studies on the regulation of Gfat2 expression, but during aging Gfat2 expression increases especially in the heart tissue and at the same time the content of UDP-HexNAc increases (Fulop 2008).

Overexpression of UGDH or its suppression by siRNA correlate with increased and reduced hyaluronan synthesis, respectively (Vigetti 2006). Increased UGDH expression has been linked to prostate cancer and also correlated there with increased hyaluronan synthesis (Wei 2009). However, when we studied several normal and cancer cell lines we found that UGDH expression did not correlate with hyaluronan synthesis. Instead, UGDH expression was high in several cancer cell lines, regardless of the activity of hyaluronan synthesis (III). I hypothesize that this may be due to the important role of UGDH in the production of glucuronides (Varki 2008). Cancer cells can remove hormones, growth inhibitors and drugs by glucuronidation to neutralize their growth restricting activities. Androgen-dependent prostate cancer cells have been shown to control their growth by using a UGDH–induced increase in the UDP-GlcUA supply for glucuronidation. At the same time, these cells synthesize very little hyaluronan, while the increased UGDH activity markedly contributes to the excretion of inactivated androgens (Wei 2009).

6.3 HYALURONAN CABLES IN KERATINOCYTES

Hyaluronan cable structures have been reported in many cell types (De la Motte 2003, De la Motte 2009a, Vigetti 2010), but not in skin cells (IV). Epidermal keratinocytes normally express a tight pericellular hyaluronan coat (Pienimäki 2001, Rilla 2004), but cytokines, ER-stress and hyperglycemic conditions induced the formation of these extended hyaluronan structures also in keratinocyte cultures (IV, Fig. 7). A special feature of the hyaluronan cables is their capability to bind leukocytes and platelets (De la Motte 2003, De la Motte 2009b). In this study, we show that non-stressed keratinocytes bind relatively low numbers of leukocytes, but binding can be doubled with IL-1β, TNFα or tunicamycin. Interestingly, the amount of secreted hyaluronan did not increase after these treatments (IV). In some cell types hyaluronan cable expression and upregulation of hyaluronan synthesis occur simultaneously (Selbi 2006b, Lauer 2009) but this was not the case in keratinocyte cultures. Overexpression of HAS3 (Selbi 2006b) stimulates, whereas overexpression of HAS2 inhibits cable formation in renal tubular epithelial cells (Selbi 2006a). The formation of cables in HAS-overexpressing

cells is obviously cell type specific and depends on factors other than total hyaluronan synthesis or specific HAS type, because in the studies of our group other cell lines overexpressing either HAS2 or HAS3 did not show cables (data not shown). Also, the present results showed no correlation between *Has* expression and hyaluronan cables because IL-1β and tunicamycin inhibited *Has* expression whereas TNFα induced it (IV).

6.4 MANNOSE AS AN ANTI-INFLAMMATORY AGENT

Mannose is a major component of many glycans. Its metabolic activation starts with the conversion to mannose 6-phosphate. Several *in vitro* and *in vivo* studies have reported the anti-inflammatory actions of mannose or mannose 6-phosphate. Mannose can reduce leukocyte attachment to venules (Stoolman 1983) and slow down their proliferation (Muchmore 1984). Mannose also inhibits the oxidative lysis of neutrophils (Rest 1988) and cytolysis mediated by natural killer cells (Werkmeister 1983). The effects of mannose have also been studied in an *in vivo* wound-healing model (Kössi 1999). Results from that study show that mannose causes a significant reduction in the number of leukocytes in the wound fluid and retards the formation of granulation tissue. Even orally administered mannose 6-phosphate improved wound healing and had an anti-inflammatory influence in the mouse (Davis 1994). There is no all-inclusive explanation for why mannose has these anti-inflammatory influences. It has been proposed that mannose 6-phophate could displace from cell-surface receptors lysosomal enzymes, which are essential for leukocyte extravasations into the inflammatory site. The present results suggest that the anti-inflammatory actions of mannose are partially mediated by reduced hyaluronan synthesis (I, II, V). The results show that mannose is an effective hyaluronan synthesis inhibitor (I, II) and it also causes reduced hyaluronan levels in the granulation tissue *in vivo* (V). We also show that mannose can prevent hyaluronan-dependent monocyte binding into cytokine-treated keratinocyte cultures (V) and that the treatment is not toxic to the cells (I). Previous studies on rats and mice have shown that mannose added to drinking water has no toxic side effects (Davis 2001, Alton 1998). In summary, mannose is a powerful hyaluronan synthesis inhibitor and it might have potential in drug development in cases where inordinate hyaluronan synthesis has to be modulated.

7 Summary and Conclusions

The main findings of this study are:

- Hyaluronan synthesis is regulated by the cellular content of its UDP-GlcNAc and UDP-GlcUA precursors
- Dynamic changes of cellular UDP-GlcNAc content feedback regulate *HAS* expression
- The normal contents of UDP-GlcNAc and UDP-GlcUA in different cell types positively correlate with *HAS1*, *HAS2* and *GFAT2* expression, but not with *HAS3*, *GFAT1* or *UGDH*
- High content of UDP-precursor sugars are required for active hyaluronan synthesis by HAS1, while HAS2 and HAS3 are less sensitive to substrate availability
- Mannose is a novel hyaluronan synthesis inhibitor and it has an anti-inflammatory effect
- Inflammatory stimulus, ER-stress and hyperglycemic conditions induce expression of hyaluronan cables in rat epidermal keratinocytes, promoting their capability to bind monocytes

This study discovered extensive connections between UDP-sugars, hyaluronan synthesis and HAS enzymes. The results show that the hyaluronan precursor sugars UDP-GlcNAc and UDP-GlcUA control hyaluronan synthesis by regulating *HAS* gene expression and acting as substrates to HAS enzymes. HAS1 especially needs high levels of substrates for active hyaluronan synthesis. The study also discovered a hyaluronan-mediated inflammatory reaction between leukocytes and keratinocytes.
In the future, studies of hyaluronan synthesis regulation should also focus on UDP-precursors. Hyaluronan synthesis seems to be very sensitive to changes in precursor contents, which provides new mechanisms to control unbalanced hyaluronan synthesis in pathological processes such as malignant tumors and inflammation. For example, mannose was found to deplete UDP-

GlcNAc, resulting in a reduced rate of hyaluronan synthesis and inhibiting granulation tissue growth and leukocyte accumulation in wounds *in vivo*. This suggests that hyaluronan synthesis is an important contributor in inflammation, and that mannose is a potential anti-inflammatory agent.

8 References

Adamia, S., Crainie, M., Kriangkum, J., Mant, M.J., Belch, A.R. & Pilarski, L.M. 2003, "Abnormal expression of hyaluronan synthases in patients with Waldenstrom's macroglobulimenia", *Seminars in oncology,* vol. 30, no. 2, pp. 165-168.

Adamia, S., Treon, S.P., Reiman, T., Tournilhac, O., McQuarrie, C., Mant, M.J., Belch, A.R. & Pilarski, L.M. 2005, "Potential impact of a single nucleotide polymorphism in the hyaluronan synthase 1 gene in Waldenstrom's macroglobulinemia", *Clinical lymphoma,* vol. 5, no. 4, pp. 253-256.

Al-Assaf, S., Navaratnam, S., Parsons, B.J. & Phillips, G.O. 2006, "Chain scission of hyaluronan by carbonate and dichloride radical anions: potential reactive oxidative species in inflammation?", *Free radical biology & medicine,* vol. 40, no. 11, pp. 2018-2027.

Alton, G., Hasilik, M., Niehues, R., Panneerselvam, K., Etchison, J.R., Fana, F. & Freeze, H.H. 1998, "Direct utilization of mannose for mammalian glycoprotein biosynthesis", *Glycobiology,* vol. 8, no. 3, pp. 285-295.

Anggiansah, C.L., Scott, D., Poli, A., Coleman, P.J., Badrick, E., Mason, R.M. & Levick, J.R. 2003, "Regulation of hyaluronan secretion into rabbit synovial joints in vivo by protein kinase C", *The Journal of physiology,* vol. 550, no. Pt 2, pp. 631-640.

Aronson, N.N.,Jr & Docherty, P.A. 1983, "Degradation of [6-3H]- and [1-14C]glucosamine-labeled asialo-alpha 1-acid glycoprotein by the perfused rat liver", *The Journal of biological chemistry,* vol. 258, no. 7, pp. 4266-4271.

Asari, A., Kanemitsu, T. & Kurihara, H. 2010, "Oral administration of high molecular weight hyaluronan (900 kDa) controls immune system via Toll-like receptor 4 in the intestinal epithelium", *The Journal of biological chemistry,* vol. 285, no. 32, pp. 24751-24758.

Baden, H.P. & Kubilus, J. 1983, "Effect of minoxidil on cultured keratinocytes", *The Journal of investigative dermatology,* vol. 81, no. 6, pp. 558-560.

Bai, K.J., Spicer, A.P., Mascarenhas, M.M., Yu, L., Ochoa, C.D., Garg, H.G. & Quinn, D.A. 2005, "The role of hyaluronan synthase 3 in ventilator-induced lung injury", *American journal of respiratory and critical care medicine,* vol. 172, no. 1, pp. 92-98.

Bandyopadhyay, S.K., de la Motte, C.A., Majors, A.K. & Strong, S.A. 2010, "Inhibition of the phosphatidylinositol-3-kinase pathway abrogates polyinosinic:polycytidylic acid-stimulated hyaluronan-mediated human mucosal smooth muscle cell binding of U937 monocytic cells", *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research,* vol. 30, no. 11, pp. 809-816.

Baranova, N.S., Nileback, E., Haller, F.M., Briggs, D.C., Svedhem, S., Day, A.J. & Richter, R.P. 2011, "The Inflammation-associated Protein TSG-6 Cross-links Hyaluronan via Hyaluronan-induced TSG-6 Oligomers", *The Journal of biological chemistry,* vol. 286, no. 29, pp. 25675-25686.

Beck-Schimmer, B., Oertli, B., Pasch, T. & Wuthrich, R.P. 1998, "Hyaluronan induces monocyte chemoattractant protein-1 expression in renal tubular epithelial cells", *Journal of the American Society of Nephrology : JASN,* vol. 9, no. 12, pp. 2283-2290.

Blass, S.L., Pure, E. & Hunter, C.A. 2001, "A role for CD44 in the production of IFN-gamma and immunopathology during infection with Toxoplasma gondii", *Journal of immunology (Baltimore, Md.: 1950),* vol. 166, no. 9, pp. 5726-5732.

Bollyky, P.L., Lord, J.D., Masewicz, S.A., Evanko, S.P., Buckner, J.H., Wight, T.N. & Nepom, G.T. 2007, "Cutting edge: high molecular weight hyaluronan promotes the suppressive effects of CD4+CD25+ regulatory T cells", *Journal of immunology (Baltimore, Md.: 1950),* vol. 179, no. 2, pp. 744-747.

Bono, P., Rubin, K., Higgins, J.M. & Hynes, R.O. 2001, "Layilin, a novel integral membrane protein, is a hyaluronan receptor", *Molecular biology of the cell*, vol. 12, no. 4, pp. 891-900.

Boukamp, P., Petrussevska, R.T., Breitkreutz, D., Hornung, J., Markham, A. & Fusenig, N.E. 1988, "Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line", *The Journal of cell biology*, vol. 106, no. 3, pp. 761-771.

Bourguignon, L.Y., Gilad, E. & Peyrollier, K. 2007, "Heregulin-mediated ErbB2-ERK signaling activates hyaluronan synthases leading to CD44-dependent ovarian tumor cell growth and migration", *The Journal of biological chemistry*, vol. 282, no. 27, pp. 19426-19441.

Bourguignon, L.Y., Ramez, M., Gilad, E., Singleton, P.A., Man, M.Q., Crumrine, D.A., Elias, P.M. & Feingold, K.R. 2006, "Hyaluronan-CD44 interaction stimulates keratinocyte differentiation, lamellar body formation/secretion, and permeability barrier homeostasis", *The Journal of investigative dermatology*, vol. 126, no. 6, pp. 1356-1365.

Bourguignon, L.Y., Wong, G., Earle, C., Krueger, K. & Spevak, C.C. 2010, "Hyaluronan-CD44 interaction promotes c-Src-mediated twist signaling, microRNA-10b expression, and RhoA/RhoC up-regulation, leading to Rho-kinase-associated cytoskeleton activation and breast tumor cell invasion", *The Journal of biological chemistry*, vol. 285, no. 47, pp. 36721-36735.

Bourguignon, L.Y., Zhu, H., Zhou, B., Diedrich, F., Singleton, P.A. & Hung, M.C. 2001, "Hyaluronan promotes CD44v3-Vav2 interaction with Grb2-p185(HER2) and induces Rac1 and Ras signaling during ovarian tumor cell migration and growth", *The Journal of biological chemistry*, vol. 276, no. 52, pp. 48679-48692.

Bowe, D.B., Sadlonova, A., Toleman, C.A., Novak, Z., Hu, Y., Huang, P., Mukherjee, S., Whitsett, T., Frost, A.R., Paterson, A.J. & Kudlow, J.E. 2006, "O-GlcNAc integrates the proteasome and transcriptome to regulate nuclear hormone receptors", *Molecular and cellular biology,* vol. 26, no. 22, pp. 8539-8550.

Brinck, J. & Heldin, P. 1999, "Expression of recombinant hyaluronan synthase (HAS) isoforms in CHO cells reduces cell migration and cell surface CD44", *Experimental cell research,* vol. 252, no. 2, pp. 342-351.

Buckwalter, J.A. & Rosenberg, L.C. 1982, "Electron microscopic studies of cartilage proteoglycans. Direct evidence for the variable length of the chondroitin sulfate-rich region of proteoglycan subunit core protein", *The Journal of biological chemistry,* vol. 257, no. 16, pp. 9830-9839.

Camenisch, T.D., Spicer, A.P., Brehm-Gibson, T., Biesterfeldt, J., Augustine, M.L., Calabro, A.,Jr, Kubalak, S., Klewer, S.E. & McDonald, J.A. 2000, "Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme", *The Journal of clinical investigation,* vol. 106, no. 3, pp. 349-360.

Campo, G.M., Avenoso, A., Campo, S., D'Ascola, A., Nastasi, G. & Calatroni, A. 2010, "Small hyaluronan oligosaccharides induce inflammation by engaging both toll-like-4 and CD44 receptors in human chondrocytes", *Biochemical pharmacology,* vol. 80, no. 4, pp. 480-490.

Campo, G.M., Avenoso, A., Campo, S., D'Ascola, A., Nastasi, G. & Calatroni, A. 2010a, "Molecular size hyaluronan differently modulates toll-like receptor-4 in LPS-induced inflammation in mouse chondrocytes", *Biochimie,* vol. 92, no. 2, pp. 204-215.

Campo, G.M., Avenoso, A., Campo, S., D'Ascola, A., Nastasi, G. & Calatroni, A. 2010b, "Small hyaluronan oligosaccharides induce inflammation by engaging both toll-like-4 and CD44 receptors in human chondrocytes", *Biochemical pharmacology,* vol. 80, no. 4, pp. 480-490.

Cayli, A., Hirschmann, F., Wirth, M., Hauser, H. & Wagner, R. 1999, "Cell lines with reduced UDP-N-acetylhexosamine pool in the presence of ammonium", *Biotechnology and bioengineering,* vol. 65, no. 2, pp. 192-200.

Chen, Q., Cai, S., Shadrach, K.G., Prestwich, G.D. & Hollyfield, J.G. 2004, "Spacrcan binding to hyaluronan and other glycosaminoglycans. Molecular and biochemical studies", *The Journal of biological chemistry,* vol. 279, no. 22, pp. 23142-23150.

Collins, S.L., Black, K.E., Chan-Li, Y., Ahn, Y.H., Cole, P.A., Powell, J.D. & Horton, M.R. 2011, "Hyaluronan Fragments Promote Inflammation by Down-Regulating the Anti-Inflammatory A2a Receptor", *American journal of respiratory cell and molecular biology,* .

Davis, J.A. & Freeze, H.H. 2001, "Studies of mannose metabolism and effects of long-term mannose ingestion in the mouse", *Biochimica et biophysica acta,* vol. 1528, no. 2-3, pp. 116-126.

Davis, R.H., Donato, J.J., Hartman, G.M. & Haas, R.C. 1994, "Anti-inflammatory and wound healing activity of a growth substance in Aloe vera", *Journal of the American Podiatric Medical Association,* vol. 84, no. 2, pp. 77-81.

Day, A.J. & Prestwich, G.D. 2002, "Hyaluronan-binding proteins: tying up the giant", *The Journal of biological chemistry,* vol. 277, no. 7, pp. 4585-4588.

De la Motte, C., Nigro, J., Vasanji, A., Rho, H., Kessler, S., Bandyopadhyay, S., Danese, S., Fiocchi, C. & Stern, R. 2009, "Platelet-derived hyaluronidase 2 cleaves hyaluronan into fragments that trigger monocyte-mediated production of proinflammatory cytokines", *The American journal of pathology,* vol. 174, no. 6, pp. 2254-2264.

De La Motte, C.A., Hascall, V.C., Calabro, A., Yen-Lieberman, B. & Strong, S.A. 1999, "Mononuclear leukocytes preferentially bind via CD44 to hyaluronan on human intestinal mucosal smooth muscle cells after virus infection or treatment with poly(I.C)", *The Journal of biological chemistry,* vol. 274, no. 43, pp. 30747-30755.

De la Motte, C.A., Hascall, V.C., Drazba, J., Bandyopadhyay, S.K. & Strong, S.A. 2003, "Mononuclear leukocytes bind to specific hyaluronan structures on colon mucosal smooth muscle cells treated with polyinosinic acid:polycytidylic acid: inter-alpha-trypsin inhibitor is crucial to structure and function", *The American journal of pathology,* vol. 163, no. 1, pp. 121-133.

Deed, R., Rooney, P., Kumar, P., Norton, J.D., Smith, J., Freemont, A.J. & Kumar, S. 1997, "Early-response gene signalling is induced by angiogenic oligosaccharides of hyaluronan in endothelial cells. Inhibition by non-angiogenic, high-molecular-weight hyaluronan", *International journal of cancer.Journal international du cancer,* vol. 71, no. 2, pp. 251-256.

DeGrendele, H.C., Estess, P. & Siegelman, M.H. 1997, "Requirement for CD44 in activated T cell extravasation into an inflammatory site", *Science (New York, N.Y.),* vol. 278, no. 5338, pp. 672-675.

DeGrendele, H.C., Estess, P., Picker, L.J. & Siegelman, M.H. 1996, "CD44 and its ligand hyaluronate mediate rolling under physiologic flow: a novel lymphocyte-endothelial cell primary adhesion pathway", *The Journal of experimental medicine,* vol. 183, no. 3, pp. 1119-1130.

Duncan, M.R. & Berman, B. 1991, "Stimulation of collagen and glycosaminoglycan production in cultured human adult dermal fibroblasts by recombinant human interleukin 6", *The Journal of investigative dermatology,* vol. 97, no. 4, pp. 686-692.

Esnault, S. & Malter, J.S. 2003, "Hyaluronic acid or TNF-alpha plus fibronectin triggers granulocyte macrophage-colony-stimulating factor mRNA stabilization in eosinophils yet engages differential intracellular pathways and mRNA binding proteins", *Journal of immunology (Baltimore, Md.: 1950),* vol. 171, no. 12, pp. 6780-6787.

Estess, P., Nandi, A., Mohamadzadeh, M. & Siegelman, M.H. 1999, "Interleukin 15 induces endothelial hyaluronan expression in vitro and promotes activated T cell extravasation through a CD44-dependent pathway in vivo", *The Journal of experimental medicine,* vol. 190, no. 1, pp. 9-19.

Evanko, S.P., Johnson, P.Y., Braun, K.R., Underhill, C.B., Dudhia, J. & Wight, T.N. 2001, "Platelet-derived growth factor stimulates the formation of versican-hyaluronan aggregates and pericellular matrix expansion in arterial smooth muscle cells", *Archives of Biochemistry and Biophysics,* vol. 394, no. 1, pp. 29-38.

Evanko, S.P., Potter-Perigo, S., Johnson, P.Y. & Wight, T.N. 2009, "Organization of hyaluronan and versican in the extracellular matrix of human fibroblasts treated with the viral mimetic poly I:C", *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society,* vol. 57, no. 11, pp. 1041-1060.

Ferrer-Martinez, A., Marotta, M., Turini, M., Mace, K. & Gomez-Foix, A.M. 2006, "Effect of sucrose and saturated-fat diets on mRNA levels of genes limiting muscle fatty acid and glucose supply in rats", *Lipids,* vol. 41, no. 1, pp. 55-62.

Fieber, C., Baumann, P., Vallon, R., Termeer, C., Simon, J.C., Hofmann, M., Angel, P., Herrlich, P. & Sleeman, J.P. 2004, "Hyaluronan-oligosaccharide-induced transcription of metalloproteases", *Journal of cell science,* vol. 117, no. Pt 2, pp. 359-367.

Fraser, J.R., Laurent, T.C. & Laurent, U.B. 1997, "Hyaluronan: its nature, distribution, functions and turnover", *Journal of internal medicine,* vol. 242, no. 1, pp. 27-33.

Fulop, N., Feng, W., Xing, D., He, K., Not, L.G., Brocks, C.A., Marchase, R.B., Miller, A.P. & Chatham, J.C. 2008, "Aging leads to increased levels of protein O-linked N-acetylglucosamine in heart, aorta, brain and skeletal muscle in Brown-Norway rats", *Biogerontology,* vol. 9, no. 3, pp. 139-151.

Gao, F., Koenitzer, J.R., Tobolewski, J.M., Jiang, D., Liang, J., Noble, P.W. & Oury, T.D. 2008, "Extracellular superoxide dismutase inhibits inflammation by preventing oxidative fragmentation of hyaluronan", *The Journal of biological chemistry,* vol. 283, no. 10, pp. 6058-6066.

Gluzman, Y. 1981, "SV40-transformed simian cells support the replication of early SV40 mutants", *Cell,* vol. 23, no. 1, pp. 175-182.

Goentzel, B.J., Weigel, P.H. & Steinberg, R.A. 2006, "Recombinant human hyaluronan synthase 3 is phosphorylated in mammalian cells", *The Biochemical journal,* vol. 396, no. 2, pp. 347-354.

Golshani, R., Hautmann, S.H., Estrella, V., Cohen, B.L., Kyle, C.C., Manoharan, M., Jorda, M., Soloway, M.S. & Lokeshwar, V.B. 2007, "HAS1 expression in bladder cancer and its relation to urinary HA test", *International journal of cancer.Journal international du cancer,* vol. 120, no. 8, pp. 1712-1720.

Gordon, S., Akopyan, G., Garban, H. & Bonavida, B. 2006, "Transcription factor YY1: structure, function, and therapeutic implications in cancer biology", *Oncogene,* vol. 25, no. 8, pp. 1125-1142.

Hall, C.L. & Turley, E.A. 1995, "Hyaluronan: RHAMM mediated cell locomotion and signaling in tumorigenesis", *Journal of neuro-oncology,* vol. 26, no. 3, pp. 221-229.

Han, I. & Kudlow, J.E. 1997, "Reduced O glycosylation of Sp1 is associated with increased proteasome susceptibility", *Molecular and cellular biology,* vol. 17, no. 5, pp. 2550-2558.

Hart, G.W., Slawson, C., Ramirez-Correa, G. & Lagerlof, O. 2011, "Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease", *Annual Review of Biochemistry,* vol. 80, pp. 825-858.

Harris, E.N., Kyosseva, S.V., Weigel, J.A. & Weigel, P.H. 2007, "Expression, processing, and glycosaminoglycan binding activity of the recombinant human 315-kDa hyaluronic acid receptor for endocytosis (HARE)", *The Journal of biological chemistry,* vol. 282, no. 5, pp. 2785-2797.

Hascall, V.C. 2000, "Hyaluronan, a common thread", *Glycoconjugate journal,* vol. 17, no. 7-9, pp. 607-616.

Hashizume, M. & Mihara, M. 2010, "High molecular weight hyaluronic acid inhibits IL-6-induced MMP production from human chondrocytes by up-regulating the ERK inhibitor, MKP-1", *Biochemical and biophysical research communications,* vol. 403, no. 2, pp. 184-189.

Hayflick, L. & Moorhead, P.S. 1961, "The serial cultivation of human diploid cell strains", *Experimental cell research,* vol. 25, pp. 585-621.

Heldin, P., Asplund, T., Ytterberg, D., Thelin, S. & Laurent, T.C. 1992, "Characterization of the molecular mechanism involved in the activation of hyaluronan synthetase by platelet-derived growth factor in human mesothelial cells", *The Biochemical journal,* vol. 283 (Pt 1), no. Pt 1, pp. 165-170.

Heldin, P., Laurent, T.C. & Heldin, C.H. 1989, "Effect of growth factors on hyaluronan synthesis in cultured human fibroblasts", *The Biochemical journal,* vol. 258, no. 3, pp. 919-922.

Hiltunen, E.L., Anttila, M., Kultti, A., Ropponen, K., Penttinen, J., Yliskoski, M., Kuronen, A.T., Juhola, M., Tammi, R., Tammi, M. & Kosma, V.M. 2002, "Elevated hyaluronan concentration without hyaluronidase activation in malignant epithelial ovarian tumors", *Cancer research,* vol. 62, no. 22, pp. 6410-6413.

Hiromura, M., Choi, C.H., Sabourin, N.A., Jones, H., Bachvarov, D. & Usheva, A. 2003, "YY1 is regulated by O-linked N-acetylglucosaminylation (O-glcNAcylation)", *The Journal of biological chemistry,* vol. 278, no. 16, pp. 14046-14052.

Hirschberg, C.B., Robbins, P.W. & Abeijon, C. 1998, "Transporters of nucleotide sugars, ATP, and nucleotide sulfate in the endoplasmic reticulum and Golgi apparatus", *Annual Review of Biochemistry,* vol. 67, pp. 49-69.

Hodge-Dufour, J., Noble, P.W., Horton, M.R., Bao, C., Wysoka, M., Burdick, M.D., Strieter, R.M., Trinchieri, G. & Pure, E. 1997, "Induction of IL-12 and chemokines by hyaluronan requires adhesion-dependent priming of resident but not elicited macrophages", *Journal of immunology (Baltimore, Md.: 1950),* vol. 159, no. 5, pp. 2492-2500.

Honda, A., Noguchi, N., Takehara, H., Ohashi, Y., Asuwa, N. & Mori, Y. 1991, "Cooperative enhancement of hyaluronic acid synthesis by combined use of IGF-I and EGF, and inhibition by tyrosine kinase inhibitor genistein, in cultured mesothelial cells from rabbit pericardial cavity", *Journal of cell science,* vol. 98 (Pt 1), no. Pt 1, pp. 91-98.

Horton, M.R., McKee, C.M., Bao, C., Liao, F., Farber, J.M., Hodge-DuFour, J., Pure, E., Oliver, B.L., Wright, T.M. & Noble, P.W. 1998, "Hyaluronan fragments synergize with interferon-gamma to induce the C-X-C chemokines mig and interferon-inducible protein-10 in mouse macrophages", *The Journal of biological chemistry,* vol. 273, no. 52, pp. 35088-35094.

Horton, M.R., Olman, M.A., Bao, C., White, K.E., Choi, A.M., Chin, B.Y., Noble, P.W. & Lowenstein, C.J. 2000, "Regulation of plasminogen activator inhibitor-1 and urokinase by hyaluronan fragments in mouse macrophages", *American journal of physiology.Lung cellular and molecular physiology,* vol. 279, no. 4, pp. L707-15.

Horton, M.R., Shapiro, S., Bao, C., Lowenstein, C.J. & Noble, P.W. 1999, "Induction and regulation of macrophage metalloelastase by hyaluronan fragments in mouse macrophages", *Journal of immunology (Baltimore, Md.: 1950),* vol. 162, no. 7, pp. 4171-4176.

Hresko, R.C., Heimberg, H., Chi, M.M. & Mueckler, M. 1998, "Glucosamine-induced insulin resistance in 3T3-L1 adipocytes is caused by depletion of intracellular ATP", *The Journal of biological chemistry,* vol. 273, no. 32, pp. 20658-20668.

Huang, T.L., Hsu, H.C., Yang, K.C. & Lin, F.H. 2011, "Hyaluronan up-regulates IL-10 expression in fibroblast-like synoviocytes from patients with tibia plateau fracture", *Journal of orthopaedic research : official publication of the Orthopaedic Research Society,* vol. 29, no. 4, pp. 495-500.

Hyc, A., Osiecka-Iwan, A., Niderla-Bielinska, J., Jankowska-Steifer, E. & Moskalewski, S. 2009, "Pro- and anti-inflammatory cytokines increase hyaluronan production by rat synovial membrane in vitro", *International journal of molecular medicine,* vol. 24, no. 4, pp. 579-585.

Isacke, C.M. & Yarwood, H. 2002, "The hyaluronan receptor, CD44", *The international journal of biochemistry & cell biology,* vol. 34, no. 7, pp. 718-721.

Itano, N., Sawai, T., Yoshida, M., Lenas, P., Yamada, Y., Imagawa, M., Shinomura, T., Hamaguchi, M., Yoshida, Y., Ohnuki, Y., Miyauchi, S., Spicer, A.P., McDonald, J.A. & Kimata, K. 1999, "Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties", *The Journal of biological chemistry,* vol. 274, no. 35, pp. 25085-25092.

Iwata, Y., Yoshizaki, A., Komura, K., Shimizu, K., Ogawa, F., Hara, T., Muroi, E., Bae, S., Takenaka, M., Yukami, T., Hasegawa, M., Fujimoto, M., Tomita, Y., Tedder, T.F. & Sato, S. 2009, "CD19, a response regulator of B lymphocytes, regulates wound healing through hyaluronan-induced TLR4 signaling", *The American journal of pathology,* vol. 175, no. 2, pp. 649-660.

Jackson, D.G. 2004, "Biology of the lymphatic marker LYVE-1 and applications in research into lymphatic trafficking and lymphangiogenesis", *APMIS : Acta Pathologica, Microbiologica, et Immunologica Scandinavica,* vol. 112, no. 7-8, pp. 526-538.

Jacobson, A., Brinck, J., Briskin, M.J., Spicer, A.P. & Heldin, P. 2000, "Expression of human hyaluronan synthases in response to external stimuli", *The Biochemical journal,* vol. 348 Pt 1, pp. 29-35.

Jameson, J.M., Cauvi, G., Sharp, L.L., Witherden, D.A. & Havran, W.L. 2005, "Gammadelta T cell-induced hyaluronan production by epithelial cells regulates inflammation", *The Journal of experimental medicine,* vol. 201, no. 8, pp. 1269-1279.

Jiang, D., Liang, J. & Noble, P.W. 2011, "Hyaluronan as an immune regulator in human diseases", *Physiological Reviews,* vol. 91, no. 1, pp. 221-264.

Jiang, D., Liang, J., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G.D., Mascarenhas, M.M., Garg, H.G., Quinn, D.A., Homer, R.J., Goldstein, D.R., Bucala, R., Lee, P.J., Medzhitov, R. & Noble, P.W. 2005, "Regulation of lung injury and repair by Toll-like receptors and hyaluronan", *Nature medicine,* vol. 11, no. 11, pp. 1173-1179.

Jones, S., Jones, S. & Phillips, A.O. 2001, "Regulation of renal proximal tubular epithelial cell hyaluronan generation: implications for diabetic nephropathy", *Kidney international,* vol. 59, no. 5, pp. 1739-1749.

Kakizaki, I., Kojima, K., Takagaki, K., Endo, M., Kannagi, R., Ito, M., Maruo, Y., Sato, H., Yasuda, T., Mita, S., Kimata, K. & Itano, N. 2004, "A novel mechanism for the inhibition of hyaluronan biosynthesis by 4-methylumbelliferone", *The Journal of biological chemistry,* vol. 279, no. 32, pp. 33281-33289.

Karousou, E., Kamiryo, M., Skandalis, S.S., Ruusala, A., Asteriou, T., Passi, A., Yamashita, H., Hellman, U., Heldin, C.H. & Heldin, P. 2010, "The activity of hyaluronan synthase 2 is regulated by dimerization and ubiquitination", *The Journal of biological chemistry,* vol. 285, no. 31, pp. 23647-23654.

Karvinen, S., Pasonen-Seppänen, S., Hyttinen, J.M., Pienimäki, J.P., Törrönen, K., Jokela, T.A., Tammi, M.I. & Tammi, R. 2003, "Keratinocyte growth factor stimulates migration and hyaluronan synthesis in the epidermis by activation of keratinocyte hyaluronan synthases 2 and 3", *Journal of Biological Chemistry,* vol. 278, no. 49, pp. 49495-49504.

Kawakami, M., Suzuki, K., Matsuki, Y., Ishizuka, T., Hidaka, T., Konishi, T., Matsumoto, M., Kataharada, K. & Nakamura, H. 1998, "Hyaluronan production in human rheumatoid fibroblastic synovial lining cells is increased by interleukin 1 beta but inhibited by transforming growth factor beta 1", *Annals of the Rheumatic Diseases,* vol. 57, no. 10, pp. 602-605.

Kennedy, C.I., Diegelmann, R.F., Haynes, J.H. & Yager, D.R. 2000, "Proinflammatory cytokines differentially regulate hyaluronan synthase isoforms in fetal and adult fibroblasts", *Journal of pediatric surgery,* vol. 35, no. 6, pp. 874-879.

Kessler, S., Rho, H., West, G., Fiocchi, C., Drazba, J. & de la Motte, C. 2008, "Hyaluronan (HA) deposition precedes and promotes leukocyte recruitment in intestinal inflammation", *Clinical and translational science,* vol. 1, no. 1, pp. 57-61.

Khan, A.I., Kerfoot, S.M., Heit, B., Liu, L., Andonegui, G., Ruffell, B., Johnson, P. & Kubes, P. 2004, "Role of CD44 and hyaluronan in neutrophil recruitment", *Journal of immunology (Baltimore, Md.: 1950),* vol. 173, no. 12, pp. 7594-7601.

Kiani, C., Chen, L., Wu, Y.J., Yee, A.J. & Yang, B.B. 2002, "Structure and function of aggrecan", *Cell research,* vol. 12, no. 1, pp. 19-32.

Kim, J.J. & Conrad, H.E. 1974, "Effect of D-glucosamine concentration on the kinetics of mucopolysaccharide biosynthesis in cultured chick embryo vertebral cartilage", *The Journal of biological chemistry,* vol. 249, no. 10, pp. 3091-3097.

Kim, Y., Lee, Y.S., Choe, J., Lee, H., Kim, Y.M. & Jeoung, D. 2008, "CD44-epidermal growth factor receptor interaction mediates hyaluronic acid-promoted cell motility by activating protein kinase C signaling involving Akt, Rac1, Phox, reactive oxygen species, focal adhesion kinase, and MMP-2", *The Journal of biological chemistry,* vol. 283, no. 33, pp. 22513-22528.

Knudson, W., Chow, G. & Knudson, C.B. 2002, "CD44-mediated uptake and degradation of hyaluronan", *Matrix biology : journal of the International Society for Matrix Biology,* vol. 21, no. 1, pp. 15-23.

Kössi, J., Peltonen, J., Ekfors, T., Niinikoski, J. & Laato, M. 1999, "Effects of hexose sugars: glucose, fructose, galactose and mannose on wound healing in the rat", *European surgical research. Europaische chirurgische Forschung. Recherches chirurgicales europeennes; European surgical research.Europaische chirurgische Forschung.Recherches chirurgicales europeennes,* vol. 31, no. 1, pp. 74-82.

Kultti, A., Pasonen-Seppänen, S., Jauhiainen, M., Rilla, K.J., Kärnä, R., Pyöria, E., Tammi, R.H. & Tammi, M.I. 2009, "4-Methylumbelliferone inhibits hyaluronan synthesis by depletion of cellular UDP-glucuronic acid and downregulation of hyaluronan synthase 2 and 3", *Experimental cell research,* vol. 315, no. 11, pp. 1914-1923.

Kuroda, K., Utani, A., Hamasaki, Y. & Shinkai, H. 2001, "Up-regulation of putative hyaluronan synthase mRNA by basic fibroblast growth factor and insulin-like growth factor-1 in human skin fibroblasts", *Journal of dermatological science,* vol. 26, no. 2, pp. 156-160.

Lauer, M.E., Fulop, C., Mukhopadhyay, D., Comhair, S., Erzurum, S.C. & Hascall, V.C. 2009, "Airway smooth muscle cells synthesize hyaluronan cable structures independent of inter-alpha-inhibitor heavy chain attachment", *The Journal of biological chemistry,* vol. 284, no. 8, pp. 5313-5323.

Laurent, T.C., Laurent, U.B. & Fraser, J.R. 1996, "Serum hyaluronan as a disease marker", *Annals of Medicine,* vol. 28, no. 3, pp. 241-253.

Lefebvre, D.C., Lai, J.C., Maeshima, N., Ford, J.L., Wong, A.S., Cross, J.L. & Johnson, P. 2010, "CD44 interacts directly with Lck in a zinc-dependent manner", *Molecular immunology,* vol. 47, no. 10, pp. 1882-1889.

Legg, J.W., Lewis, C.A., Parsons, M., Ng, T. & Isacke, C.M. 2002, "A novel PKC-regulated mechanism controls CD44 ezrin association and directional cell motility", *Nature cell biology,* vol. 4, no. 6, pp. 399-407.

Liu, D., Liu, T., Li, R. & Sy, M.S. 1998, "Mechanisms regulating the binding activity of CD44 to hyaluronic acid", *Frontiers in bioscience : a journal and virtual library,* vol. 3, pp. d631-6.

Love, D.C. & Hanover, J.A. 2005, "The hexosamine signaling pathway: deciphering the "O-GlcNAc code"", *Science's STKE : signal transduction knowledge environment,* vol. 2005, no. 312, pp. re13.

Maeshima, N., Poon, G.F., Dosanjh, M., Felberg, J., Lee, S.S., Cross, J.L., Birkenhead, D. & Johnson, P. 2011, "Hyaluronan binding identifies the most proliferative activated and memory T cells", *European journal of immunology,* vol. 41, no. 4, pp. 1108-1119.

Maier, K.G., Han, X., Sadowitz, B., Gentile, K.L., Middleton, F.A. & Gahtan, V. 2010, "Thrombospondin-1: a proatherosclerotic protein augmented by hyperglycemia", *Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter,* vol. 51, no. 5, pp. 1238-1247.

Majors, A.K., Austin, R.C., de la Motte, C.A., Pyeritz, R.E., Hascall, V.C., Kessler, S.P., Sen, G. & Strong, S.A. 2003, "Endoplasmic reticulum stress induces hyaluronan deposition and leukocyte adhesion", *The Journal of biological chemistry,* vol. 278, no. 47, pp. 47223-47231.

Majumdar, G., Harrington, A., Hungerford, J., Martinez-Hernandez, A., Gerling, I.C., Raghow, R. & Solomon, S. 2006, "Insulin dynamically regulates calmodulin gene expression by sequential o-glycosylation and phosphorylation of sp1 and its subcellular compartmentalization in liver cells", *The Journal of biological chemistry,* vol. 281, no. 6, pp. 3642-3650.

Makkonen, K.M., Pasonen-Seppänen, S., Törrönen, K., Tammi, M.I. & Carlberg, C. 2009, "Regulation of the hyaluronan synthase 2 gene by convergence in cyclic AMP response element-binding protein and retinoid acid receptor signaling", *The Journal of biological chemistry,* vol. 284, no. 27, pp. 18270-18281.

Marshall, S., Bacote, V. & Traxinger, R.R. 1991, "Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance", *The Journal of biological chemistry,* vol. 266, no. 8, pp. 4706-4712.

Marshall, S., Nadeau, O. & Yamasaki, K. 2005, "Glucosamine-induced activation of glycogen biosynthesis in isolated adipocytes. Evidence for a rapid allosteric control mechanism within the hexosamine biosynthesis pathway", *The Journal of biological chemistry,* vol. 280, no. 12, pp. 11018-11024.

Maytin, E.V., Chung, H.H. & Seetharaman, V.M. 2004, "Hyaluronan participates in the epidermal response to disruption of the permeability barrier in vivo", *The American journal of pathology,* vol. 165, no. 4, pp. 1331-1341.

McKee, C.M., Penno, M.B., Cowman, M., Burdick, M.D., Strieter, R.M., Bao, C. & Noble, P.W. 1996, "Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages. The role of HA size and CD44", *The Journal of clinical investigation,* vol. 98, no. 10, pp. 2403-2413.

Meran, S., Luo, D.D., Simpson, R., Martin, J., Wells, A., Steadman, R. & Phillips, A.O. 2011, "Hyaluronan facilitates transforming growth factor-beta1-dependent proliferation via CD44 and epidermal growth factor receptor interaction", *The Journal of biological chemistry,* vol. 286, no. 20, pp. 17618-17630.

Milner, C.M. & Day, A.J. 2003, "TSG-6: a multifunctional protein associated with inflammation", *Journal of cell science,* vol. 116, no. Pt 10, pp. 1863-1873.

Mitsui, Y., Gotoh, M., Nakama, K., Yamada, T., Higuchi, F. & Nagata, K. 2008, "Hyaluronic acid inhibits mRNA expression of proinflammatory cytokines and cyclooxygenase-2/prostaglandin E(2) production via CD44 in interleukin-1-stimulated subacromial synovial fibroblasts from patients with rotator cuff disease", *Journal of orthopaedic research : official publication of the Orthopaedic Research Society,* vol. 26, no. 7, pp. 1032-1037.

Monslow, J., Williams, J.D., Fraser, D.J., Michael, D.R., Foka, P., Kift-Morgan, A.P., Luo, D.D., Fielding, C.A., Craig, K.J., Topley, N., Jones, S.A., Ramji, D.P. & Bowen, T. 2006, "Sp1 and Sp3 mediate constitutive transcription of the human hyaluronan synthase 2 gene", *The Journal of biological chemistry,* vol. 281, no. 26, pp. 18043-18050.

Monslow, J., Williams, J.D., Norton, N., Guy, C.A., Price, I.K., Coleman, S.L., Williams, N.M., Buckland, P.R., Spicer, A.P., Topley, N., Davies, M. & Bowen, T. 2003, "The human hyaluronan synthase genes: genomic structures, proximal promoters and polymorphic microsatellite markers", *The international journal of biochemistry & cell biology,* vol. 35, no. 8, pp. 1272-1283.

Morales, T.I. & Hascall, V.C. 1988, "Correlated metabolism of proteoglycans and hyaluronic acid in bovine cartilage organ cultures", *The Journal of biological chemistry,* vol. 263, no. 8, pp. 3632-3638.

Muchmore, A.V., Decker, J.M., Blaese, R.M. & Nilsson, B. 1984, "Purification and characterization of a mannose-containing disaccharide obtained from human pregnancy urine. A new immunoregulatory saccharide", *The Journal of experimental medicine,* vol. 160, no. 6, pp. 1672-1685.

Murai, T., Maruyama, Y., Mio, K., Nishiyama, H., Suga, M. & Sato, C. 2010, "Low cholesterol triggers membrane microdomain-dependent CD44 shedding and suppresses tumor cell migration", *The Journal of biological chemistry,* .

Nagy, N., Freudenberger, T., Melchior-Becker, A., Rock, K., Ter Braak, M., Jastrow, H., Kinzig, M., Lucke, S., Suvorava, T., Kojda, G., Weber, A.A., Sorgel, F., Levkau, B., Ergun, S. & Fischer, J.W. 2010, "Inhibition of hyaluronan synthesis accelerates murine atherosclerosis: novel insights into the role of hyaluronan synthesis", *Circulation,* vol. 122, no. 22, pp. 2313-2322.

Naor, D. & Nedvetzki, S. 2003, "CD44 in rheumatoid arthritis", *Arthritis research & therapy,* vol. 5, no. 3, pp. 105-115.

Noble, P.W., Lake, F.R., Henson, P.M. & Riches, D.W. 1993, "Hyaluronate activation of CD44 induces insulin-like growth factor-1 expression by a tumor necrosis factor-alpha-dependent mechanism in murine macrophages", *The Journal of clinical investigation,* vol. 91, no. 6, pp. 2368-2377.

Oguchi, T. & Ishiguro, N. 2004, "Differential stimulation of three forms of hyaluronan synthase by TGF-beta, IL-1beta, and TNF-alpha", *Connective tissue research,* vol. 45, no. 4-5, pp. 197-205.

Ohkawara, Y., Tamura, G., Iwasaki, T., Tanaka, A., Kikuchi, T. & Shirato, K. 2000, "Activation and transforming growth factor-beta production in eosinophils by hyaluronan", *American journal of respiratory cell and molecular biology,* vol. 23, no. 4, pp. 444-451.

Ohno, S., Tanimoto, K., Fujimoto, K., Ijuin, C., Honda, K., Tanaka, N., Doi, T., Nakahara, M. & Tanne, K. 2001, "Molecular cloning of rabbit hyaluronic acid synthases and their expression patterns in synovial membrane and articular cartilage", *Biochimica et biophysica acta,* vol. 1520, no. 1, pp. 71-78.

Oki, T., Yamazaki, K., Kuromitsu, J., Okada, M. & Tanaka, I. 1999, "cDNA cloning and mapping of a novel subtype of glutamine:fructose-6-phosphate amidotransferase (GFAT2) in human and mouse", *Genomics,* vol. 57, no. 2, pp. 227-234.

Özcan, S., Andrali, S.S. & Cantrell, J.E. 2010, "Modulation of transcription factor function by O-GlcNAc modification", *Biochimica et biophysica acta,* vol. 1799, no. 5-6, pp. 353-364.

Panneerselvam, K. & Freeze, H.H. 1996, "Mannose enters mammalian cells using a specific transporter that is insensitive to glucose", *The Journal of biological chemistry,* vol. 271, no. 16, pp. 9417-9421.

Pasonen-Seppänen, S., Karvinen, S., Törrönen, K., Hyttinen, J.M., Jokela, T., Lammi, M.J., Tammi, M.I. & Tammi, R. 2003, "EGF upregulates, whereas TGF-beta downregulates, the hyaluronan synthases Has2 and Has3 in organotypic keratinocyte cultures: correlations with epidermal proliferation and differentiation", *The Journal of investigative dermatology,* vol. 120, no. 6, pp. 1038-1044.

Pasonen-Seppänen, S.M., Maytin, E.V., Törrönen, K.J., Hyttinen, J.M., Hascall, V.C., MacCallum, D.K., Kultti, A.H., Jokela, T.A., Tammi, M.I. & Tammi, R.H. 2008, "All-trans retinoic acid-induced hyaluronan production and hyperplasia are partly mediated by EGFR signaling in epidermal keratinocytes", *The Journal of investigative dermatology,* vol. 128, no. 4, pp. 797-807.

Passi, A., Sadeghi, P., Kawamura, H., Anand, S., Sato, N., White, L.E., Hascall, V.C. & Maytin, E.V. 2004, "Hyaluronan suppresses epidermal differentiation in organotypic cultures of rat keratinocytes", *Experimental cell research,* vol. 296, no. 2, pp. 123-134.

Pauloin, T., Dutot, M., Joly, F., Warnet, J.M. & Rat, P. 2009, "High molecular weight hyaluronan decreases UVB-induced apoptosis and inflammation in human epithelial corneal cells", *Molecular vision,* vol. 15, pp. 577-583.

Pienimäki, J.P., Rilla, K., Fulop, C., Sironen, R.K., Karvinen, S., Pasonen, S., Lammi, M.J., Tammi, R., Hascall, V.C. & Tammi, M.I. 2001, "Epidermal growth factor activates hyaluronan synthase 2 in epidermal keratinocytes and increases pericellular and intracellular hyaluronan", *Journal of Biological Chemistry,* vol. 276, no. 23, pp. 20428-20435.

Prehm, P. 1984, "Hyaluronate is synthesized at plasma membranes", *The Biochemical journal,* vol. 220, no. 2, pp. 597-600.

Pummill, P.E. & DeAngelis, P.L. 2002, "Evaluation of critical structural elements of UDP-sugar substrates and certain cysteine residues of a vertebrate hyaluronan synthase", *The Journal of biological chemistry,* vol. 277, no. 24, pp. 21610-21616.

Rafi, A., Nagarkatti, M. & Nagarkatti, P.S. 1997, "Hyaluronate-CD44 interactions can induce murine B-cell activation", *Blood,* vol. 89, no. 8, pp. 2901-2908.

Rest, R.F., Farrell, C.F. & Naids, F.L. 1988, "Mannose inhibits the human neutrophil oxidative burst", *Journal of leukocyte biology,* vol. 43, no. 2, pp. 158-164.

Rilla, K., Pasonen-Seppänen, S., Rieppo, J., Tammi, M. & Tammi, R. 2004, "The hyaluronan synthesis inhibitor 4-methylumbelliferone prevents keratinocyte activation and epidermal hyperproliferation induced by epidermal growth factor", *The Journal of investigative dermatology,* vol. 123, no. 4, pp. 708-714.

Rilla, K., Siiskonen, H., Spicer, A.P., Hyttinen, J.M., Tammi, M.I. & Tammi, R.H. 2005, "Plasma membrane residence of hyaluronan synthase is coupled to its enzymatic activity", *Journal of Biological Chemistry,* .

Rilla, K., Tiihonen, R., Kultti, A., Tammi, M. & Tammi, R. 2008, "Pericellular hyaluronan coat visualized in live cells with a fluorescent probe is scaffolded by plasma membrane protrusions", *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society,* vol. 56, no. 10, pp. 901-910.

Rugheimer, L., Carlsson, C., Johnsson, C. & Hansell, P. 2008, "Renal hyaluronan content during experimental uncontrolled diabetes in rats", *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society,* vol. 59, no. 1, pp. 115-128.

Ryll, T., Valley, U. & Wagner, R. 1994, "Biochemistry of growth inhibition by ammonium ions in mammalian cells", *Biotechnology and bioengineering,* vol. 44, no. 2, pp. 184-193.

Röck, K., Fischer, K. & Fischer, J.W. 2010, "Hyaluronan used for intradermal injections is incorporated into the pericellular matrix and promotes proliferation in human skin fibroblasts in vitro", *Dermatology (Basel, Switzerland),* vol. 221, no. 3, pp. 219-228.

Saavalainen, K., Pasonen-Seppänen, S., Dunlop, T.W., Tammi, R., Tammi, M.I. & Carlberg, C. 2005, "The human hyaluronan synthase 2 gene is a primary retinoic acid and epidermal growth factor responding gene", *The Journal of biological chemistry,* vol. 280, no. 15, pp. 14636-14644.

Saavalainen, K., Tammi, M.I., Bowen, T., Schmitz, M.L. & Carlberg, C. 2007, "Integration of the activation of the human hyaluronan synthase 2 gene promoter by common cofactors of the transcription factors retinoic acid receptor and nuclear factor kappaB", *The Journal of biological chemistry,* vol. 282, no. 15, pp. 11530-11539.

Sainio, A., Jokela, T., Tammi, M.I. & Järvelainen, H. 2010, "Hyperglycemic conditions modulate connective tissue reorganization by human vascular smooth muscle cells through stimulation of hyaluronan synthesis", *Glycobiology,* vol. 20, no. 9, pp. 1117-1126.

Sakr, S.W., Potter-Perigo, S., Kinsella, M.G., Johnson, P.Y., Braun, K.R., Goueffic, Y., Rosenfeld, M.E. & Wight, T.N. 2008, "Hyaluronan accumulation is elevated in cultures of low density lipoprotein receptor-deficient cells and is altered by manipulation of cell cholesterol content", *The Journal of biological chemistry,* vol. 283, no. 52, pp. 36195-36204.

Sampson, P.M., Rochester, C.L., Freundlich, B. & Elias, J.A. 1992, "Cytokine regulation of human lung fibroblast hyaluronan (hyaluronic acid) production. Evidence for cytokine-regulated hyaluronan (hyaluronic acid) degradation and human lung fibroblast-derived hyaluronidase", *The Journal of clinical investigation,* vol. 90, no. 4, pp. 1492-1503.

Sayo, T., Sugiyama, Y., Takahashi, Y., Ozawa, N., Sakai, S., Ishikawa, O., Tamura, M. & Inoue, S. 2002, "Hyaluronan synthase 3 regulates hyaluronan synthesis in cultured human keratinocytes", *The Journal of investigative dermatology,* vol. 118, no. 1, pp. 43-48.

Scheibner, K.A., Lutz, M.A., Boodoo, S., Fenton, M.J., Powell, J.D. & Horton, M.R. 2006, "Hyaluronan fragments act as an endogenous danger signal by engaging TLR2", *Journal of immunology (Baltimore, Md.: 1950),* vol. 177, no. 2, pp. 1272-1281.

Schleicher, E.D. & Weigert, C. 2000, "Role of the hexosamine biosynthetic pathway in diabetic nephropathy", *Kidney international.Supplement,* vol. 77, pp. S13-8.

Selbi, W., Day, A.J., Rugg, M.S., Fulop, C., de la Motte, C.A., Bowen, T., Hascall, V.C. & Phillips, A.O. 2006, "Overexpression of hyaluronan synthase 2 alters hyaluronan distribution and function in proximal tubular epithelial cells", *Journal of the American Society of Nephrology : JASN,* vol. 17, no. 6, pp. 1553-1567.

Selbi, W., de la Motte, C.A., Hascall, V.C., Day, A.J., Bowen, T. & Phillips, A.O. 2006b, "Characterization of hyaluronan cable structure and function in renal proximal tubular epithelial cells", *Kidney international,* vol. 70, no. 7, pp. 1287-1295.

Sesma, J.I., Esther, C.R.,Jr, Kreda, S.M., Jones, L., O'Neal, W., Nishihara, S., Nicholas, R.A. & Lazarowski, E.R. 2009, "Endoplasmic reticulum/golgi nucleotide sugar transporters contribute to the cellular release of UDP-sugar signaling molecules", *The Journal of biological chemistry,* vol. 284, no. 18, pp. 12572-12583.

Shi, T., Duan, Z.H., Papay, R., Pluskota, E., Gaivin, R.J., de la Motte, C.A., Plow, E.F. & Perez, D.M. 2006, "Novel alpha1-adrenergic receptor signaling pathways: secreted factors and interactions with the extracellular matrix", *Molecular pharmacology,* vol. 70, no. 1, pp. 129-142.

Shi, Y., Lee, J.S. & Galvin, K.M. 1997, "Everything you have ever wanted to know about Yin Yang 1.....", *Biochimica et biophysica acta,* vol. 1332, no. 2, pp. F49-66.

Shimada, M., Yanai, Y., Okazaki, T., Noma, N., Kawashima, I., Mori, T. & Richards, J.S. 2008, "Hyaluronan fragments generated by sperm-secreted hyaluronidase stimulate cytokine/chemokine production via the TLR2 and TLR4 pathway in cumulus cells of ovulated COCs, which may enhance fertilization", *Development (Cambridge, England),* vol. 135, no. 11, pp. 2001-2011.

Shimizu, M., Yasuda, T., Nakagawa, T., Yamashita, E., Julovi, S.M., Hiramitsu, T. & Nakamura, T. 2003, "Hyaluronan inhibits matrix metalloproteinase-1 production by rheumatoid synovial fibroblasts stimulated by proinflammatory cytokines", *The Journal of rheumatology,* vol. 30, no. 6, pp. 1164-1172.

Solomon, S.S., Majumdar, G., Martinez-Hernandez, A. & Raghow, R. 2008, "A critical role of Sp1 transcription factor in regulating gene expression in response to insulin and other hormones", *Life Sciences,* vol. 83, no. 9-10, pp. 305-312.

Soltes, L., Mendichi, R., Kogan, G., Schiller, J., Stankovska, M. & Arnhold, J. 2006, "Degradative action of reactive oxygen species on hyaluronan", *Biomacromolecules,* vol. 7, no. 3, pp. 659-668.

Sommarin, Y. & Heinegard, D. 1983, "Specific interaction between cartilage proteoglycans and hyaluronic acid at the chondrocyte cell surface", *The Biochemical journal,* vol. 214, no. 3, pp. 777-784.

Soule, H.D., Vazguez, J., Long, A., Albert, S. & Brennan, M. 1973, "A human cell line from a pleural effusion derived from a breast carcinoma", *Journal of the National Cancer Institute,* vol. 51, no. 5, pp. 1409-1416.

Stern, R. 2003, "Devising a pathway for hyaluronan catabolism: are we there yet?", *Glycobiology,* vol. 13, no. 12, pp. 105R-115R.

Stoolman, L.M. & Rosen, S.D. 1983, "Possible role for cell-surface carbohydrate-binding molecules in lymphocyte recirculation", *The Journal of cell biology,* vol. 96, no. 3, pp. 722-729.

Stuhlmeier, K.M. & Pollaschek, C. 2004, "Differential effect of transforming growth factor beta (TGF-beta) on the genes encoding hyaluronan synthases and utilization of the p38 MAPK pathway in TGF-beta-induced hyaluronan synthase 1 activation", *The Journal of biological chemistry,* vol. 279, no. 10, pp. 8753-8760.

Sugahara, K.N., Murai, T., Nishinakamura, H., Kawashima, H., Saya, H. & Miyasaka, M. 2003, "Hyaluronan oligosaccharides induce CD44 cleavage and promote cell migration in CD44-expressing tumor cells", *The Journal of biological chemistry,* vol. 278, no. 34, pp. 32259-32265.

Sugiyama, Y., Shimada, A., Sayo, T., Sakai, S. & Inoue, S. 1998, "Putative hyaluronan synthase mRNA are expressed in mouse skin and TGF-beta upregulates their expression in cultured human skin cells", *The Journal of investigative dermatology,* vol. 110, no. 2, pp. 116-121.

Suzuki, K., Yamamoto, T., Usui, T., Suzuki, K., Heldin, P. & Yamashita, H. 2003, "Expression of hyaluronan synthase in intraocular proliferative diseases: regulation of expression in human vascular endothelial cells by transforming growth factor-beta", *Japanese journal of ophthalmology,* vol. 47, no. 6, pp. 557-564.

Sweeney, C., Mackintosh, D. & Mason, R.M. 1993, "UDP-sugar metabolism in Swarm rat chondrosarcoma chondrocytes", *The Biochemical journal,* vol. 290 (Pt 2), no. Pt 2, pp. 563-570.

Takahashi, Y., Li, L., Kamiryo, M., Asteriou, T., Moustakas, A., Yamashita, H. & Heldin, P. 2005, "Hyaluronan fragments induce endothelial cell differentiation in a CD44- and CXCL1/GRO1-dependent manner", *The Journal of biological chemistry,* vol. 280, no. 25, pp. 24195-24204.

Takigawa, M., Tajima, K., Pan, H.O., Enomoto, M., Kinoshita, A., Suzuki, F., Takano, Y. & Mori, Y. 1989, "Establishment of a clonal human chondrosarcoma cell line with cartilage phenotypes", *Cancer research,* vol. 49, no. 14, pp. 3996-4002.

Tammi, R., Ågren, U.M., Tuhkanen, A.L. & Tammi, M. 1994, "Hyaluronan metabolism in skin", *Progress in histochemistry and cytochemistry,* vol. 29, no. 2, pp. 1-81.

Tammi, R., MacCallum, D., Hascall, V.C., Pienimäki, J.P., Hyttinen, M. & Tammi, M. 1998, "Hyaluronan bound to CD44 on keratinocytes is displaced by hyaluronan decasaccharides and not hexasaccharides", *The Journal of biological chemistry,* vol. 273, no. 44, pp. 28878-28888.

Tammi, R., Pasonen-Seppänen, S., Kolehmainen, E. & Tammi, M. 2005, "Hyaluronan synthase induction and hyaluronan accumulation in mouse epidermis following skin injury", *The Journal of investigative dermatology; The Journal of investigative dermatology,* vol. 124, no. 5, pp. 898-905.

Tammi, R., Ripellino, J.A., Margolis, R.U. & Tammi, M. 1988, "Localization of epidermal hyaluronic acid using the hyaluronate binding region of cartilage proteoglycan as a specific probe", *The Journal of investigative dermatology,* vol. 90, no. 3, pp. 412-414.

Tammi, R., Säämänen, A.M., Maibach, H.I. & Tammi, M. 1991, "Degradation of newly synthesized high molecular mass hyaluronan in the epidermal and dermal compartments of human skin in organ culture", *The Journal of investigative dermatology,* vol. 97, no. 1, pp. 126-130.

Tammi, R.H. & Tammi, M.I. 2009, "Hyaluronan accumulation in wounded epidermis: a mediator of keratinocyte activation", *The Journal of investigative dermatology,* vol. 129, no. 8, pp. 1858-1860.

Tammi, R.H., Kultti, A., Kosma, V.M., Pirinen, R., Auvinen, P. & Tammi, M.I. 2008, "Hyaluronan in human tumors: pathobiological and prognostic messages from cell-associated and stromal hyaluronan", *Seminars in cancer biology,* vol. 18, no. 4, pp. 288-295.

Tammi, R.H., Tammi, M.I., Hascall, V.C., Hogg, M., Pasonen, S. & MacCallum, D.K. 2000, "A preformed basal lamina alters the metabolism and distribution of hyaluronan in epidermal keratinocyte "organotypic" cultures grown on collagen matrices", *Histochemistry and cell biology,* vol. 113, no. 4, pp. 265-277.

Taylor, K.R., Trowbridge, J.M., Rudisill, J.A., Termeer, C.C., Simon, J.C. & Gallo, R.L. 2004, "Hyaluronan fragments stimulate endothelial recognition of injury through TLR4", *The Journal of biological chemistry,* vol. 279, no. 17, pp. 17079-17084.

Taylor, K.R., Yamasaki, K., Radek, K.A., Di Nardo, A., Goodarzi, H., Golenbock, D., Beutler, B. & Gallo, R.L. 2007, "Recognition of hyaluronan released in sterile injury involves a unique receptor complex dependent on Toll-like receptor 4, CD44, and MD-2", *The Journal of biological chemistry,* vol. 282, no. 25, pp. 18265-18275.

Termeer, C., Benedix, F., Sleeman, J., Fieber, C., Voith, U., Ahrens, T., Miyake, K., Freudenberg, M., Galanos, C. & Simon, J.C. 2002, "Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4", *The Journal of experimental medicine,* vol. 195, no. 1, pp. 99-111.

Termeer, C.C., Hennies, J., Voith, U., Ahrens, T., Weiss, J.M., Prehm, P. & Simon, J.C. 2000, "Oligosaccharides of hyaluronan are potent activators of dendritic cells", *Journal of immunology (Baltimore, Md.: 1950),* vol. 165, no. 4, pp. 1863-1870.

Tien, J.Y. & Spicer, A.P. 2005, "Three vertebrate hyaluronan synthases are expressed during mouse development in distinct spatial and temporal patterns", *Developmental dynamics : an official publication of the American Association of Anatomists,* vol. 233, no. 1, pp. 130-141.

Tomiya, N., Ailor, E., Lawrence, S.M., Betenbaugh, M.J. & Lee, Y.C. 2001, "Determination of nucleotides and sugar nucleotides involved in protein glycosylation by high-performance anion-exchange chromatography: sugar nucleotide contents in cultured insect cells and mammalian cells", *Analytical Biochemistry,* vol. 293, no. 1, pp. 129-137.

Toole, B.P. & Hascall, V.C. 2002, "Hyaluronan and tumor growth", *American Journal of Pathology; The American journal of pathology,* vol. 161, no. 3, pp. 745-747.

Toole, B.P. 2000, "Hyaluronan is not just a goo!", *The Journal of clinical investigation,* vol. 106, no. 3, pp. 335-336.

Toole, B.P. 2004, "Hyaluronan: from extracellular glue to pericellular cue", *Nature reviews.Cancer,* vol. 4, no. 7, pp. 528-539.

Turley, E.A., Noble, P.W. & Bourguignon, L.Y. 2002, "Signaling properties of hyaluronan receptors", *The Journal of biological chemistry,* vol. 277, no. 7, pp. 4589-4592.

Uchiyama, T., Sakuta, T. & Kanayama, T. 2005, "Regulation of hyaluronan synthases in mouse uterine cervix", *Biochemical and biophysical research communications,* vol. 327, no. 3, pp. 927-932.

Uldry, M., Ibberson, M., Hosokawa, M. & Thorens, B. 2002, "GLUT2 is a high affinity glucosamine transporter", *FEBS letters,* vol. 524, no. 1-3, pp. 199-203.

Varki, A., Cummings, R., Esko, J., Freeze, H., Stanley, P., Bertozzi, C., Hart, G.W. & Etzler, M. (eds) 2008, *Essentials of Glycobiology*, 2nd edn, Cold spring harbor Laboratory Press, Cold spring harbor, new york.

Vigetti, D., Clerici, M., Deleonibus, S., Karousou, E., Viola, M., Moretto, P., Heldin, P., Hascall, V.C., De Luca, G. & Passi, A. 2011, "Hyaluronan synthesis is inhibited by adenosine monophosphate activated protein kinase through the regulation of hyaluronan synthase 2 activity in human aortic smooth muscle cells", *The Journal of biological chemistry,* .

Vigetti, D., Genasetti, A., Karousou, E., Viola, M., Clerici, M., Bartolini, B., Moretto, P., De Luca, G., Hascall, V.C. & Passi, A. 2009, "Modulation of hyaluronan synthase activity in cellular membrane fractions", *The Journal of biological chemistry,* vol. 284, no. 44, pp. 30684-30694.

Vigetti, D., Genasetti, A., Karousou, E., Viola, M., Moretto, P., Clerici, M., Deleonibus, S., De Luca, G., Hascall, V.C. & Passi, A. 2010, "Proinflammatory cytokines induce hyaluronan synthesis and monocyte adhesion in human endothelial cells through hyaluronan synthase 2 (HAS2) and the nuclear factor-kappaB (NF-kappaB) pathway", *The Journal of biological chemistry,* vol. 285, no. 32, pp. 24639-24645.

Vigetti, D., Ori, M., Viola, M., Genasetti, A., Karousou, E., Rizzi, M., Pallotti, F., Nardi, I., Hascall, V.C., De Luca, G. & Passi, A. 2006, "Molecular cloning and characterization of UDP-glucose dehydrogenase from the amphibian xenopus laevis and its involvement in hyaluronan synthesis", *Journal of Biological Chemistry,* .

Vigetti, D., Rizzi, M., Viola, M., Karousou, E., Genasetti, A., Clerici, M., Bartolini, B., Hascall, V.C., De Luca, G. & Passi, A. 2009, "The effects of 4-methylumbelliferone on hyaluronan synthesis, MMP2 activity, proliferation, and motility of human aortic smooth muscle cells", *Glycobiology,* vol. 19, no. 5, pp. 537-546.

Voelcker, V., Gebhardt, C., Averbeck, M., Saalbach, A., Wolf, V., Weih, F., Sleeman, J., Anderegg, U. & Simon, J. 2008, "Hyaluronan fragments induce cytokine and metalloprotease upregulation in human melanoma cells in part by signalling via TLR4", *Experimental dermatology,* vol. 17, no. 2, pp. 100-107.

Wallet, M.A., Wallet, S.M., Guiulfo, G., Sleasman, J.W. & Goodenow, M.M. 2010, "IFNgamma primes macrophages for inflammatory activation by high molecular weight hyaluronan", *Cellular immunology,* vol. 262, no. 2, pp. 84-88.

Wang, A. & Hascall, V.C. 2004, "Hyaluronan structures synthesized by rat mesangial cells in response to hyperglycemia induce monocyte adhesion", *The Journal of biological chemistry,* vol. 279, no. 11, pp. 10279-10285.

Wang, C.T., Lin, Y.T., Chiang, B.L., Lin, Y.H. & Hou, S.M. 2006, "High molecular weight hyaluronic acid down-regulates the gene expression of osteoarthritis-associated cytokines and enzymes in fibroblast-like synoviocytes from patients with early osteoarthritis", *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society,* vol. 14, no. 12, pp. 1237-1247.

Wei, Q., Galbenus, R., Raza, A., Cerny, R.L. & Simpson, M.A. 2009, "Androgen-stimulated UDP-glucose dehydrogenase expression limits prostate androgen availability without impacting hyaluronan levels", *Cancer research,* vol. 69, no. 6, pp. 2332-2339.

Weigel, P.H. & DeAngelis, P.L. 2007, "Hyaluronan synthases: a decade-plus of novel glycosyltransferases", *The Journal of biological chemistry,* vol. 282, no. 51, pp. 36777-36781.

Weigel, P.H., Kyossev, Z. & Torres, L.C. 2006, "Phospholipid dependence and liposome reconstitution of purified hyaluronan synthase", *The Journal of biological chemistry,* vol. 281, no. 48, pp. 36542-36551.

Werkmeister, J.A., Roder, J.C., Curry, C. & Pross, H.F. 1983, "The effect of unphosphorylated and phosphorylated sugar moieties on human and mouse natural killer cell activity: is there selective inhibition at the level of target recognition and lytic acceptor site?", *Cellular immunology,* vol. 80, no. 1, pp. 172-186.

Wice, B.M., Trugnan, G., Pinto, M., Rousset, M., Chevalier, G., Dussaulx, E., Lacroix, B. & Zweibaum, A. 1985, "The intracellular accumulation of UDP-N-acetylhexosamines is concomitant with the inability of human colon cancer cells to differentiate", *The Journal of biological chemistry,* vol. 260, no. 1, pp. 139-146.

Wilkinson, T.S., Bressler, S.L., Evanko, S.P., Braun, K.R. & Wight, T.N. 2006, "Overexpression of hyaluronan synthases alters vascular smooth muscle cell phenotype and promotes monocyte adhesion", *Journal of cellular physiology,* vol. 206, no. 2, pp. 378-385.

Yamada, Y., Itano, N., Hata, K., Ueda, M. & Kimata, K. 2004, "Differential regulation by IL-1beta and EGF of expression of three different hyaluronan synthases in oral mucosal epithelial cells and fibroblasts and dermal fibroblasts: quantitative analysis using real-time RT-PCR", *The Journal of investigative dermatology,* vol. 122, no. 3, pp. 631-639.

Yamawaki, H., Hirohata, S., Miyoshi, T., Takahashi, K., Ogawa, H., Shinohata, R., Demircan, K., Kusachi, S., Yamamoto, K. & Ninomiya, Y. 2009, "Hyaluronan receptors involved in cytokine induction in monocytes", *Glycobiology,* vol. 19, no. 1, pp. 83-92.

Yang, B., Hall, C.L., Yang, B.L., Savani, R.C. & Turley, E.A. 1994, "Identification of a novel heparin binding domain in RHAMM and evidence that it modifies HA mediated locomotion of ras-transformed cells", *Journal of cellular biochemistry,* vol. 56, no. 4, pp. 455-468.

Yasuda, T. 2010, "Hyaluronan inhibits prostaglandin E2 production via CD44 in U937 human macrophages", *The Tohoku journal of experimental medicine,* vol. 220, no. 3, pp. 229-235.

Yevdokimova, N.Y. 2006, "Elevated level of ambient glucose stimulates the synthesis of high-molecular-weight hyaluronic acid by human mesangial cells. The involvement of transforming growth factor beta1 and its activation by thrombospondin-1", *Acta Biochimica Polonica,* vol. 53, no. 2, pp. 383-393.

Yu, Q. & Stamenkovic, I. 1999, "Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion", *Genes & development,* vol. 13, no. 1, pp. 35-48.

Yu, Q. & Stamenkovic, I. 2000, "Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis", *Genes & development,* vol. 14, no. 2, pp. 163-176.

Zhang, X.L., Selbi, W., de la Motte, C., Hascall, V. & Phillips, A.O. 2005, "Bone morphogenic protein-7 inhibits monocyte-stimulated TGF-beta1 generation in renal proximal tubular epithelial cells", *Journal of the American Society of Nephrology : JASN,* vol. 16, no. 1, pp. 79-89.

Zhang, Y., Thant, A.A., Machida, K., Ichigotani, Y., Naito, Y., Hiraiwa, Y., Senga, T., Sohara, Y., Matsuda, S. & Hamaguchi, M. 2002, "Hyaluronan-CD44s signaling regulates matrix metalloproteinase-2 secretion in a human lung carcinoma cell line QG90", *Cancer research,* vol. 62, no. 14, pp. 3962-3965.

Zhao, H., Leu, S.W., Shi, L., Dedaj, R., Zhao, G., Garg, H.G., Shen, L., Lien, E., Fitzgerald, K.A., Shiedlin, A., Shen, H., Quinn, D.A. & Hales, C.A. 2010, "TLR4 is a negative regulator in noninfectious lung inflammation", *Journal of immunology (Baltimore, Md.: 1950),* vol. 184, no. 9, pp. 5308-5314.

Zheng, L., Riehl, T.E. & Stenson, W.F. 2009, "Regulation of colonic epithelial repair in mice by Toll-like receptors and hyaluronic acid", *Gastroenterology,* vol. 137, no. 6, pp. 2041-2051.

Zhou, B., McGary, C.T., Weigel, J.A., Saxena, A. & Weigel, P.H. 2003, "Purification and molecular identification of the human hyaluronan receptor for endocytosis", *Glycobiology,* vol. 13, no. 5, pp. 339-349.

Ågren, U.M., Tammi, R. & Tammi M. 1997, "Reactive oxygen species contribute to epidermal hyaluronan catabolism in human skin organ culture", *Free radical biology and medicine,* vol. 23, no. 7, pp.996-1001.

Tiina Jokela

Regulation of Hyaluronan Synthesis by UDP-sugars

The thesis showed that the rate of hyaluronan (HA) synthesis in different cell types correlated with the cellular content of UDP-GlcNAc and UDP-GlcUA. UDP-GlcNAc controlled HA synthesis as a critical substrate for HA synthases (HAS1-3) but also by regulating HAS expression through O-GlcNAc modification of transcription factors. Mannose was found to inhibit HA synthesis by UDP-GlcNAc depletion. This novel inhibitor blocked HA-dependent monocyte binding to keratinocytes, induced by inflammation, and prevented leukocyte accumulation and growth in wound tissue.

UNIVERSITY OF
EASTERN FINLAND

Publications of the University of Eastern Finland
Dissertations in Health Sciences

ISBN 978-952-61-0625-0