

Why the platelet is inflammatory: a story of hyaluronidases

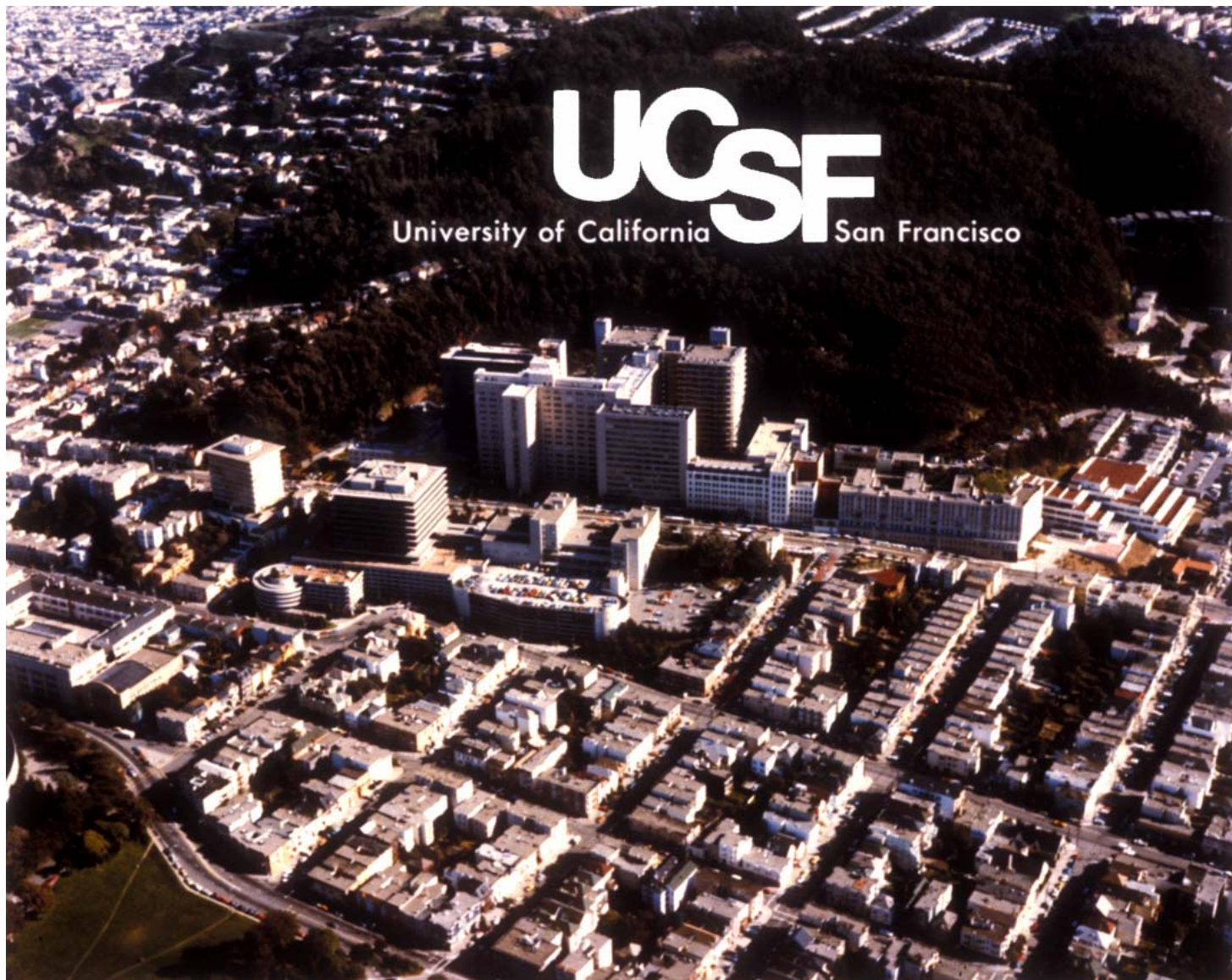
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UCSF

University of California San Francisco



HA

High MW extracellular matrix (ECM)
polymer.

Only non-sulfated GAG.

Only GAG not associated with a
core protein to form a proteoglycan.

Only GAG not synthesized in Golgi.

Hyaluronan (HA)

Straight chain of the repeating disaccharide

$(\beta\text{- N-acetylglucosamine- } \beta\text{- glucuronic acid})_n$

Importance of the beta-linkage

beta-glucose polymer = cellulose

alpha-glucose polymer = glycogen

beta-N-acetyl-glucosamine polymer = chitin

beta polymers provide **structure**

alpha polymers provide **food and energy**

○
globular protein (MW 50,000)

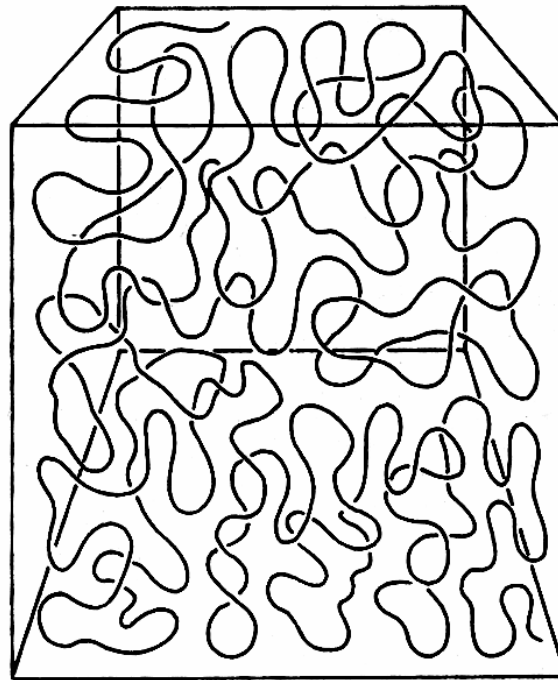


glycogen



spectrin

collagen



hyaluronic acid

← 300 nm →

HA functions:

- 1. hydrates**
- 2. opens tissue spaces for cell movement**
- 3. confers cell motility**
- 4. diminishes cell-cell, cell-matrix interactions**
- 5. promotes morphogenesis,
impedes differentiation**

The cultured myoblast model:

**Cells plated on HA do not undergo
differentiation,
but do so when plated on plastic.**

[UC-eLinks](#)

Hyaluronic acid bonded to cell culture surfaces inhibits the program of myogenesis.

Kujawa MJ, Pechak DG, Fiszman MY, Caplan AI.

Primary isolates of chick leg muscle myoblasts cultured on hyaluronic acid substrates have been examined by transmission electron microscopy for evidence of myoblast fusion and subsequent differentiation. Even though these cells form close contacts, no evidence of multinucleated myotubes is found in these cultures. Two-dimensional SDS-polyacrylamide gel electrophoresis shows that the muscle macromolecular biosynthetic program is not initiated in these hyaluronic acid fusion-blocked cells. Further, these fusion-blocked myoblasts continue replicating while cultured on hyaluronic acid surfaces. The inhibition of both fusion and the myogenic expressional program is reversed by replating these myoblasts onto a denatured collagen (gelatin) substrate; both the synthesis of muscle-specific proteins and the formation of multinucleated myotubes are observed when these subcultured cells are introduced onto gelatin substrates. These observations indicate that the hyaluronic acid inhibition of fusion is not permanent and is manifested in a way different from other fusion blockers in that hyaluronic acid inhibits both fusion and the myogenic expressional program.

PMID: 3943658 [PubMed - indexed for MEDLINE]

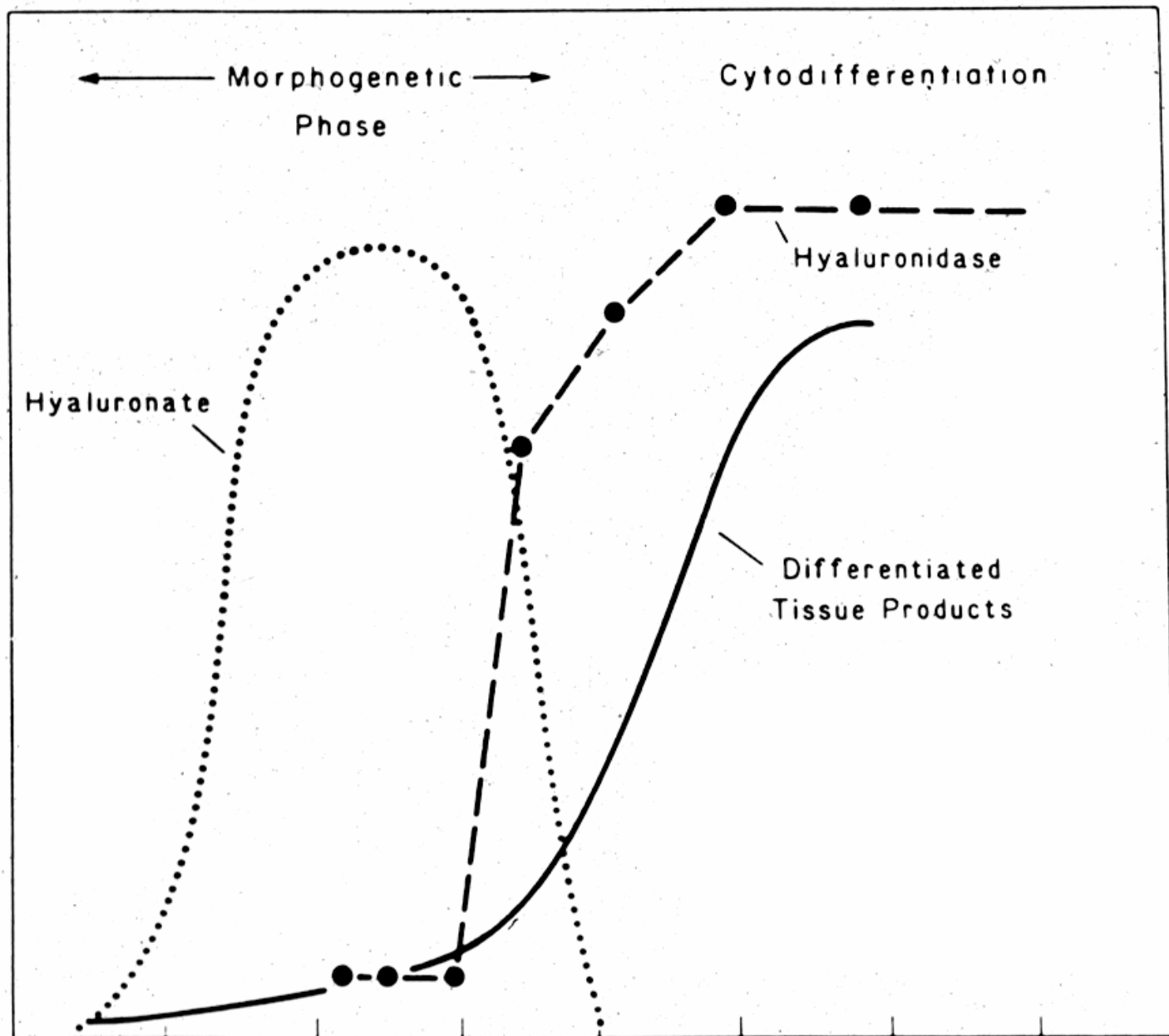


Fig. 1. Correlation of hyaluronate and hyaluronidase levels with morphogenesis and differentiation (adapted from the scheme of Zwilling [1968] for embryonic limb development).

Stem cells are associated with HA

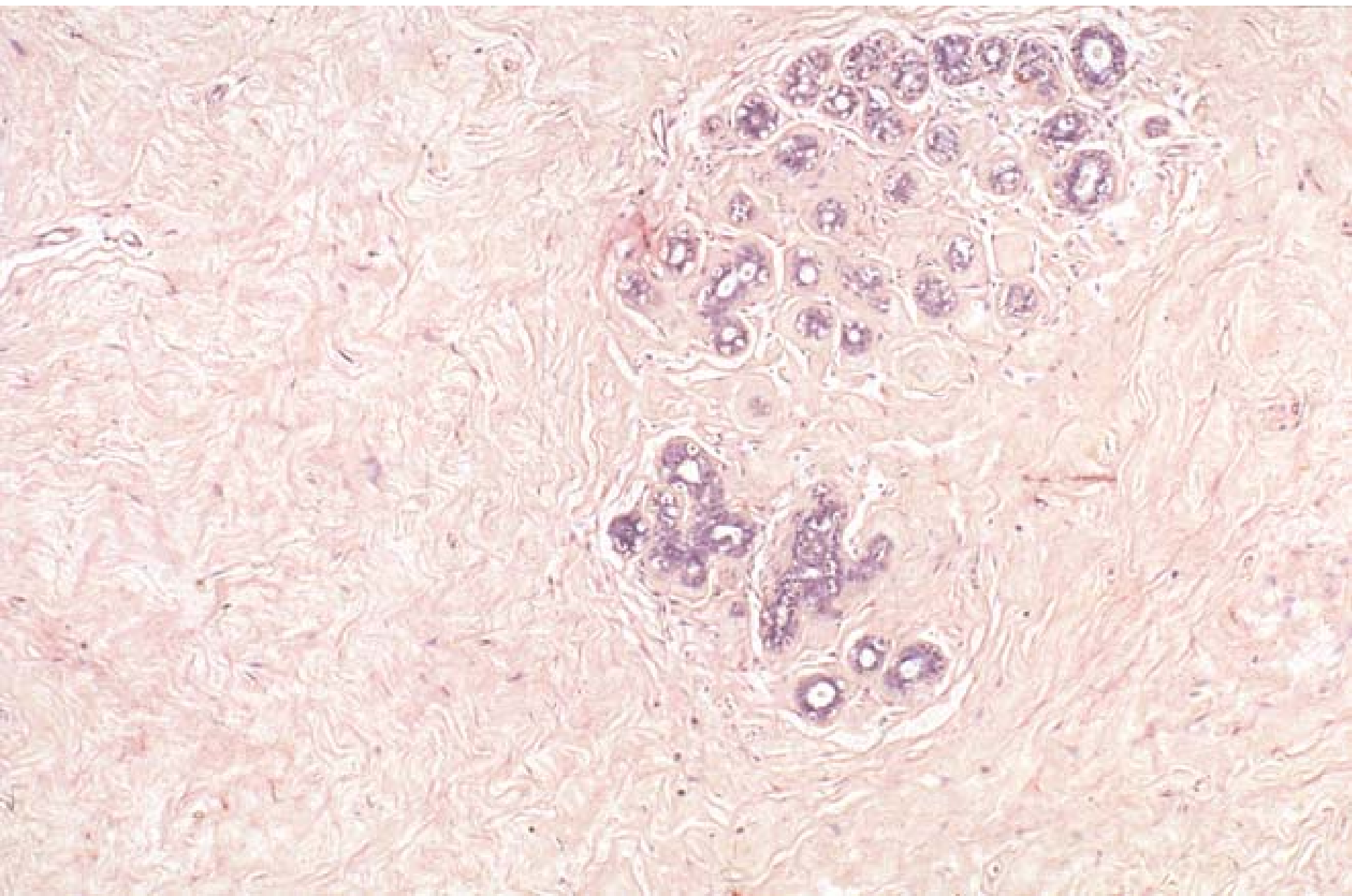
Both normal and cancer stem cells are
contained within an HA-rich niche.

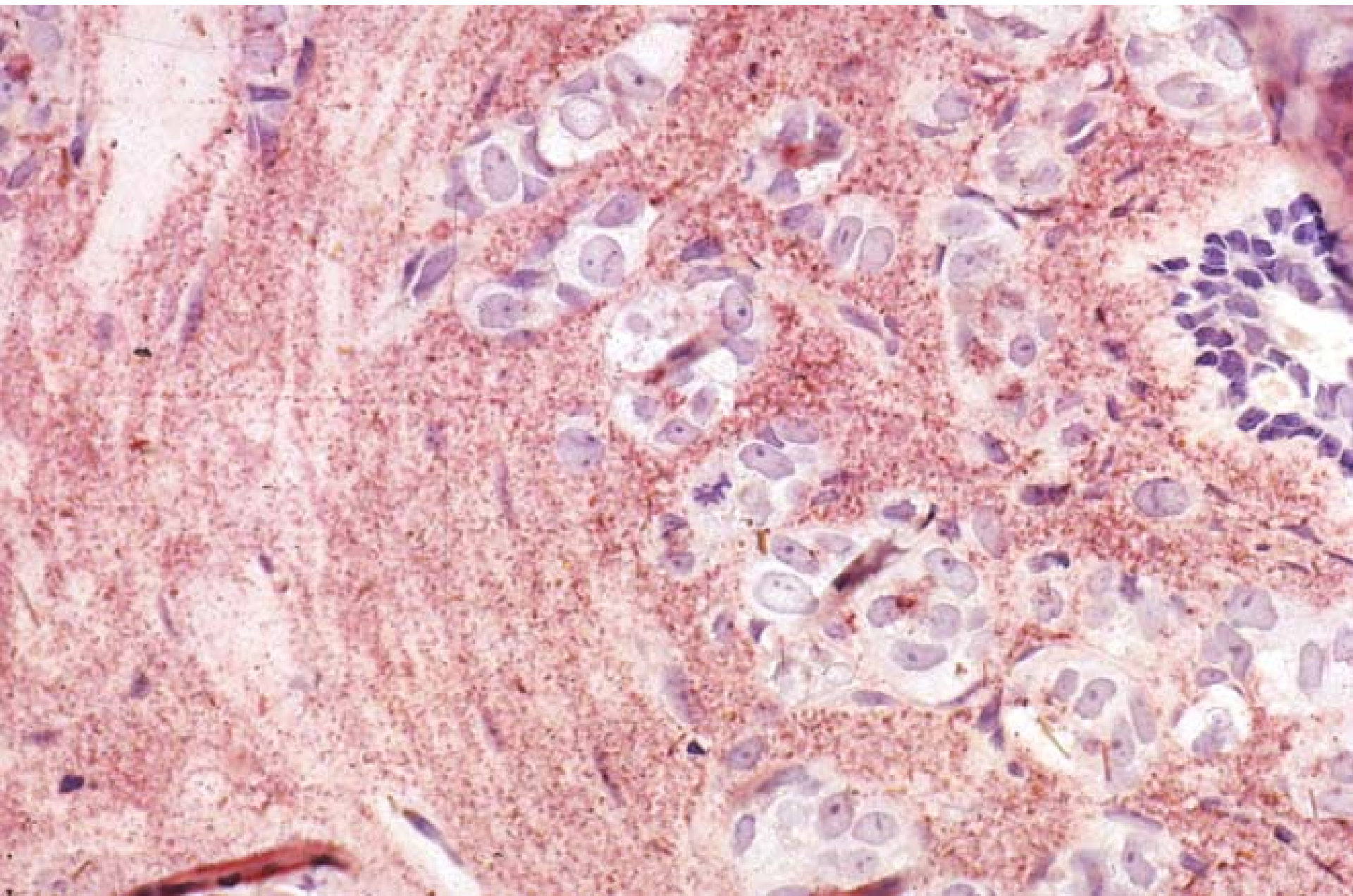
CD44 is the predominant receptor for HA.

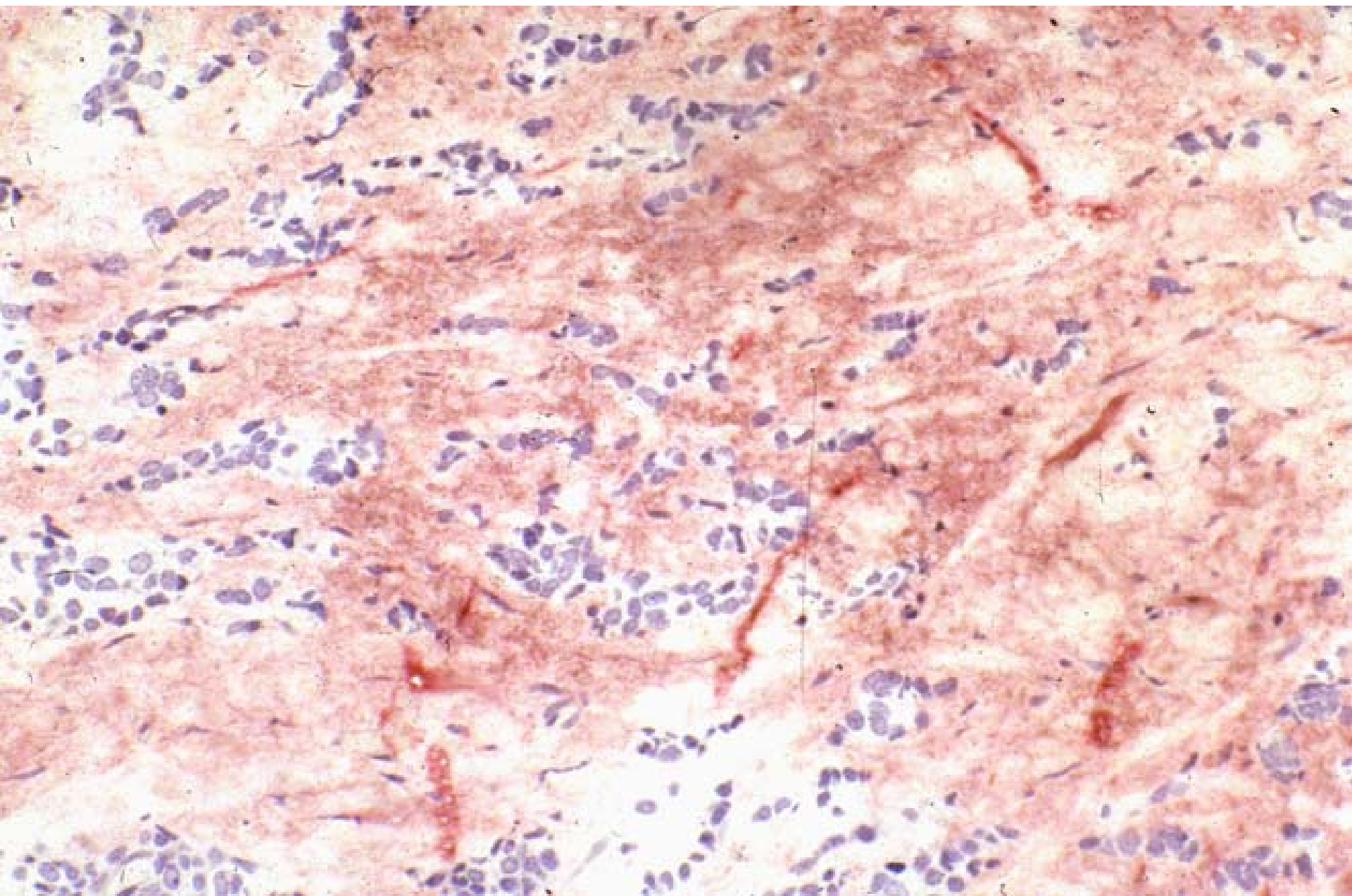
All stem cells to-date express CD44
isoforms on their cell surfaces.

HA and cancer

1. Malignancies are associated with an HA-rich environment.
2. Levels of HA correlate with poor prognosis.







HA has a very high rate of turnover

In the 70 kg individual,
there are 15 g of HA.

5 g turnover daily.

The hyaluronidase enzymes
are responsible for this rapid
HA turnover.

HA is rapidly degraded, but
reforms very quickly

Vertebrate hyaluronidases are difficult to isolate.

They require the constant presence of detergents and protease inhibitors throughout the isolation procedure.

Until recently, the sperm enzyme, PH-20, was the only vertebrate hyaluronidase that had been identified.

These were, until recently, neglected enzymes.

Purification of hyaluronidase from human plasma

Purification Step	Volume (ml)	Protein (mg/ml)	Specific Activity (NFU/mg)	X-fold Purification
Starting Material	2,100	86	0.058	1.0
Detergent Phase	650	1.3	3.62	63
SP-Sepharose	60	0.85	50	875
Hydroxyapatite	1.0	0.0225	86,355	1.5×10^6

HYAL1 (hyaluronidase-1)

55 kDa glycoprotein

49 kDa protein & 6 kDa carbohydrate

ng/ml in blood and urine

Sequencing of HYAL1 peptides

**the amino terminal and
an internal tryptic peptide.**

Sequences were entered into the Gene Bank

Search for:

Human tumor suppressor (LUCA-1) mRNA, complete cds

SEQUENCE INFORMATION

- GenBank entry: [U03056](#)
- Sequence length: 2517 bases
- G+C content: 58 %
- Coding sequence: 617..1924
- Product: [PID_g532974](#); LUCA-1.

SEQUENCE DATA

```
>U03056 Human tumor suppressor (LUCA-1) mRNA, complete cds
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CATTGGATCTTCTCCAGTGGCTGCCAGGATTTCTGGTGAAGAGACAGGAAGGCTCCC
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A new enzyme family

**As a result of Human Genome Project
and the EST (Expressed Sequence
Tags) data banks,**

**it was possible to identify six members of the
hyaluronidase gene family.**

Hyal2_h 1 -----HRAAGPGPTVYALVYAVAW-----AMELKPTAPFIFTGRPFVVAWVDVPTQDCGPRRLK
Hyal2_m 1 -----HRAAGLPITITLALVYEVAV-----AGELKPKPFPFIFTGRPFVVAWVPTQDCGPRRLK
PH20_h 1 -----MGVLKPKHIFRFSVKSSEGVSVQVFTTLLIPC-----CLTNFRAPRPFIVNVALWVAWNPSEFCGLGRFD
PH20_m 1 -----MGELRPFKHLFWGSGFVESGGTFQVTLITLLIPC-----SLTVDRAPRPFILSNNTTLWVWNPSEFCGLGRFD
Hyal1_h 1 -----MAAHLIPICALFLLTL-----LDMAQGGFRGPLLPNRPFTTVWNTQWCLERHG
Hyal1_m 1 MLGLTQHAQKVWRMKPFSPEVSPGSSPATAGHLRLISTLFLTL-----LELAQVCRGSSVSNRPFTTVWNGDTWCLTEYG
Hyal4_h 1 -----MKVLEGGQLKLCVVPQVHLTSWLLIFFILKSISSCLKPARLPVYQKKEPIAAWNPFTDCLLTKYN
Hyal4_m 1 -----MQLLEGGQLRLCVPQVHLTSWLLIFFILKSISSCLKPARLPVYQKKEPIAAWNPFTDCLLTKYN
Hyalp1_h 1 -----MCNFWANLGVLPFLLILL-TQAALKPAMPVVIKSPQENIFWAAWNPFTDCLLTKYN
Hyalp1_m 1 -----MFYQWVTLQGLVFLVLLVAPAAKLPAMPVVIKSDHPPNFWAAWNPFTDCLLTKYN
Hyal3_h 1 -----MTTQLGPAVLGV-----ALCLGQQLLPVGV-----PSSARCAHFG
Hyal3_m 1 -----MIMHLGLHMVGL-----TLCLMHGQALLQVPEHPSVWVPSARCAHFG

Hyal2_h 53 VPLDLNAPDVQASFNEGFFVNQNIETIFYYRRLGLYPRFDSAA-GRSVHGGVPMVSTWAKRMLQKRVEHYRTQESAGLAV
Hyal2_m 53 VPLDLNAPDVQATFNEGFFVNQNIETIFYYRRLGLYPRFDSAA-GRSVHGGVPMVSTWAKRMLQKRVEHYRTQESAGLAV
PH20_h 66 EPLDMSLFSFIGSPRINATGGQVTFYVDRGLGYPIYDISITCVTVNGGIPQKISLQDHLDKAKKIDITYM-PVDNGLAV
66 DPDLDSFSLIGSPRKTATGGQVTFYVDRGLGYPIYDISITCVTVNGGIPQKISLQDHLDKAKKIDITYM-PDDKGLAV
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77 VDDVDSVFDVVANKEQSFQGSNNMTIFYYRRLGLYPRFDSAA-GRSVHGGVPMVSTWAKRMLQKRVEHYRTQESAGLAV
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Hyal3_h 48 VHLPLNALGITANRGQHFHGGNNMTIFYYRRLGLYPRFDSAA-GRSVHGGVPMVSTWAKRMLQKRVEHYRTQESAGLAV
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Hyal2_m 362 QYCSRAQCHGHC--CVPGNPSASTLHL--STNSFRFLVPGHAPGEPQLRPVGLSWADIDHLQTHFRCCYQLWSGEGCQ
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Hyal2_m 439 RNYKGAAGSHLTSLLALAALFTWTTL-----
PH20_h 448 DVKDTDAVDVCIADGVCIADLPKP-F--METEPQIFYNASPS---TLSATMFIVSILFLIISSVASL
PH20_m 448 DVKNVQDVNVVCGDNNVICAKVEPNFAFYLLPGKSLFMTTLGHVLYHLPQDIFVFPKRLTVSTP----
Hyal1_h 431 RKSMM-----
Hyal1_m 459 KRGM-----
Hyal4_h 448 EIKTADGCGSGVSPSPGSLMTLCLLLLASYSIQL-----
Hyal4_m 448 EMTASGSPGSLSSSSSVITLCLLLLAGYQSIQL-----
Hyalp1_h 437 D-HSSDLLRVNNKAPTINFLNLLVFLIMASSVILKKILALTTMPIFS-----
Hyalp1_m 439 S-HSPNLQK--NKAPASGLNSAVIVGMALFVILMN---YFPYIYNGNFSLKPLKRRREIFL-----
Hyal3_h -----
Hyal3_m -----



The hyaluronidase gene family

Chromosomal Orientation OF Hyaluronidase Genes



Hyaluronidase Genes and their gene products:

	<u>Gene</u>	<u>Protein</u>
3p21.3	HYAL1	Hyal-1
	HYAL2	Hyal-2
	HYAL3	Hyal-3
7q31.3	HYAL4	Hyal-4
	SPAM1	PH-20
	HYALP1	None

The hyaluronidase gene family

Each member of this gene family is transcriptionally active, and has a unique profile of tissue expression.

Sequences of human plasma hyaluronidase and the mouse ortholog

There is 80% identity between the two sequences, even though divergence between man and mouse occurred 80 million years ago.

Human	1	-----MAAHLLPICAFLLTLLDAAQGF	RGPIIPNRPF	TTVWNANTQWCLE								
Mouse	1	MKPFSPPEVSPGSSPATAGHLLRISTLFLTLLEAAQVC	RGSVVSNRPF	ITVWNAGDTHWCLT								
Human	46	RHGVDVDVSVFDVVANPGQTFRGPD	MTIFYSSQLGTYPYYTPTGEPVFGGLPQNASL	IAH								
Mouse	61	EYGVVDVDVSVFDVVANKEQSFQGSN	MTIFYREELGTYPYYTPTGEPVFGGLPQNASL	VTH								
Human	106	LARTFQDILEAAAPAPDFSGLAVIDWEAWRPRWAFNWD	AKDIYRQRSRA	LVQAQHPDWPAP								
Mouse	121	LARTFQDIKAAAPPEPDFSGLAVIDWEAWRPRWAFNWD	SKDIYRQRSME	LVQAQHPDWPET								
Human	166	QVEAVAQDQFQGAARAWHAGTLQLGRA	LRPRGLWGYGFPDCYN	YDFLSPNYTGQCPSGT								
Mouse	181	LVEAAAKNQFQGAARAWHAGTLQLGQV	LRPRGLWGYGFPDCYN	NDFLSLNYTRQCPVFP								
Human	226	RAQNDQLGWLWGQS	RALYPSIYMPAVLEGTGKSQHYVQHRVA	EAFRVAVAAGDPNLPVLP								
Mouse	241	RDQNDQLGWLWNQS	YALYPSIYLPAAALMGTEKSQMYVRHRVQ	EALRVAIVSRDPHYVPMP								
Human	286	YVQIFYT	TNHF	LPLQ	ELEHSLGESAAQGAAGV	VLWVSWENT	RTKES	COAIKEYMD	TLG			
Mouse	301	YVQIFY	MTDYL	LPLQ	ELEHSLGESAAQGVAG	AVLWLSS	DKTST	KES	COAIKAYMD	STLG		
Human	346	PFINVTSCALLCSQA	LC	SGHGRCVRE	TSH	PKALLL	LN	PASFSI	QLTP	CG	PLSL	EGALS
Mouse	361	PFINVTSCALLCSQA	LC	SGHGRCVRE	PSY	PEALLT	LN	PASFSI	ELTH	DGR	PPSL	NGTLS
Human	406	LEDOAQMAEF	CRCY	PGWQAP	WCERKS	MW						
Mouse	421	LKDRAQMAKF	CRCY	RGWRG	KWCD	KRCM-						

Human HYAL1 and *C. elegans* Hyaluronidase

HYAL1	1	...MAGH	LLPICALP	LTLLDM	AOGP	.RG	PLV	PNRP	PFTT	VWN	ANTQW	CLERHGV	DVDVSV
<i>C.elegans</i>	1	MVIVWYHQ	LLLVLLIF	FIGAAK	AGYIGS	GA	SQ	PNRT	.DV	VWM	VP	SWTC	KNEYSIDVE..K
HYAL1	56	FDV	VANPG	OTERG	.PD	MT	IFY	SSQL	GTYP	PYYT	PTGE	..PV	F
<i>C.elegans</i>	58	YGILQ	NED	QHPV	G	GKQFA	IPY	EH	S	F	GKI	PY	FKAQNESD
HYAL1	113	TLAA	IPAP	DFSG	DAVID	WEA	WR	R	WAFN	W	DTK	DIY	RQRS
<i>C.elegans</i>	118	INET	IPDEN	PNGT	DAVIDI	IEE	PR	M	WEL	S	WG	P	FSVYKTESIR
HYAL1	173	DQFQ	GA	AARAW	MAGT	TLQ	LG	AL	R	R	RGL	W	G
<i>C.elegans</i>	178	RDY	EKA	ACQ	K	PF	ETL	R	LG	K	R	L	R
HYAL1	233	GWL	WGQ	S	R	AL	Y	P	S	I	Y	M	.PAV
<i>C.elegans</i>	236	HWL	WG	E	S	T	A	L	F	P	S	I	Y
HYAL1	292	D...	T	NH	F	L	P	L	D	E	L	E	H
<i>C.elegans</i>	295	NPYY	T	P	D	D	E	F	Y	S	K	Q	N
HYAL1	349	LNVT	SG	ALL	C	S	Q	A	L	C	S	G	H
<i>C.elegans</i>	353	QLTD	R	N	L	D	K	C	R	M	E	R	C
HYAL1	409	QAQ	MA	V	E	F	K	C	R	C	Y	P	G
<i>C.elegans</i>	387	F	A	C	R	C	E	R	P	Y	F	G
HYAL1												
<i>C.elegans</i>	440	APNQ	F	Y	S	R	T	G	G	D	I	K	L

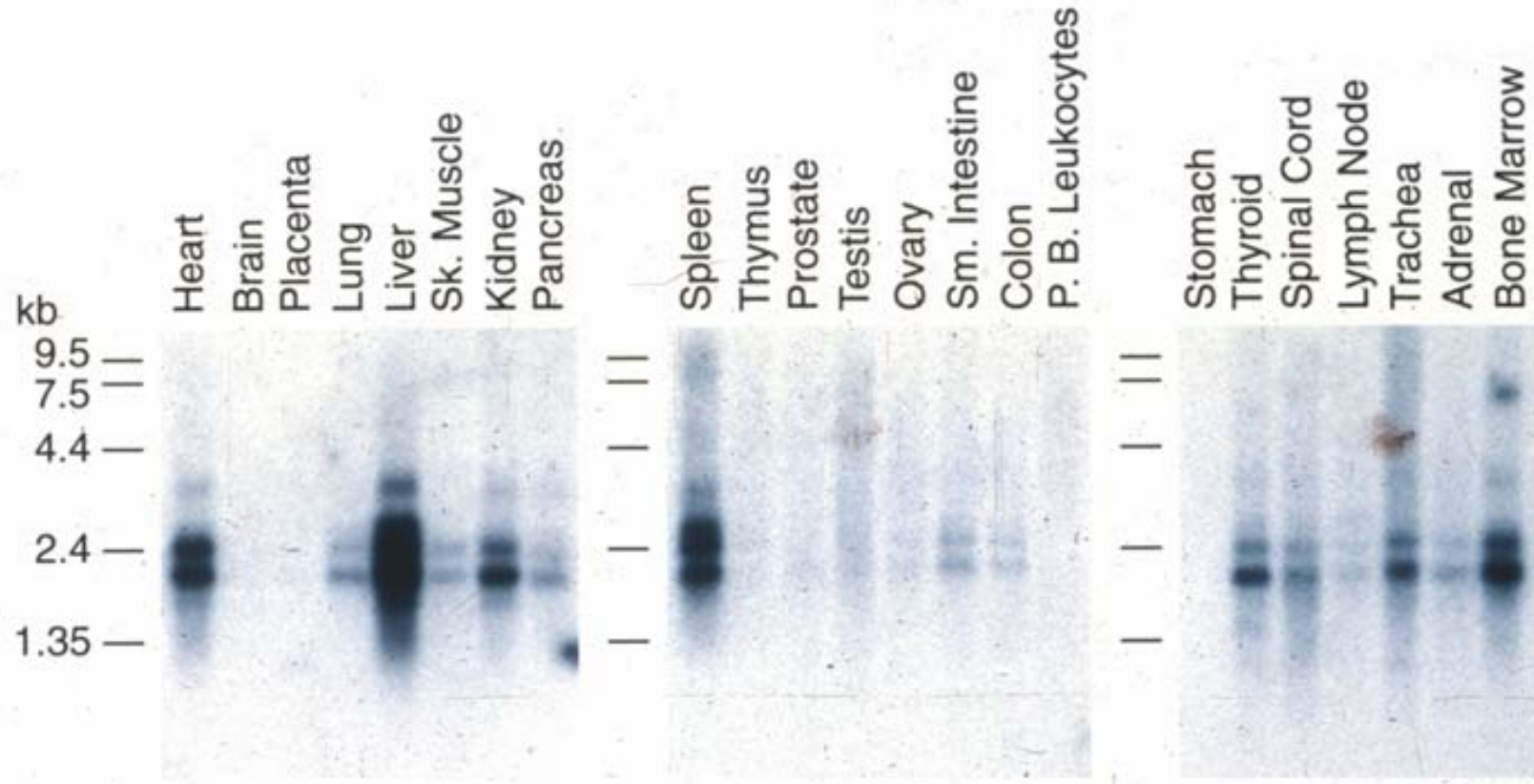
HYAL1 and HYAL2

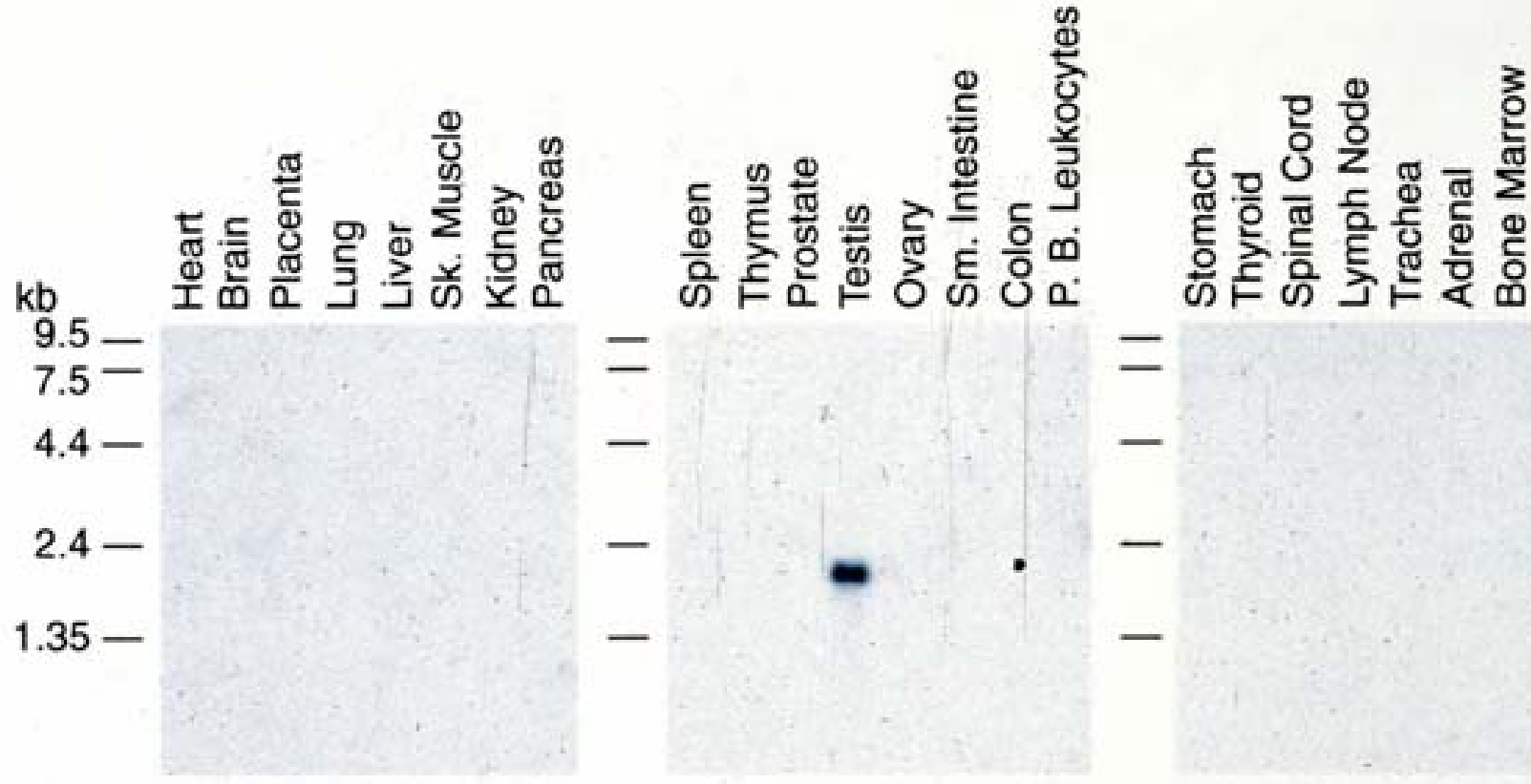
These are the predominant hyaluronidases in the catabolism of HA in vertebrate tissues, and responsible for rapid HA turnover.

Hyaluronidase-1 (HYAL1)

HYAL1, the only active
hyaluronidase in blood and urine.

It is a candidate tumor suppressor
gene product (LuCa-1).





SPAM1
(PH-20)

Activity of HYAL1

This enzyme degrades HA down to tetrasaccharide fragments.

hyaluronidase-2

HYAL2

HYAL2 is GPI-linked on the surface of plasma membranes

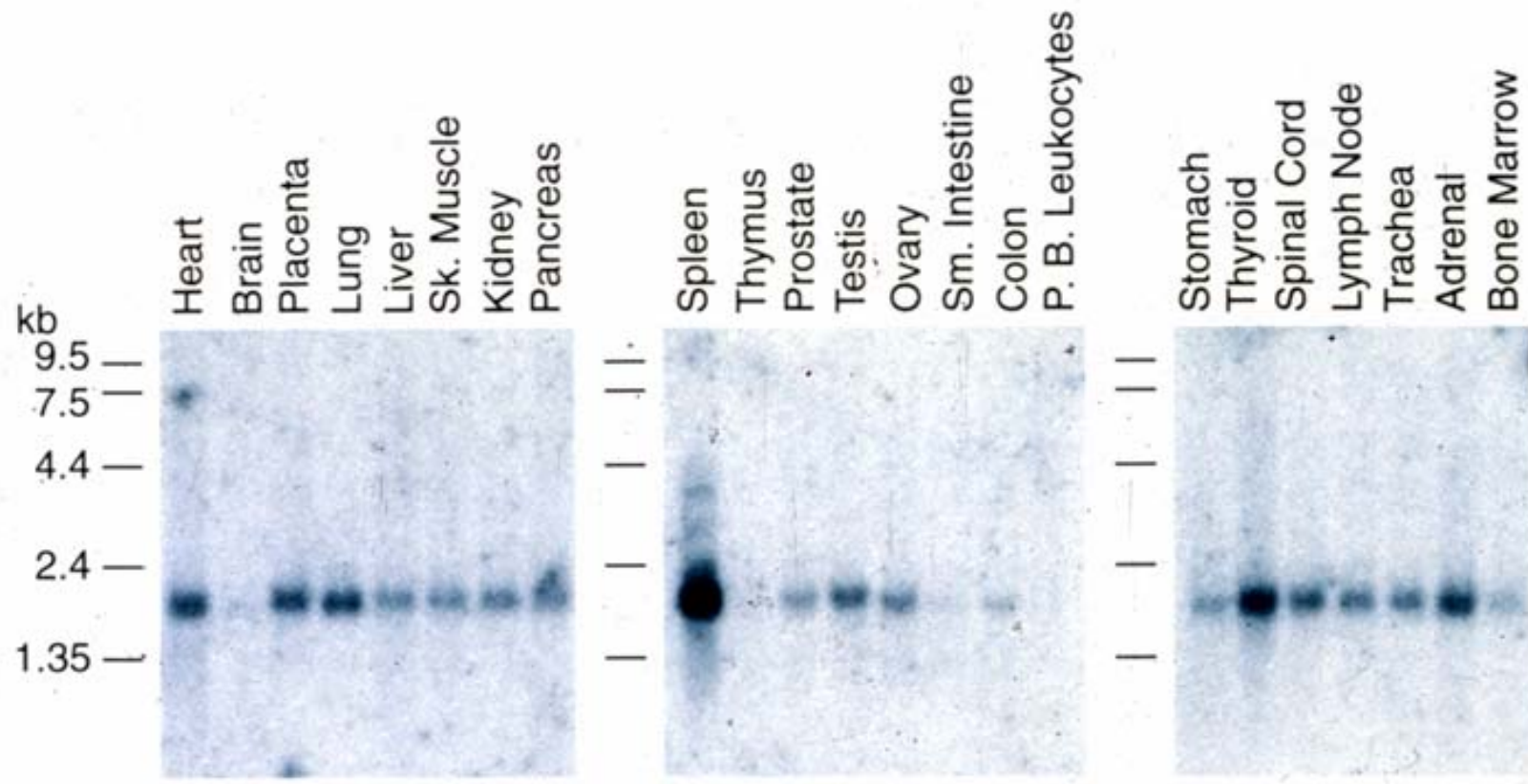
GPI- = glycosylphosphatidyl-inositol

GPI-linked hyaluronidases

HYAL2

HYAL4

PH-20



HYAL2

HYAL2 has size-restricted reaction products

HYAL2 cuts high MW HA to about
20 kDa
50 disaccharides
100 sugars

Substrate-specificity of HYAL2

Both HYAL1 and HYAL2 degrade high MW HA,
but HYAL2 slows considerably with HA chains
smaller than 50 disaccharides.

This is a matter of conformation of the HA
substrate. Chains smaller than 50 disaccharides are
unable to take on a 3 D conformation.

Size-specific functions of HA chains (a new concept)

1. Hi MW HA chains reflect healthy tissues, are anti-angiogenic, anti-inflammatory, immunosuppressant
2. The intermediate-sized chains are highly angiogenic, very inflammatory and immuno-stimulatory
3. Tetrasaccharides suppress apoptosis and induce production of heat shock proteins.

A new concept:

Various sizes of HA chains occur in the vertebrate body.

Each size appears to have a different biological function.



Available online at www.sciencedirect.com



European Journal of Cell Biology 85 (2006) 699–715

European Journal of Cell Biology

www.elsevier.de/ejcb

Hyaluronan fragments: An information-rich system

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Size-specific functions of HA

High MW HA reflects intact healthy tissues.

Intermediate size HA chains are a response to stress. (HYAL2 products)

The smallest fragments ameliorate the stress response
(HYAL1 products)

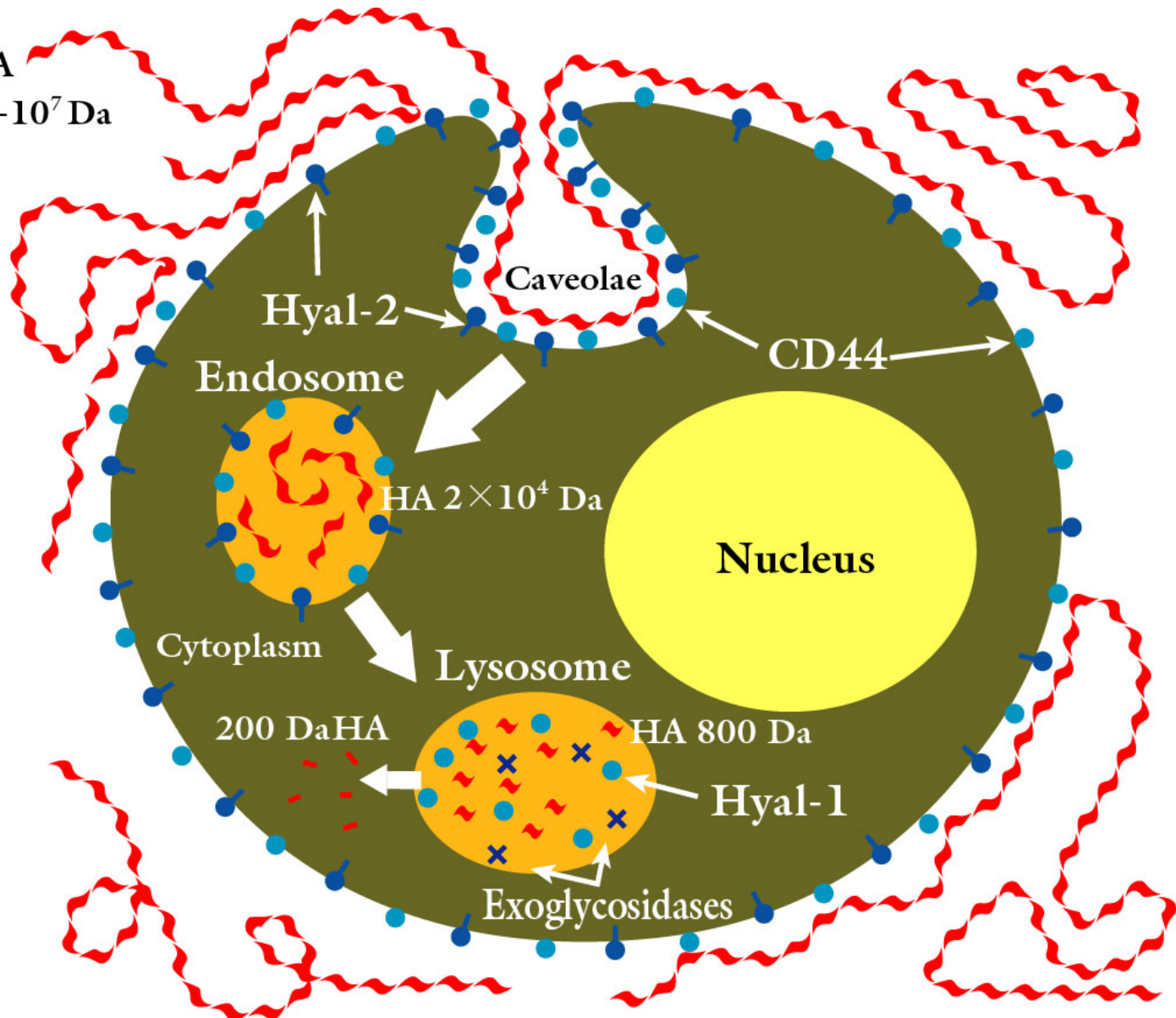
New catabolic scheme

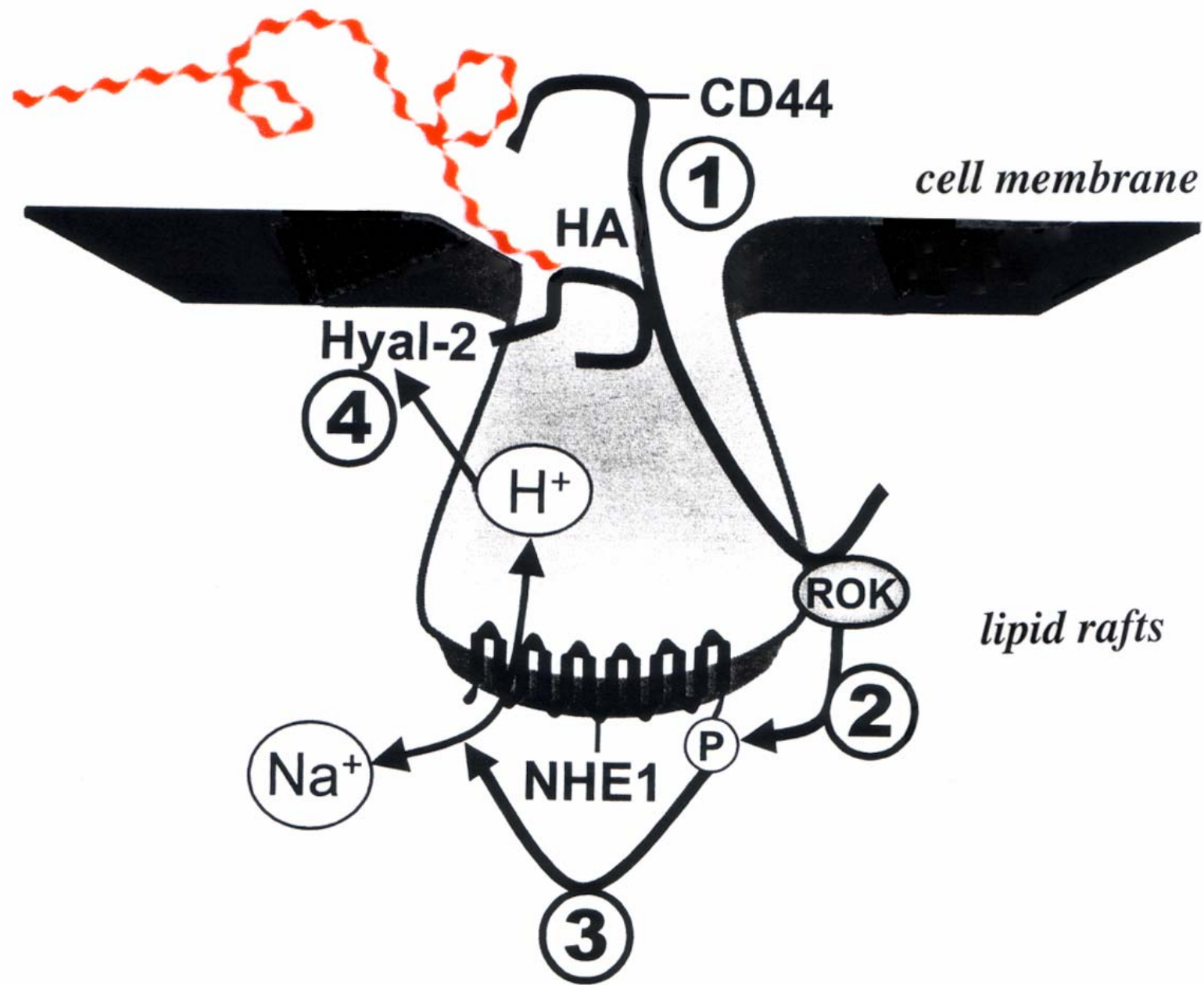
The enzymatic and chemical
catabolism of HA
in vertebrate tissues.

Degradation occurs in quantum
steps, with different size HA
chains generated at each step.

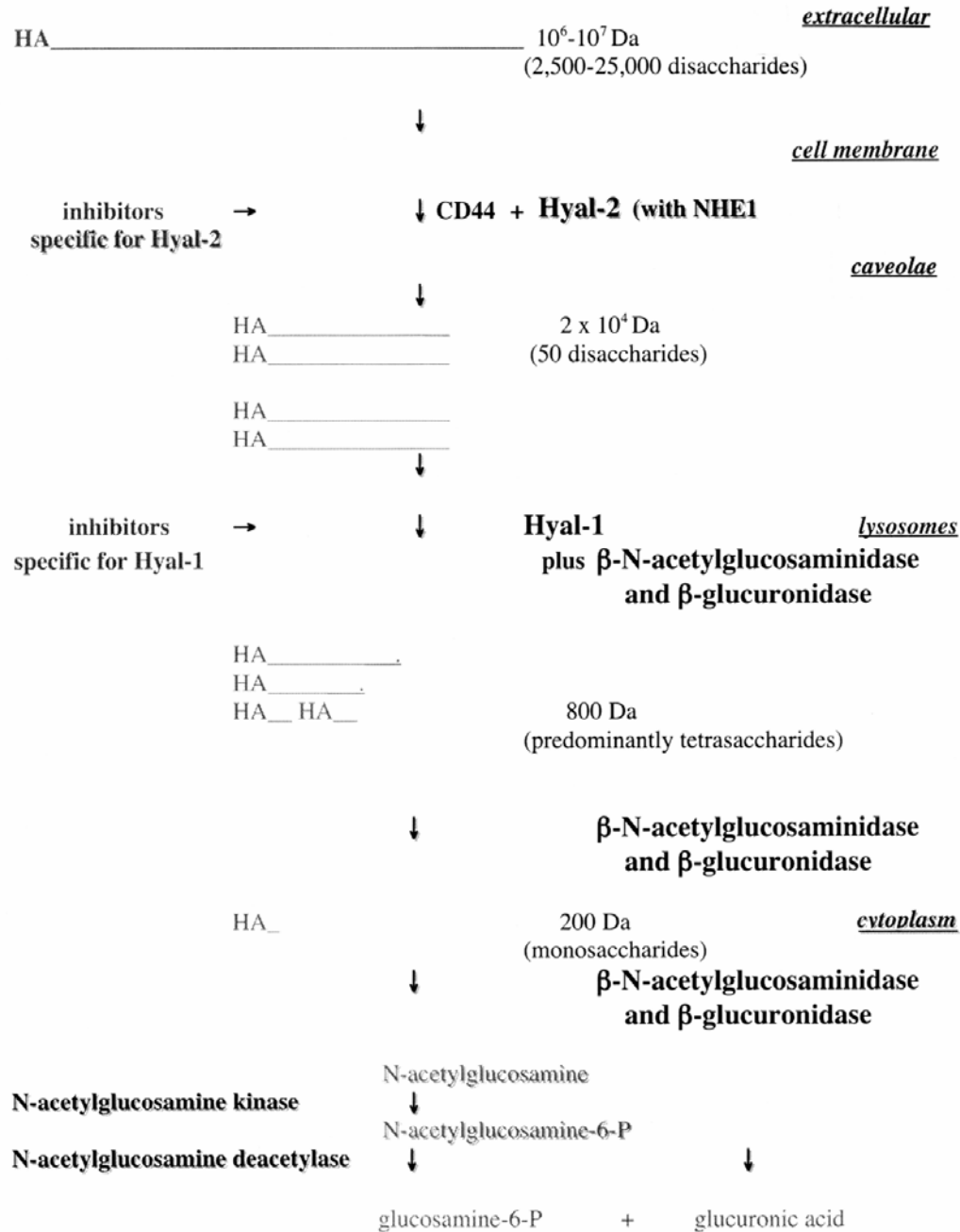
Catabolic Scheme for HA

HA
 10^6 - 10^7 Da





SCHEME FOR HYALURONAN CATABOLISM





Eur. J. Cell Biol. 83 (2004) 317–325
<http://www.elsevier.com/locate/ejcb>



Hyaluronan catabolism: a new metabolic pathway

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San Francisco, San Francisco, CA, USA

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Accepted June 1, 2004

Examination of platelets:

A new study involving
hyaluronidases and HA in
platelet preparations

with **Carol de la Motte** of the Cleveland Clinic

Degradation of HA in somatic tissues

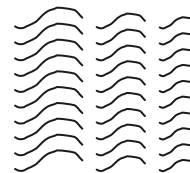
Enzyme:

HYAL2

HYAL1



HA



kDa
sugars

10,000
~50,000

~20
~100

0.8
4

Degradation of HA by platelets

HYAL2 enzyme only

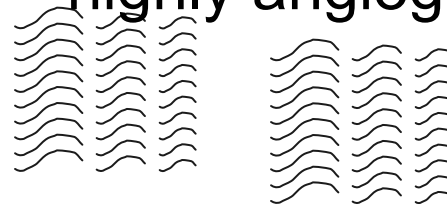
HA high MW → intermediate size

Anti-inflammatory
Anti-angiogenic

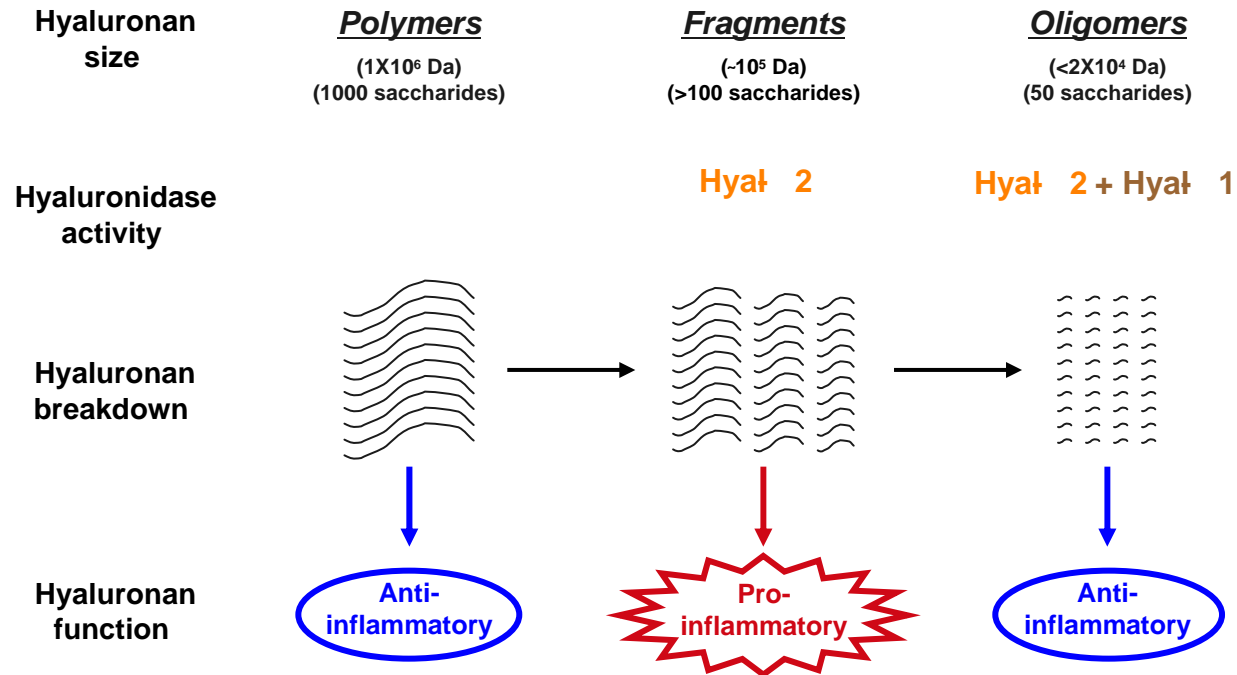


10,000 kDa
50,000 sugars

highly inflammatory
highly angiogenic



~ 20 kDa
~100 sugars



The anti- or pro-inflammatory functions of HA are related to chain size. High MW polymer is degraded by Hyal2 enzyme to a size that is very inflammatory and angiogenic, and is further degraded by Hyal1 to tetrasaccharides.

Platelet-rich clots contains HYAL2

The platelet-rich clot, obtained both *in vitro* and *in vivo*, contains HYAL2 (red) as well as platelet-specific CD42b, the von Willebrand receptor (green). These overlap (yellow).

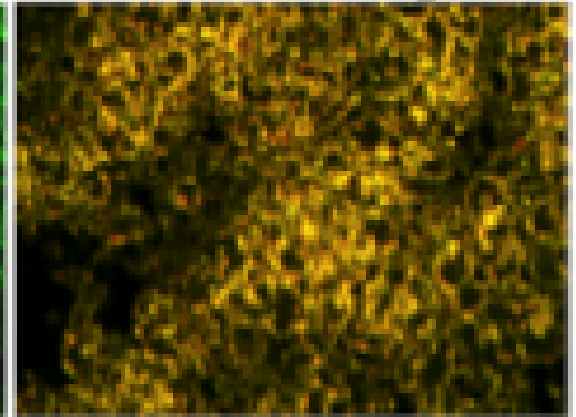
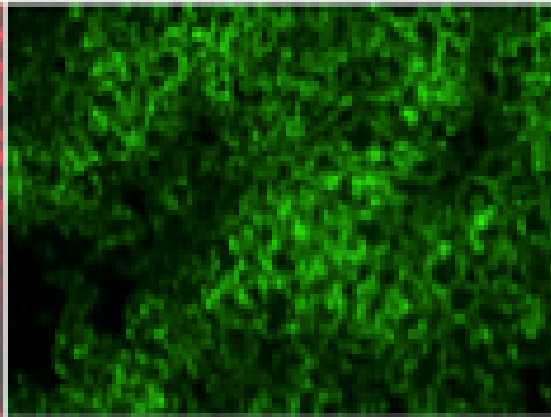
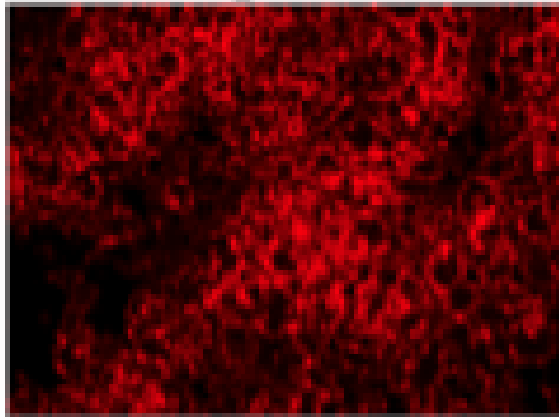
Platelets in a clot

Hyal2

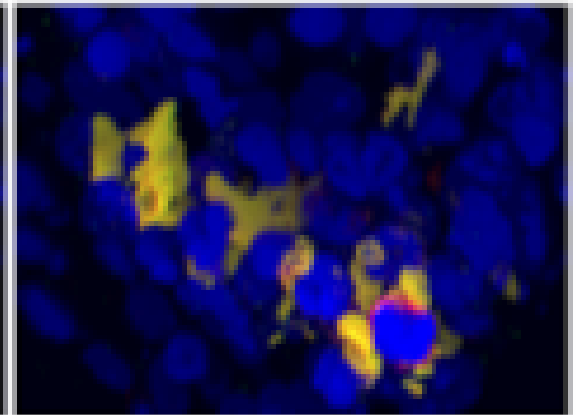
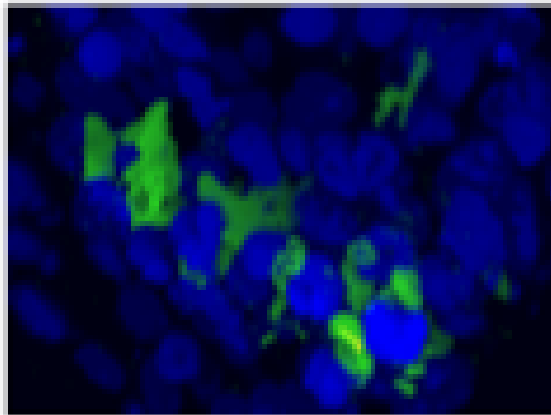
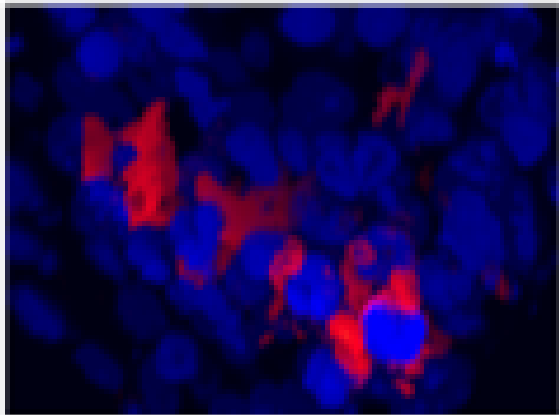
CD42b

overlay

in vitro



in vivo



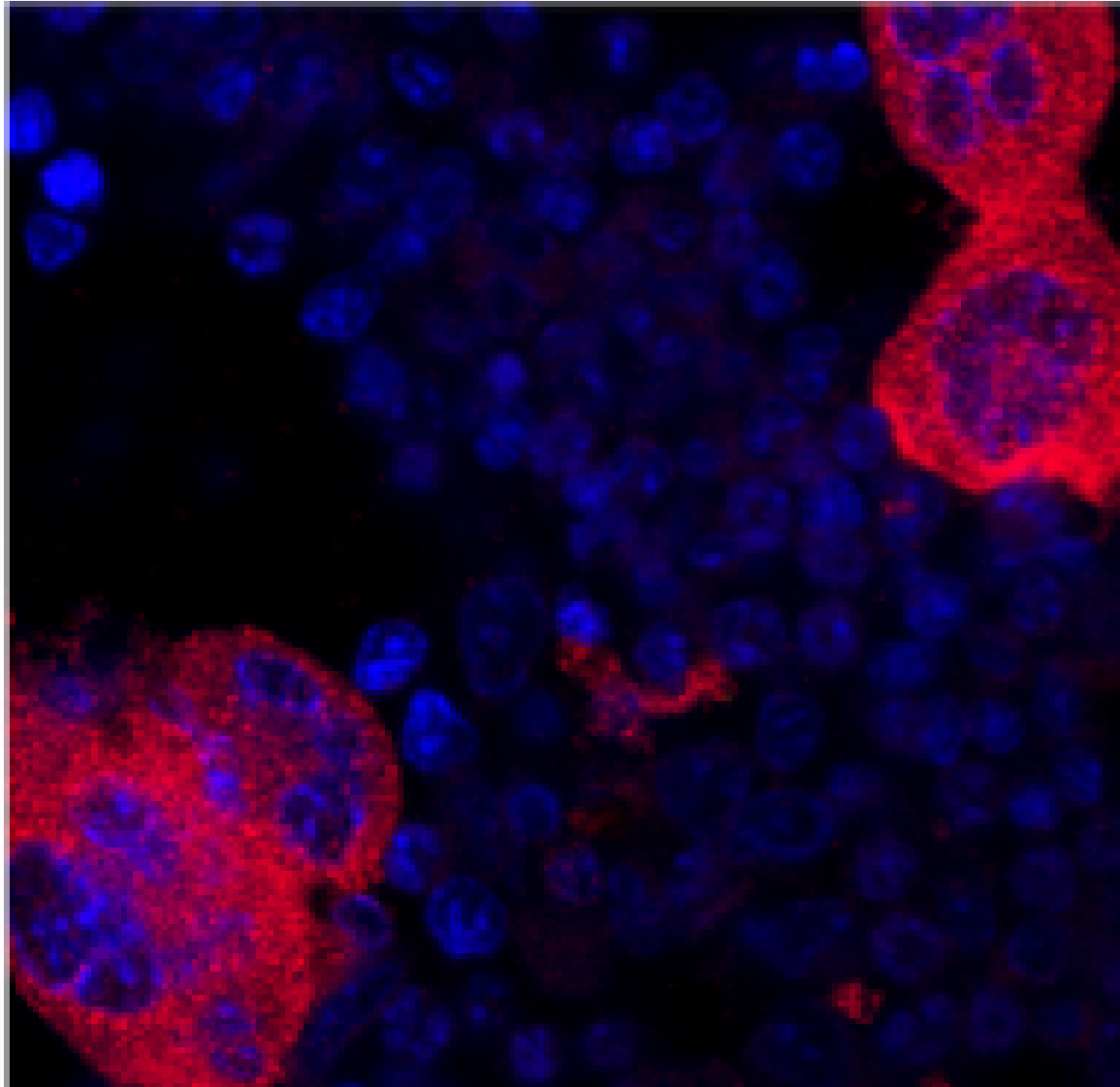
Megakaryocytes also contain HYAL2

HYAL2 (red) in cytoplasm of
megakaryocytes.

Other cells of bone marrow are negative.

Nuclei stain blue (DAPI).

Bone Marrow Megakaryocytes

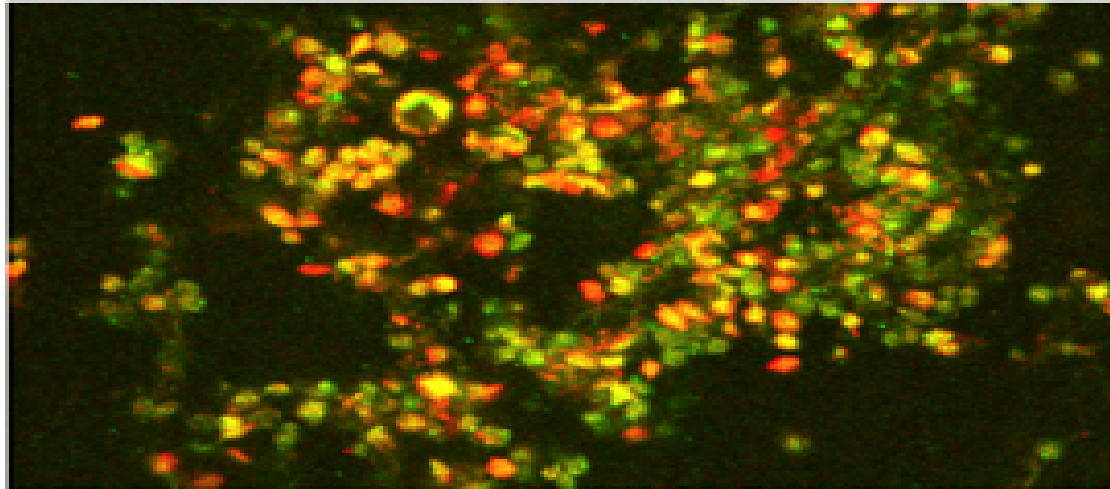


Platelets and megakaryocytes also contain HA !!

This has never been shown previously.

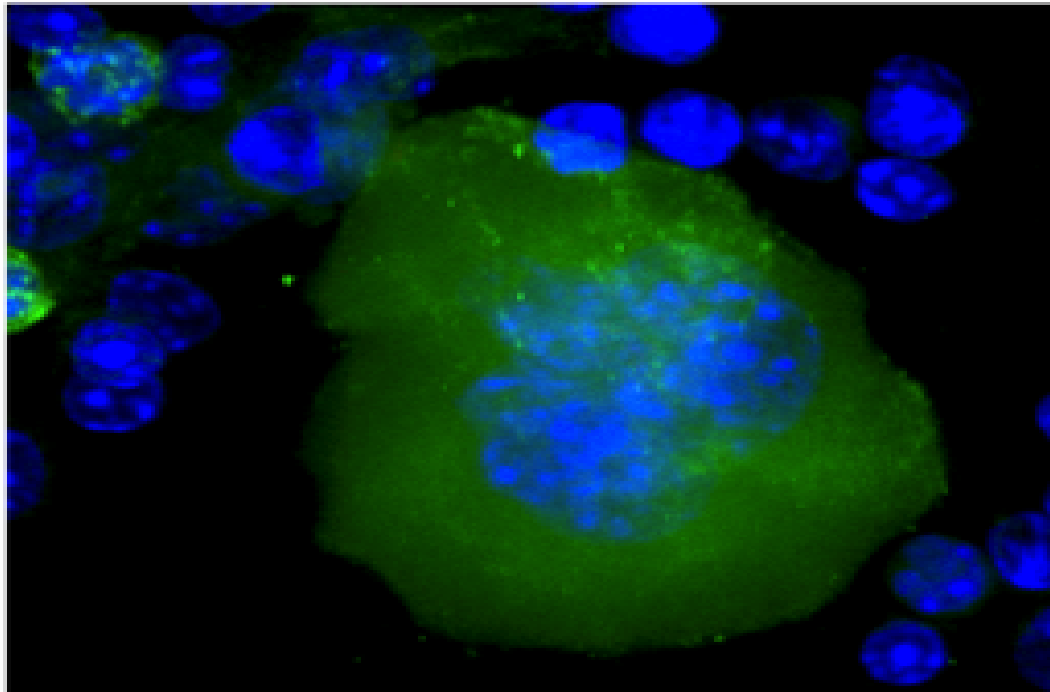
1. Platelets contain both HA and HYAL2.
2. Megakaryocytes may be “mega” cells because of the HA they contain, and the enormous solvent volume or water-of-hydration.

Hyaluronan and Hyal2 in platelets



A

Hyaluronan in megakaryocytes

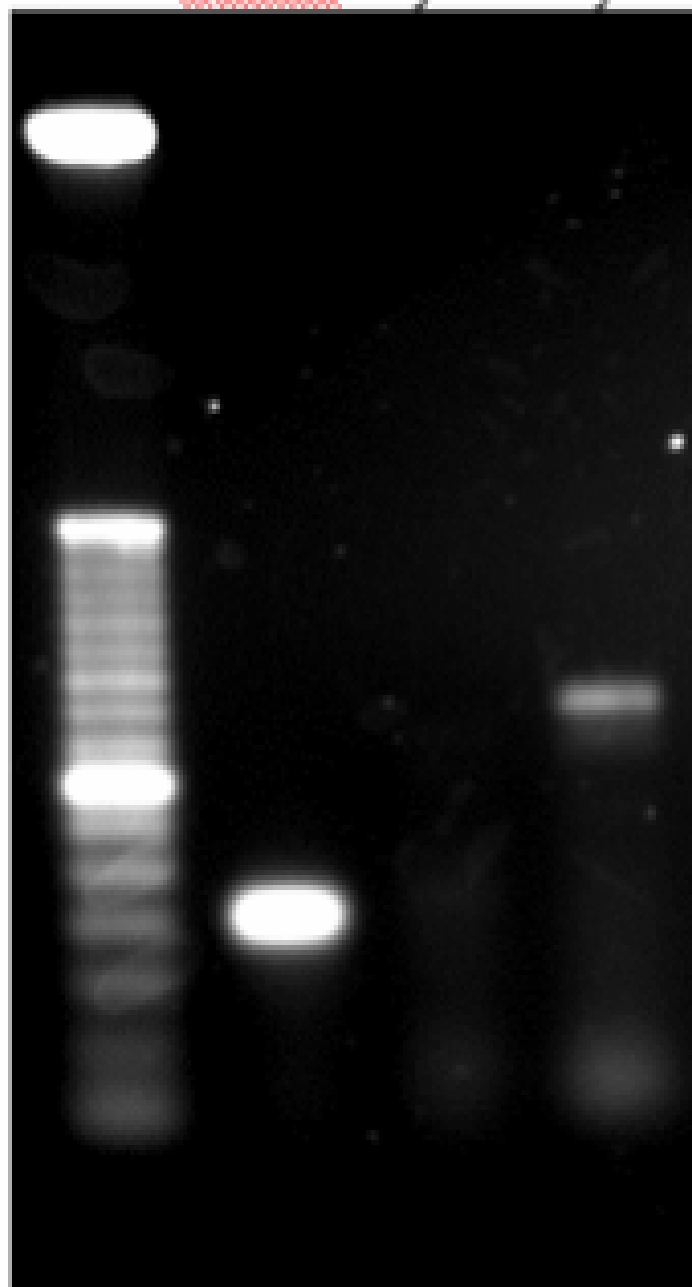


B

RT-PCR study of HYAL mRNAs in platelets

Platelet preparations contain HYAL2 mRNA with no evidence for presence of HYAL1.

Std b-actin Hyal1 Hyal2



Expected Products

← 446bp-Hyal2

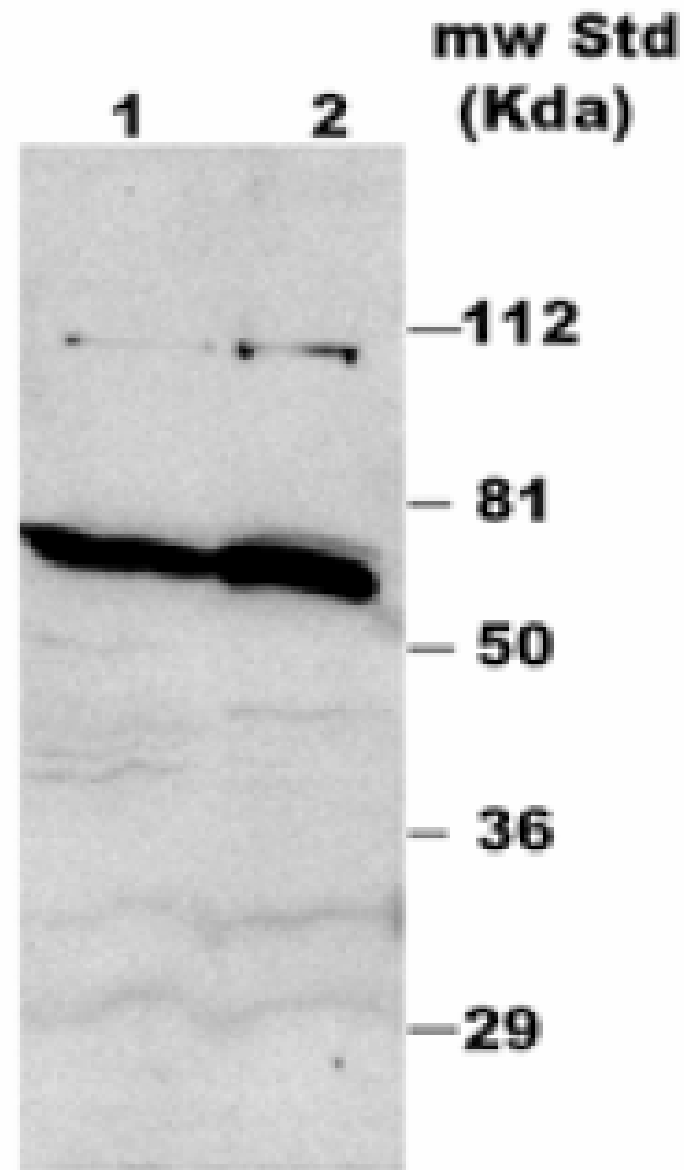
← 400bp-Hyal1

← 204bp-b-actin

Western blot analyses of Hyals in platelets

Platelets contain Hyal2 protein, with no
evidence for Hyal1.

Platelet Hyal2 protein



Incubation of hmw HA
with platelets
generates signaling sized
inflammatory fragments

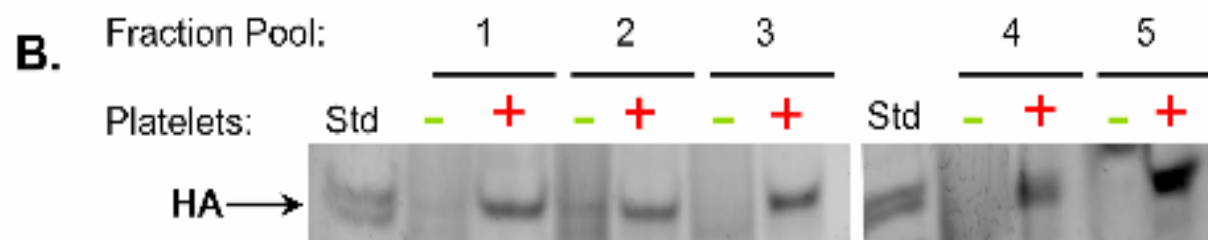
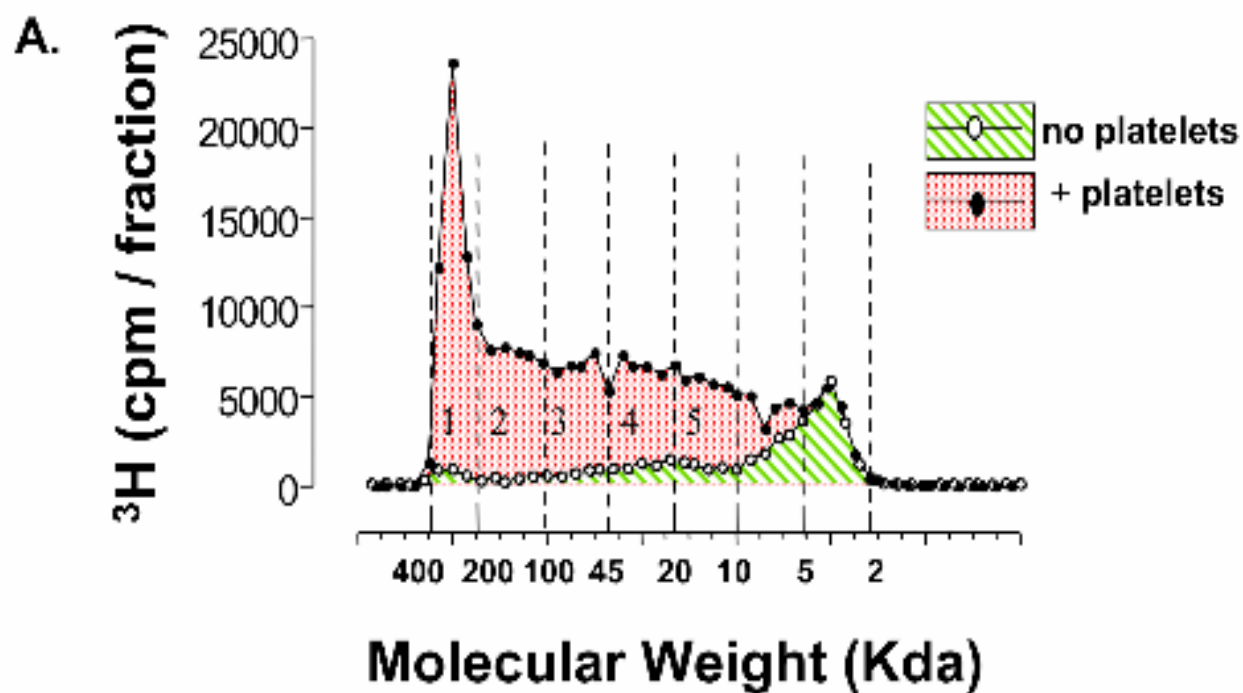


Figure 8

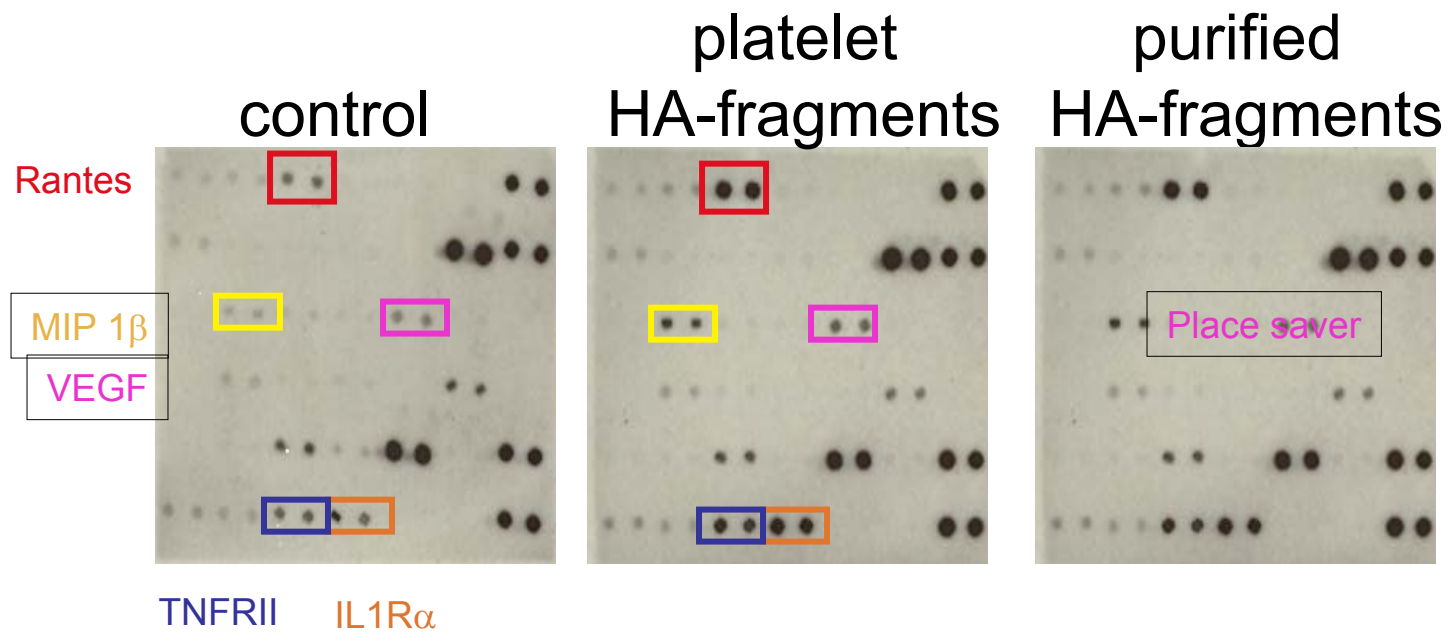


Figure 9

Inflammatory cytokines

Rantes: Chemotactic cytokine for memory T cells and eosinophils, member of 1L8 super family

MIP 1 β : Macrophage inflammatory protein 1 beta, member of the C-C subfamily of chemokines

VEGF: Vascular endothelial growth factor, of PDGF family

TNFRII: Tumor necrosis factor receptor II

IL1R α : Interleukin 1 receptor alpha

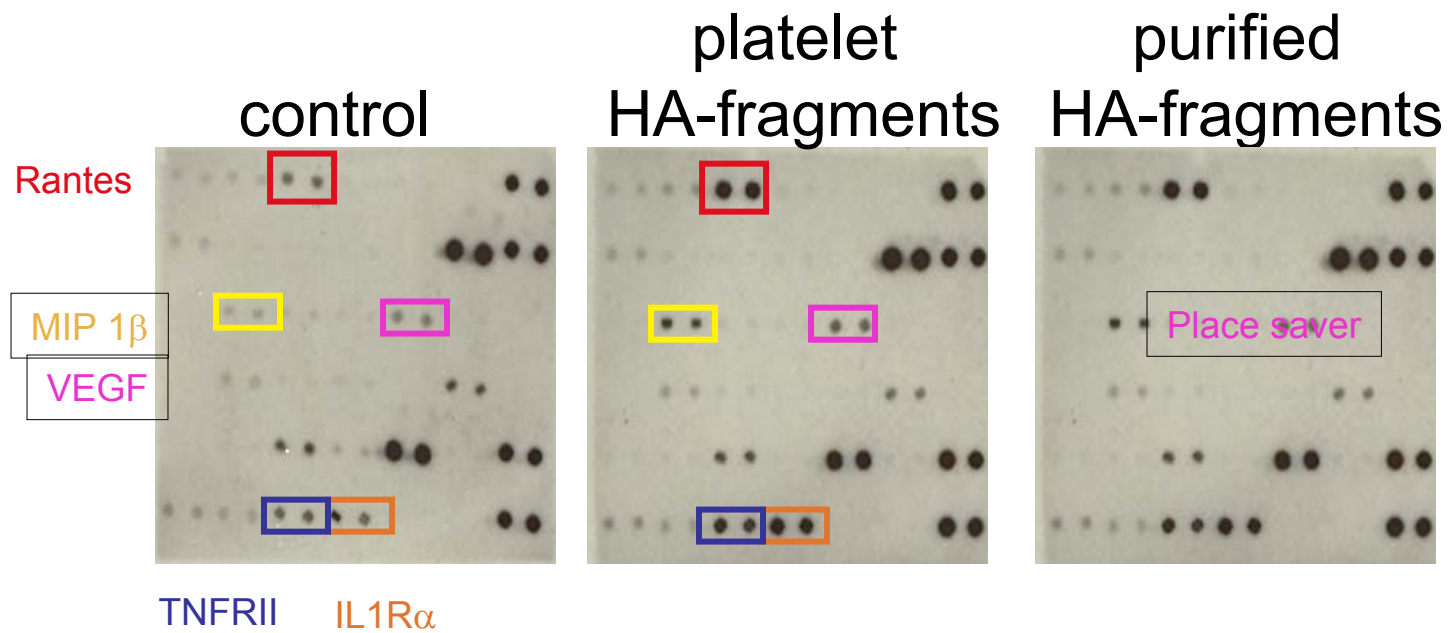


Figure 9

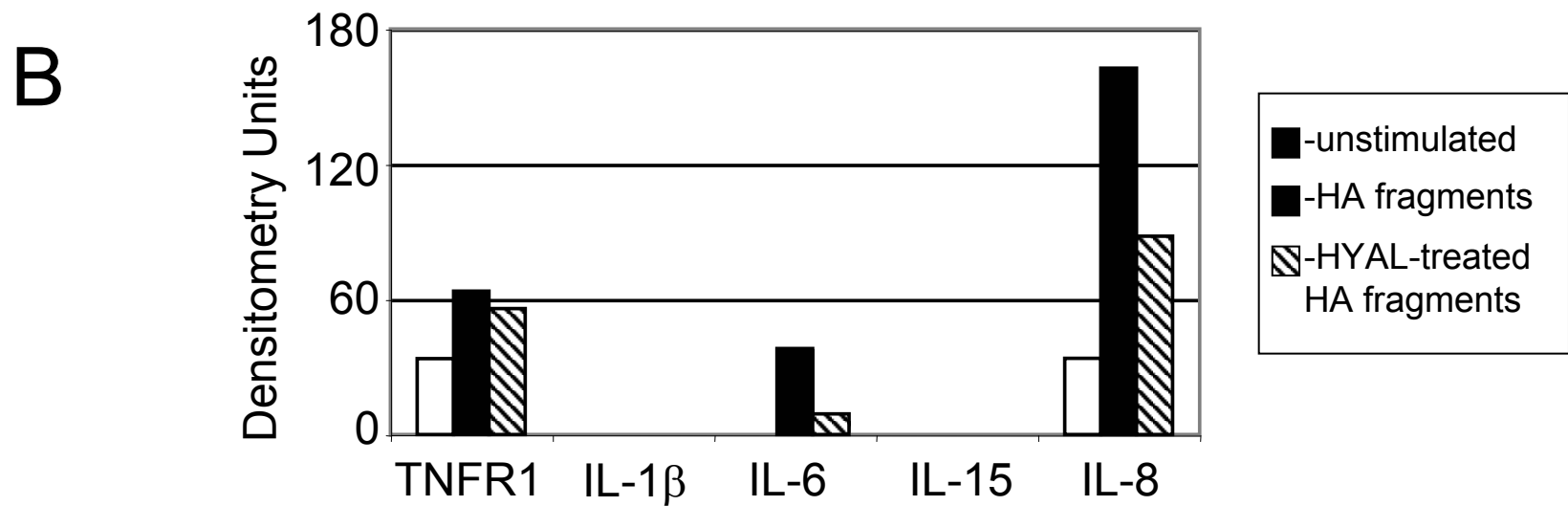
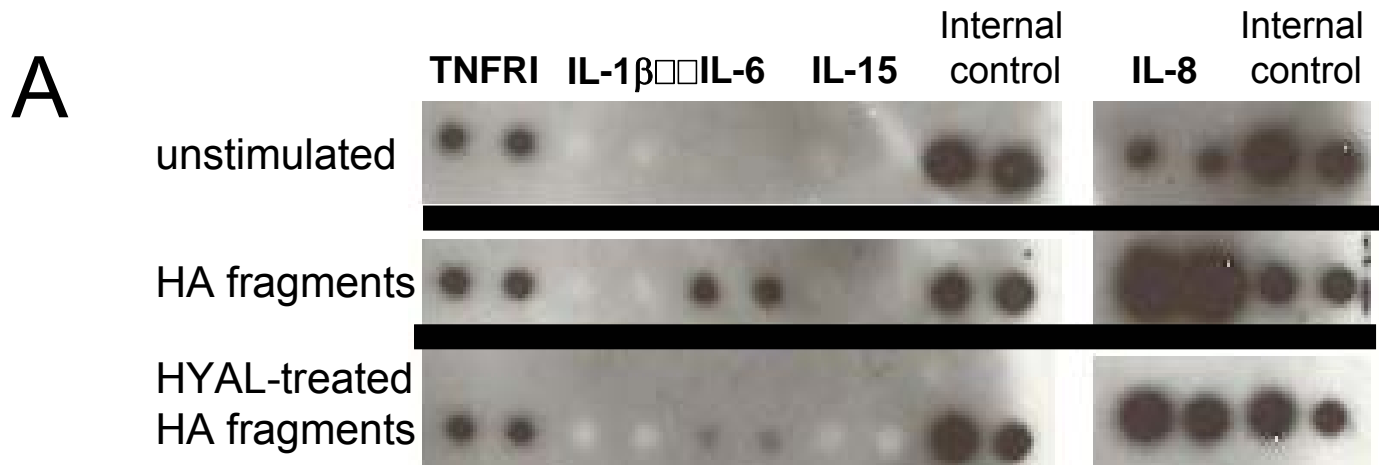
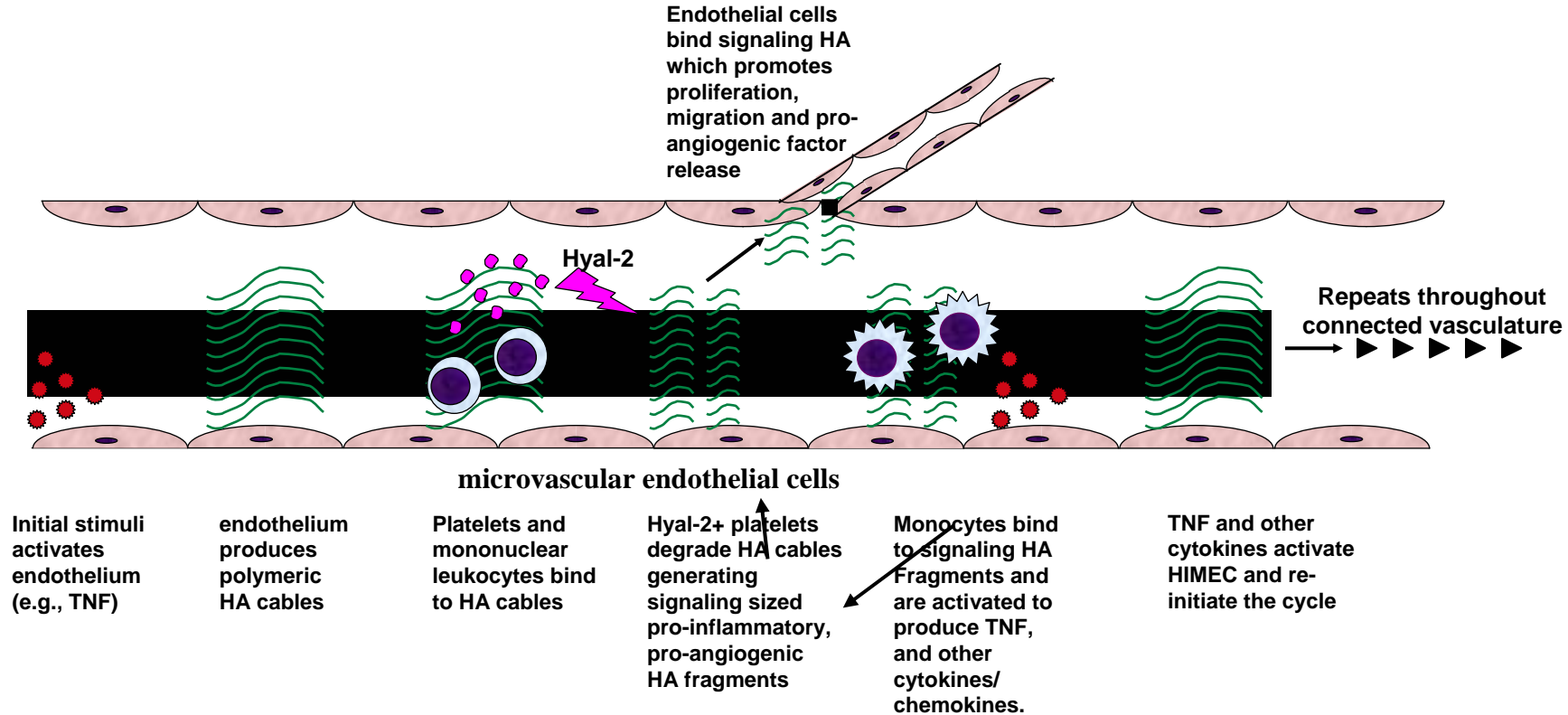


Figure 9

Angiogenesis



Inflammation

Figure 10

HA fragments are also
immunogenic

J. Biol. Chem., 282, 18265–18275, 2007 ¶

Division of Dermatology, UC, San Diego, ¶

E-mail: rgallo@vapop.ucsd.edu. ¶

A unique complex of TLR4, MD-2, and CD44 recognizes hyaluronan. Immunoprecipitation experiments confirm the physical association of TLR4 and CD44. Taken together, our results define a previously unknown mechanism for initiation of sterile inflammation that involves recognition of released hyaluronan fragments as an endogenous signal of tissue injury. ¶

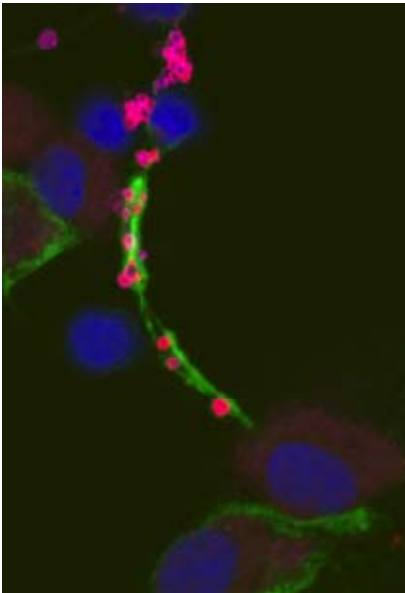
THE HA CABLE STORY

TNF- α stimulates synthesis of HA cables in endothelial cells

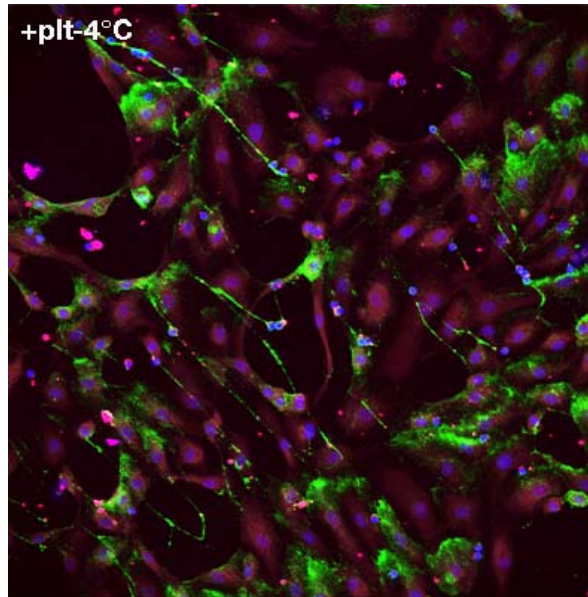
Platelets bind to such HA cables.

At low power, platelets bind at 4°
and degrade these cables at 37°

a



b



c

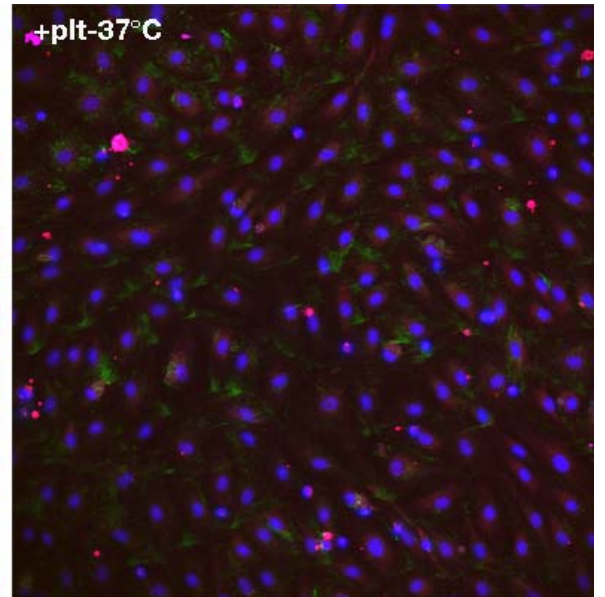
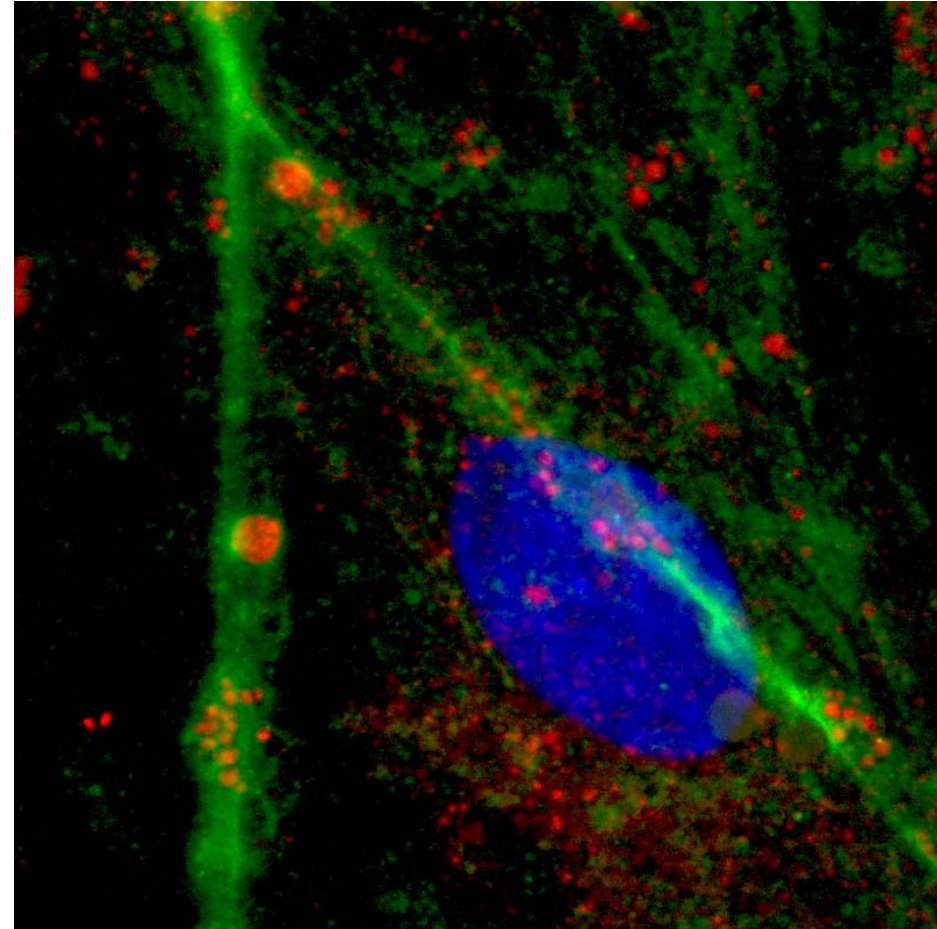
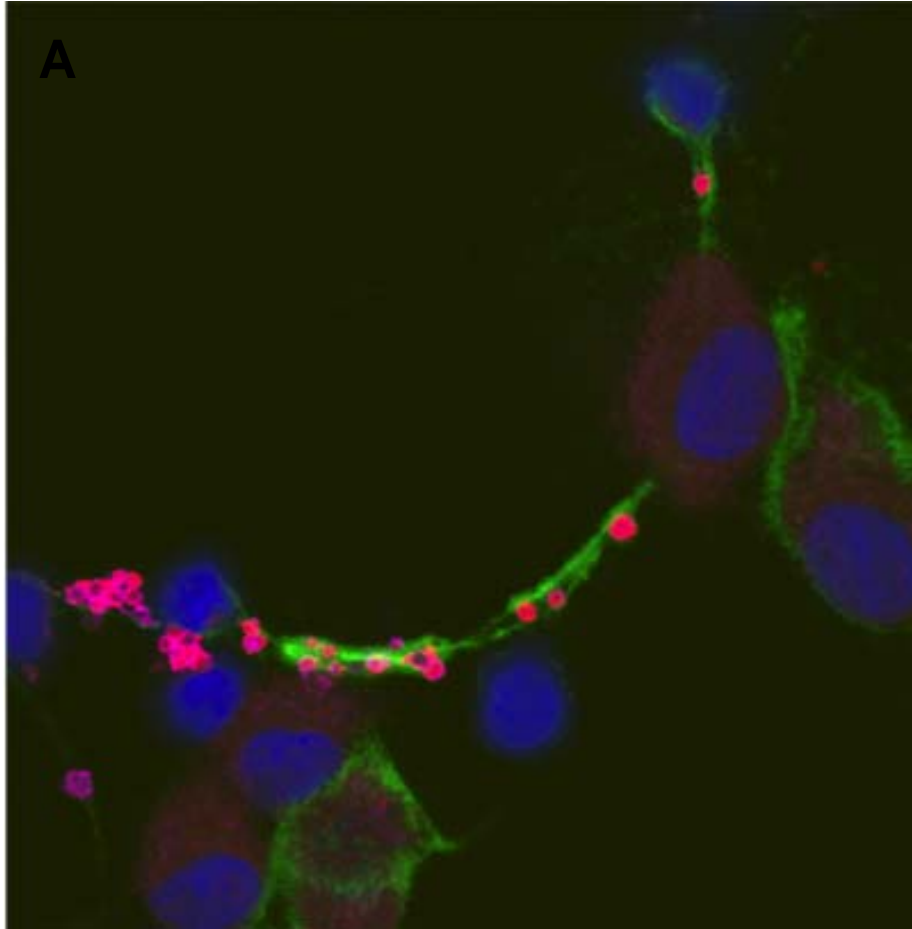


Figure 7



- A. CD42b-positive platelets (magenta) bound to HA cables (green) produced *in vitro* by $\text{TNF}\alpha$ -stimulated smooth muscle cells.
- B. High power view of HYAL 2-positive human platelets (red) in contact with HA cables (green). Nucleus of a smooth muscle cell is stained with DAPI (blue).

Functions of HA cables are unknown

We propose that these cables, a high MW form of HA, are deposited at the onset of inflammation.

They may be homeostatic mechanisms to control intensity of the inflammatory response or an attempt to hold it in abeyance.

HA cables

HA cables, anomalously, in the attempt to mollify the inflammatory reaction, may actually lead to chronicity, and in some cases, participate in prolonging the autoimmune response.

Mother Nature, in her abundant wisdom, occasionally over-reacts, creating ever-new disease mechanisms.

WOUND HEALING

Wound healing

Wound healing represents a cascade of carefully controlled reactions in which the products of one reaction can provide the substrates for the subsequent reaction.

This is similar to the coagulation or the complement cascade.

HA, a critical component of the wound healing cascade

HA and its attendant metabolic reactions play a central role in the process of wound healing. HA, its various size fragments, HA synthases, the Hyals, hyaluronidase inhibitors, as well as cytokines that modulate their expression are previously unrecognized participants in wound healing.

The process of normal wound healing

1. Clot formation, with a platelet plug and fibrinogen conversion to fibrin
2. Wound edema (HA)
3. Acute inflammatory cells (first line of defense)
4. Chronic inflammatory cells (lymphocytes and monocytes)
5. Endothelial cell migration, proliferation(angiogenesis)
6. Fibroblast invasion and proliferation
collagen III followed by collagen I deposition

Wound healing (con't)

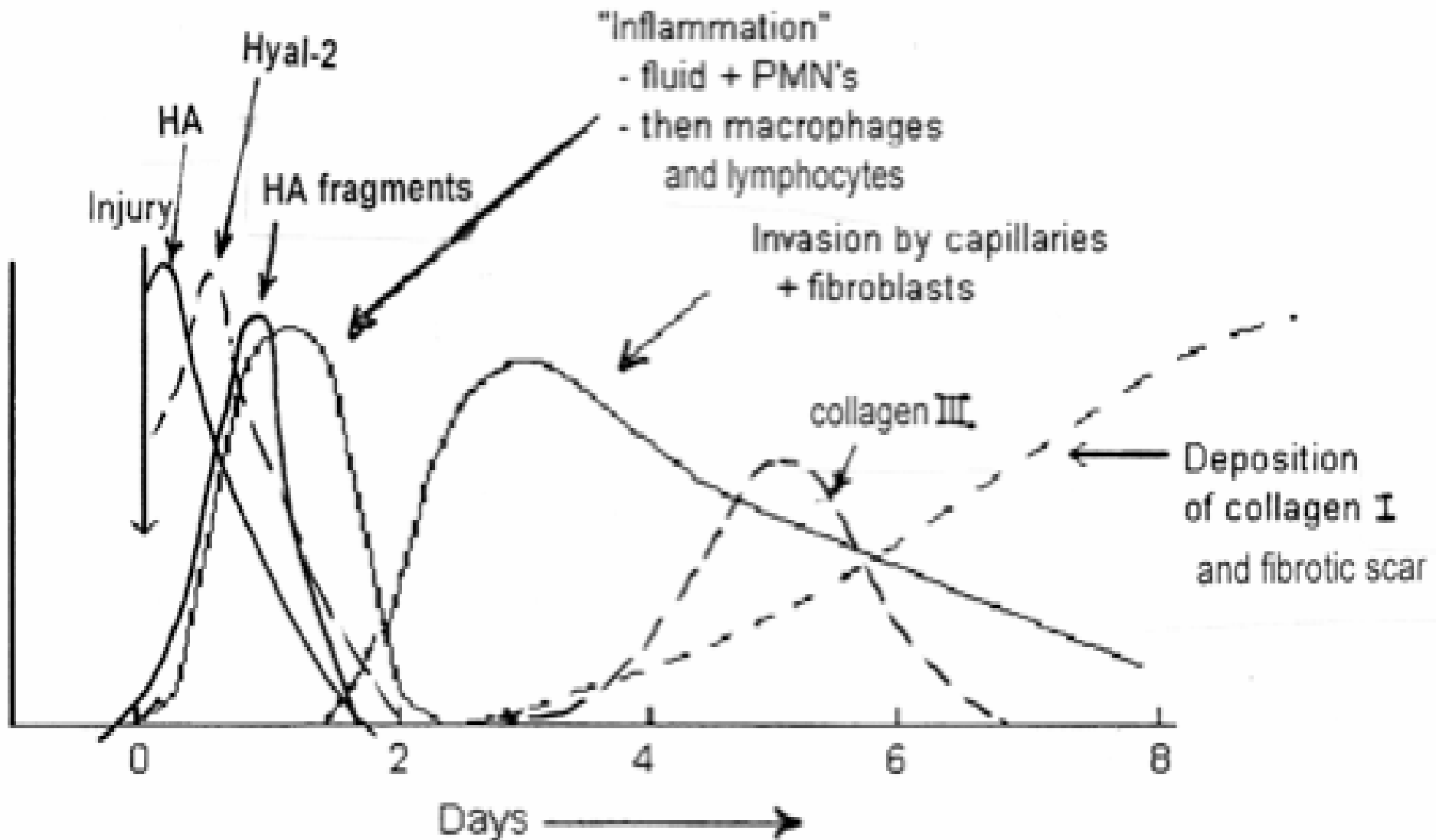
Formation of the platelet plug is the first step in wound healing.

Inflammatory cells are another component of normal wound healing.

Platelets may provide the signal for inflammatory cells to migrate to the wound

Time frame for events in the wound healing cascade + 4

SCHEMATIC OF WOUND HEALING :



HYALURONIDASE INHIBITORS

Unopposed hyaluronidase
activity would create
great havoc in tissues.

There must be mechanisms for
modulation of activity. Little is
known about such control
modulators.

Hyaluronidase inhibitors

1. All tissues have such inhibitors.
2. Acute-phase proteins contain such inhibitors.
3. This is a virtually unexplored area of biology.

As is true for many biological systems,
stimulation occurs by release from
inhibition.

Going faster, not by stepping on the
accelerator, but by taking foot off the brake.

A more prompt increase in HA can occur by inhibiting
HA degradation than by stimulating HA synthesis.

This is a survival strategy and explains rapid
rates of HA turnover under normal conditions.

Circulating HA increases dramatically under certain stress situations

1. Sepsis, particularly gram-negative sepsis
2. Shock, blood loss, after major surgery
3. Severe burns

We propose that an inhibitor
of HYAL2 exists in platelets.

We wish to isolate and
characterize this putative
inhibitor.

That is why I am here in
Bratislava, in addition to
visiting my good friend,
Dr. Laco Soltes

SUMMARY

Platelets are inflammatory cells, in part, because of fragmentation of HA by HYAL2.

Unlike any other tissue, platelets express HYAL2, with no evidence for HYAL1.

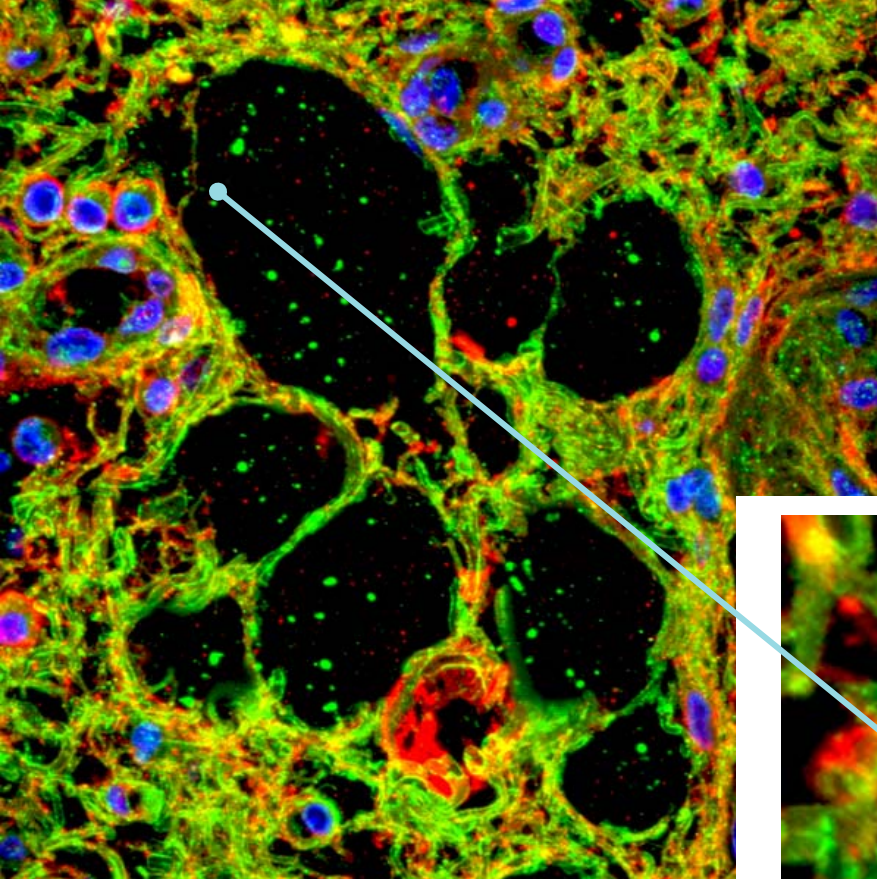
A new patho-physiological mechanism of disease

Accumulations of platelet aggregates and microthrombi induce an inflammatory response because of Hyal2-catalyzed fragmentation of HA.

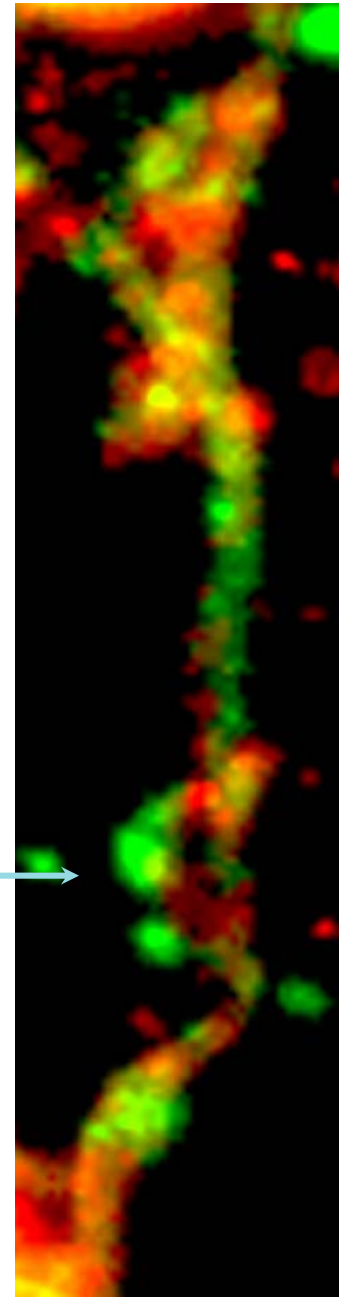
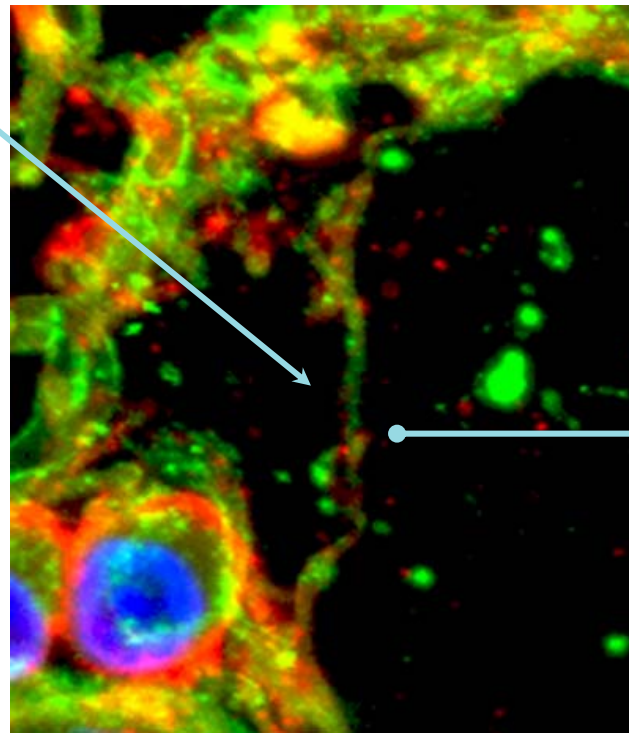
Systematically, review all human pathologies, identifying those associated with platelet plugs, and microthrombi, associated with inflammatory cells.

Disorders associated with microthrombi and inflammatory cells

1. Inflammatory bowel disease
(ulcerative colitis, Crohn's disease)
2. Rickettsial infections
3. Atypical hemolytic uremic syndrome (HUS)
4. HELLP syndrome (eclampsia, pre-eclampsia)
5. Certain autoimmune disorders (lupus, scleroderma)
6. Psoriasis, and other dermatological disorders
7. Malignant hypertension, pulmonary hypertension
8. Atheromatous tears (stroke, M.I.'s)
and many others.....
9. Various vasculitis disorders



HA (green) and
fibrinogen (red) in
murine IBD model.



Other aspects:

Additional hyaluronidase-related phenomena from this laboratory have not been written, or are not yet published.



The work is not done until the paper is done.

Acknowledgements:

STERN laboratory (UC San Francisco):

Gregory Frost

Anthony Csoka

Svetlana Shuster

Tim Wong

Carol de la Motte, Cleveland Clinic, OH

Mark Jedrzejewski, Children's Hospital Oakland, CA

Purification and Characterization of Human Serum Hyaluronidase

Alaa M. Afify,¹ Michael Stern, Markus Guntenhöner,² and Robert Stern³

*Department of Pathology, School of Medicine, and the Department of Oral & Maxillofacial Surgery,
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Received July 31, 1992, and in revised form May 19, 1993

Hyaluronidase from fresh human serum was purified to apparent homogeneity in a two-step procedure. Potent serum inhibitors of hyaluronidase activity were removed during the course of the purification. Isolation of the enzyme was expedited by the use of a newly devised ELISA-like assay. Enzyme activity was measured by following the rates of hydrolysis of hyaluronan (HA) adsorbed onto microtiter wells. Following enzymatic digestion, the remaining HA was measured using a cartilage-derived biotinylated HA-binding protein and an avidin-peroxidase reaction. Molecular sieve chromatography yielded a doublet of proteins with apparent molecular sizes of 42 and 50 kDa. The molecular size of the major band of protein obtained on sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions was 59 kDa. Under reducing conditions, however, the size increased to 72 kDa. The pH optimum of the enzyme was 3.7. Sodium chloride concentrations greater than 100 mM were inhibitory. Activity of the serum enzyme was further characterized with a new HA-substrate gel procedure. The serum enzyme activity is different from the liver-derived activity. The tissue source of this circulating enzyme is unknown. © 1993 Academic Press, Inc.

Interest has grown recently in the proteoglycans and the glycosaminoglycans of the extracellular matrix (ECM),⁴ now recognized to be not only structural moieties

but also to be involved in cellular growth control (1-4). Prominent among the glycosaminoglycans is hyaluronan (HA), whose levels are elevated during embryologic development (5), in wound healing (6-8), whenever rapid tissue regeneration and repair occur, and during tumorigenesis (9). The turnover rate of plasma HA in vertebrates is exceedingly rapid, with a median $t_{1/2}$ of 5-7 min (10). Despite the obvious importance of HA in basic biological processes, little is known about the reactions involved in the catabolism of this carbohydrate copolymer, largely because of the lack of rapid, reliable, and sensitive assays.

Several assays for hyaluronidase activity have been described (11-16), but each of these procedures has an inherent difficulty, in either a lack of sensitivity or specificity or difficulty in generating the appropriate reagents. We recently developed an ELISA-like assay for the hyaluronidase reaction based on a cartilage-derived biotinylated HA-binding peptide (17, 18). Hyaluronan adherent to microtiter wells is exposed to samples with hyaluronidase and the remaining HA measured using the biotinylated HA-binding peptide (HABP) that then reacts with avidin peroxidase. This technique permits rapid assessment of activity and facilitates development of an isolation procedure. The hyaluronidase present in fresh human serum was purified to apparent homogeneity in a two-step procedure. The function and mode of action of a circulating acid-active hyaluronidase continues to be an enigma, as is the tissue source for this activity.

MATERIALS AND METHODS

Materials

DEAE-cellulose (DE-52, Whatman) was obtained from Fisher Scientific. Hyaluronan prepared from human umbilical cords (CN Im;

PBS, phosphate-buffered saline; FPLC, fast performance liquid chromatography; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis; HAc, acetic acid; SDS, sodium dodecyl sulfate; ELISA, enzyme-linked immunosorbent assay.

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² Present address: Fachbereich, Zahnmedizin, Philipps-Universität, Marburg/Lahn Hessen, Germany.

³ To whom correspondence and requests for reprints should be addressed at Department of Pathology, University of California, San Francisco, CA 94143-0506.

⁴ Abbreviations used: ECM, extracellular matrix; HA, hyaluronan; HABP, HA-binding protein; C4S, chondroitin 4-sulfate; C6S, chondroitin 6-sulfate; DS, dermatan sulfate; HS, heparan sulfate; H, heparin.

Tissue distribution of hyaluronidase paralogs as determined by Northern blot analysis

Hyal-1 Widely expressed, except in brain.

Not expressed during early development

Hyal-2 Similar expression pattern to Hyal-1.

Highly expressed during early development

**Hyal-3 Widely expressed, but at low levels, higher in testis
and bone marrow**

(a stem cell connection?)

**Hyal-4 Expressed primarily in placenta and in skeletal
muscle. This may be a chondroitinase!**

**Hyal-5 Wide, but low level expression,
a pseudogene in humans (PHYAL1).**

PH-20 Normally expressed only in testis.



JNI ONCOLOGY LECTURES

June 14, 2007, 15:30

JNI/Erasmus MC, room BE 425

Dr. Molewaterplein 50, Rotterdam.

Robert Stern

*Department of Pathology, School of Medicine
University of California, San Francisco, USA.*

Why the Platelet is Inflammatory

To contact the speaker or for more info:
Dr Carl Verkoelen, c.verkoelen@erasmusmc.nl

UNIVERSITÄT LEIPZIG

Medizinische Fakultät

Interdisziplinäres Zentrum für Klinische Forschung Leipzig

IZKF-Gastvortrag

Why the Platelet is Inflammatory

Gastreferent

Prof. Dr. Robert Stern

Department of Pathology, School of Medicine/University of California, San Francisco,
USA

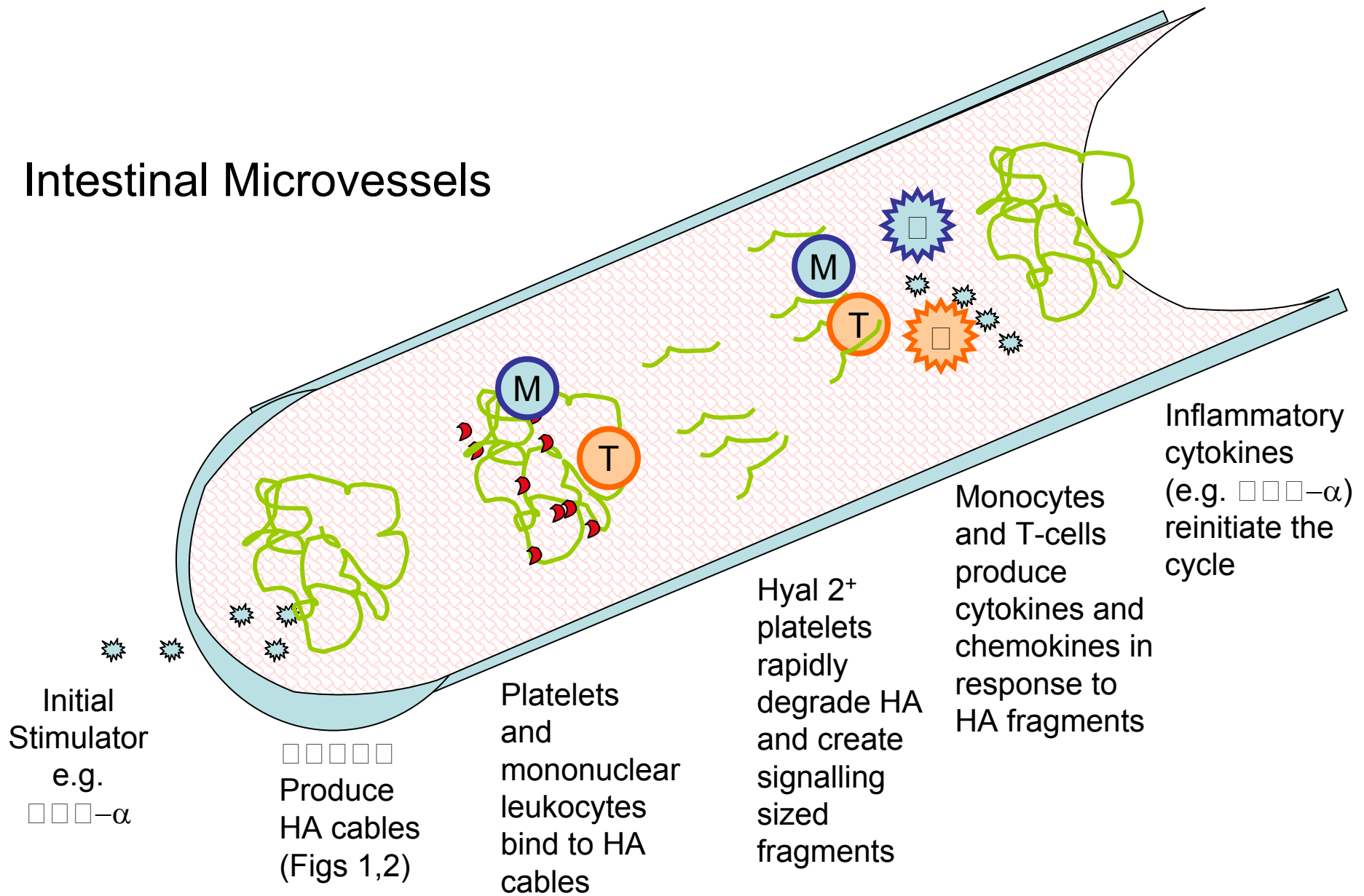
QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Another mechanism for inhibition of rapid HA catabolism:

Under conditions of severe stress, as in septicemia, shock, blood loss, massive burns, major surgery, and inflammation, there is the appearance of large amounts of HA, in the circulation, and locally, as in cable formation.

Hyal-2 is not shuttled to the cell surface, as occurs normally, but under severe stress, remains in endosomes. High MW HA then accumulates, and normal steady state turnover does not occur.

Intestinal Microvessels



Why the platelet is inflammatory: a story of hyaluronidases

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Hyaluronidase is a misnomer

These enzymes are also able to degrade
chondroitin and chondroitin sulfates,
albeit to a slower degree.

Chondroitinases

Are there other sequences in the
Human Genome that code for
pure chondroitinases?



Available online at www.sciencedirect.com



ANALYTICAL
BIOCHEMISTRY

Analytical Biochemistry 347 (2005) 42–48

www.elsevier.com/locate/yabio

An assay for bacterial and eukaryotic chondroitinases using a chondroitin sulfate-binding protein

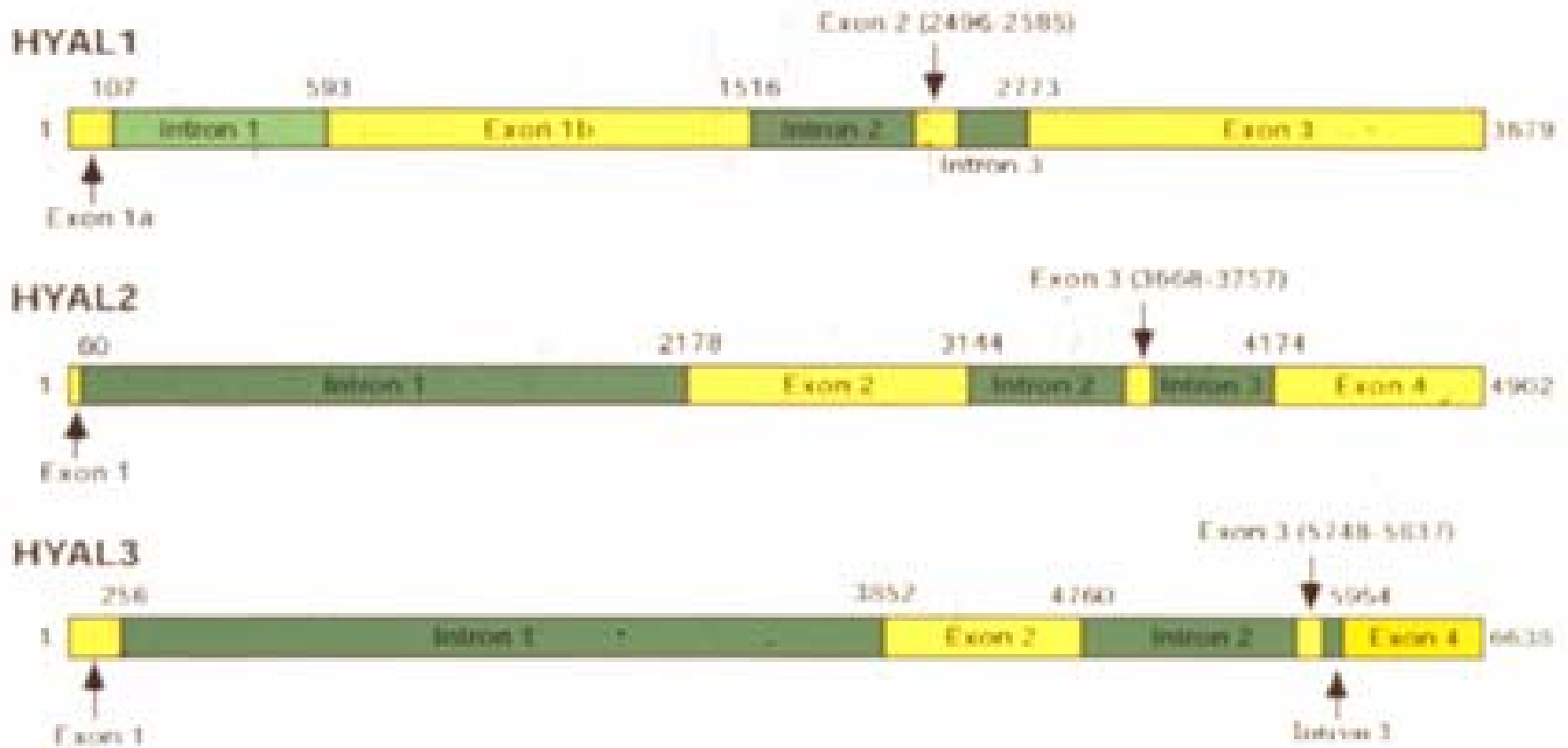
Hans-Georg Wisniewski ^{a,*}, Moshe H. Sweet ^a, Robert Stern ^b

^a *Department of Microbiology, School of Medicine, New York University, New York, NY 10016, USA*

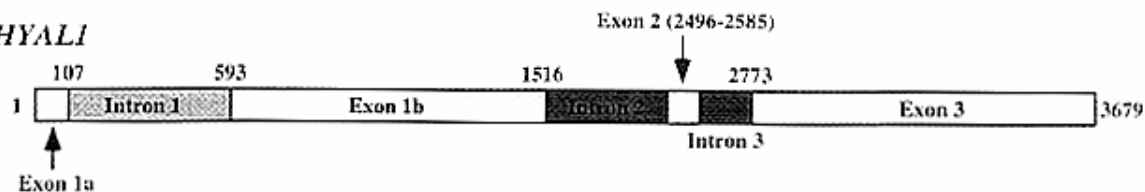
^b *Department of Pathology, School of Medicine, University of California, San Francisco, CA 94143, USA*

Received 20 April 2005

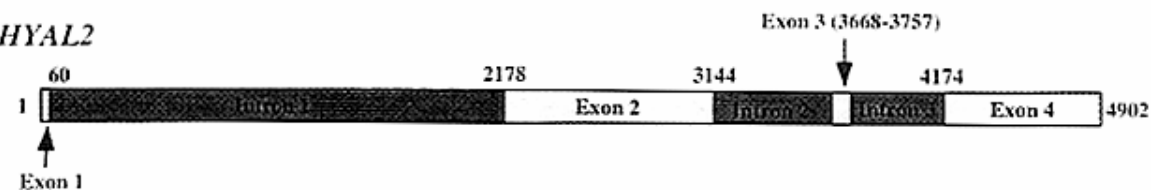
Genomic structure of the hyaluronidase genes



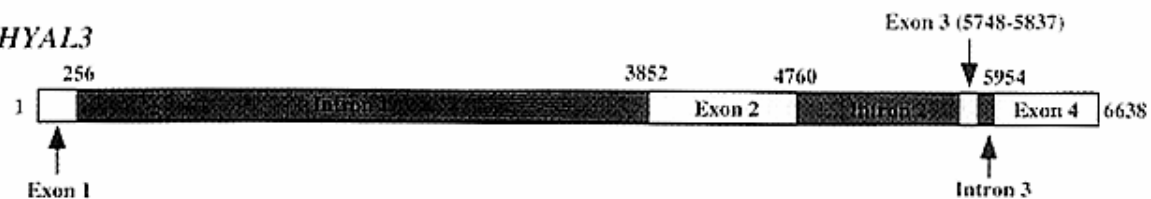
HYAL1



HYAL2



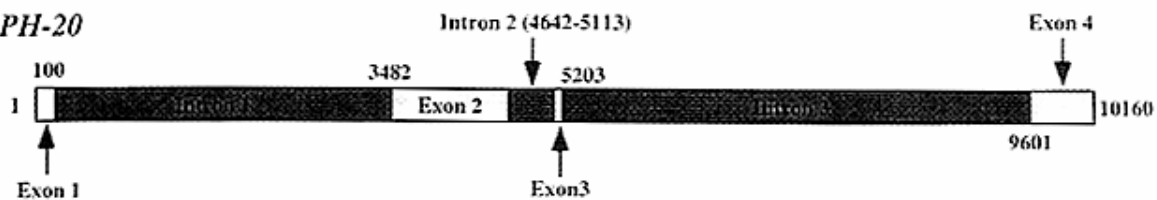
HYAL3



HYAL4



PH-20



HYALP1 (pseudogene)

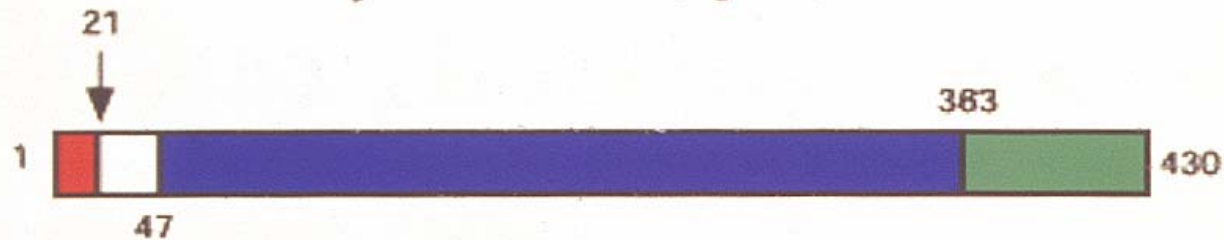


Hyaluronidase Domains

Bee Venom Hyaluronidase



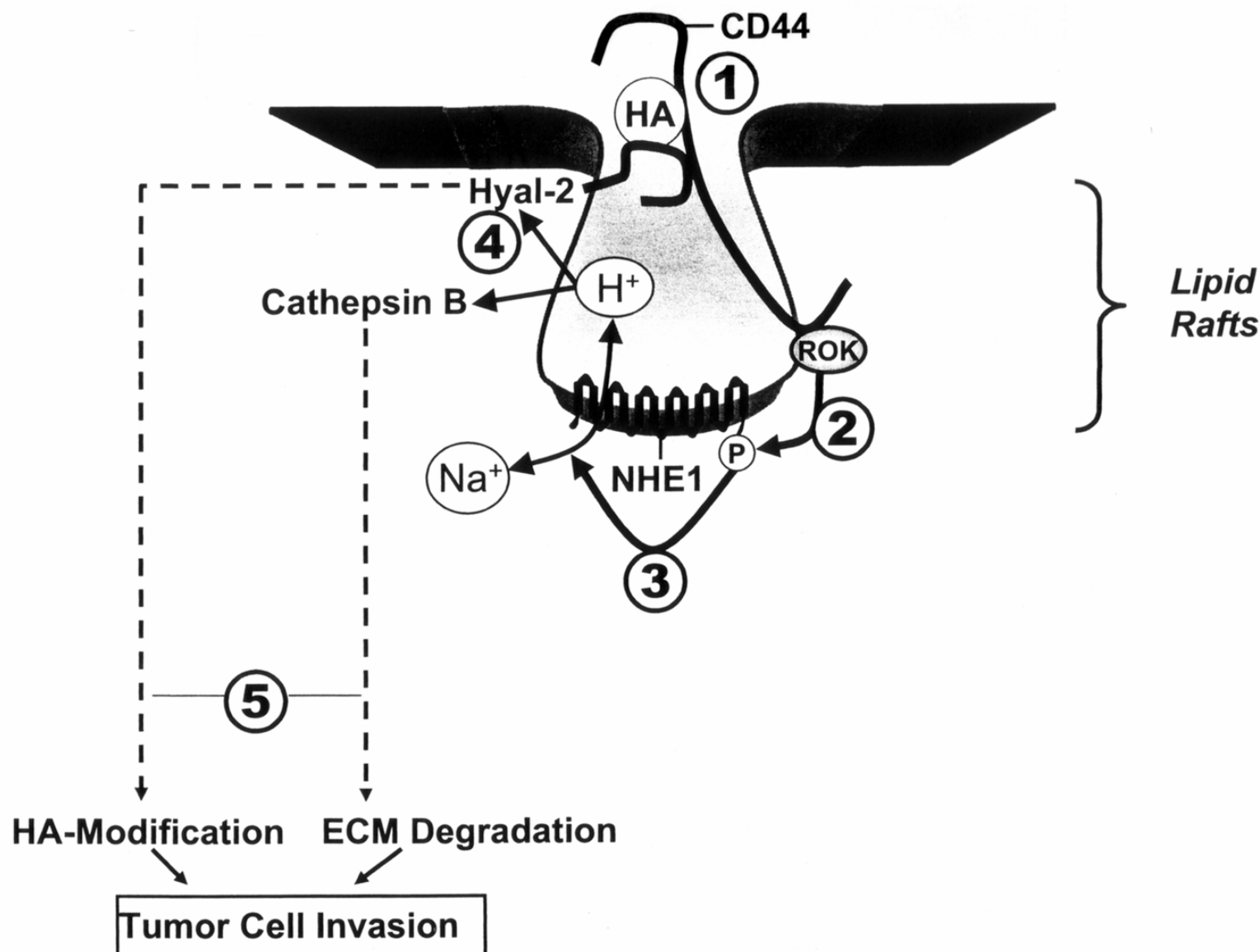
Hyaluronidase 1 (Hyal-1)



PH-20 (SPAM1)



CD44-NHE1 signaling causes pH changes and activates Hyal-2, and cathepsin B



Addendum

Halozyme Corporation
founded by Gregory Frost
has expressed hyaluronidases
as their commercial products.

HALO

Commercial success of Halozyme

They have entered into a \$680 million dollar agreement with Roche Corporation, as an adjunct for drug administration.

Market report: ¶

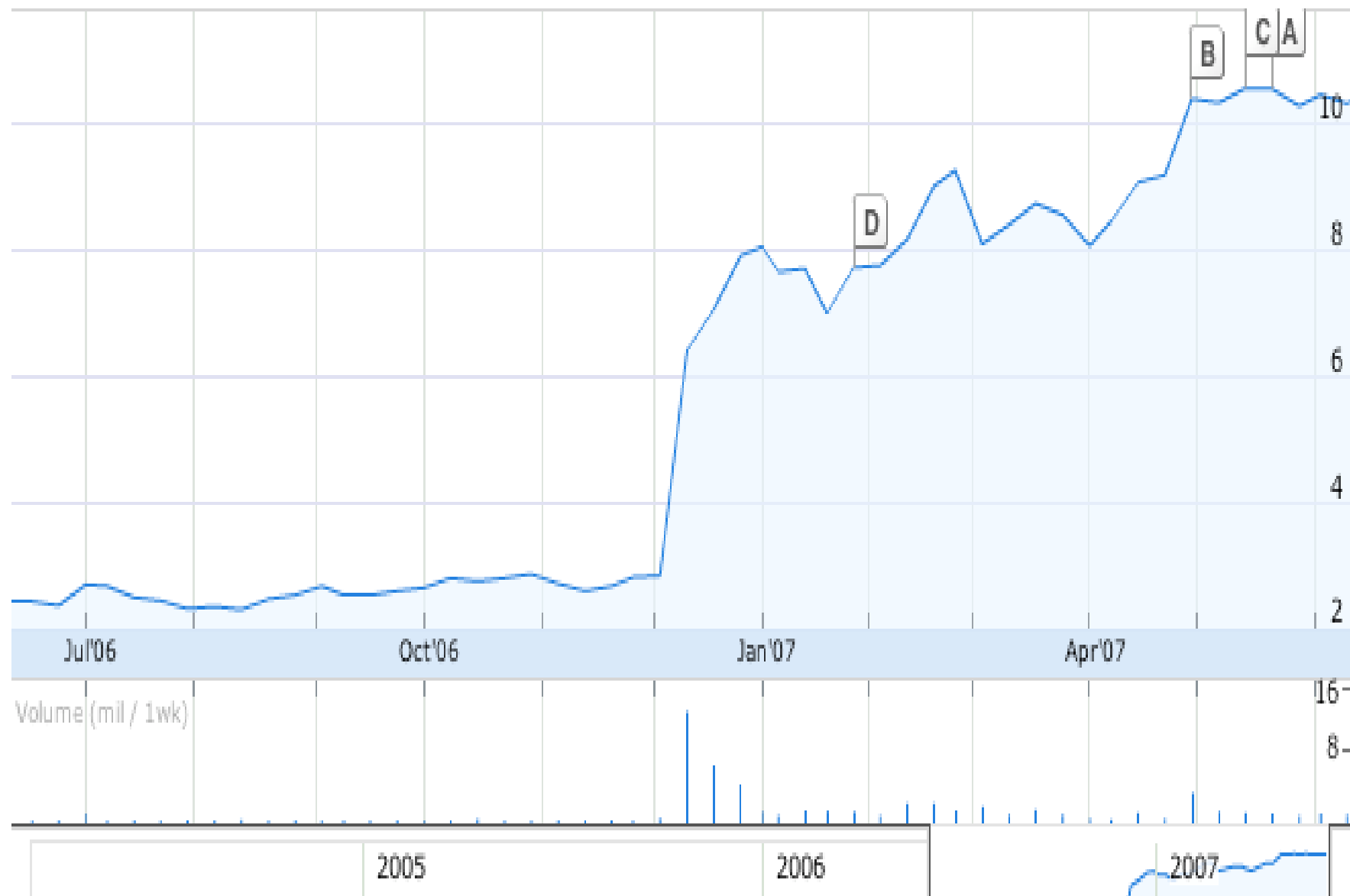
Last December, Halozyme entered into a collaboration agreement with Switzerland-based Roche, which makes several leading biologics including the antiviral drug Pegasys and the monoclonal antibody-based cancer drugs Avastin and Herceptin. The agreement gave Roche an exclusive worldwide license to develop and market co-formulations of rHuHyaluronidase and 13 of its own drugs. In return, Roche agreed to pay \$20 million up front, invest \$11 million in equity, and pay \$111 million in milestones for its first three targets. ¶

In February this year, the deal was expanded to allow the company to use rHuHyaluronidase in combination with proprietary and non-proprietary small molecule drugs. Roche agreed to pay Halozyme \$10 million up front, invest \$20 million in equity, and make other milestone payments. The deal could be worth nearly \$650 million; → ¶

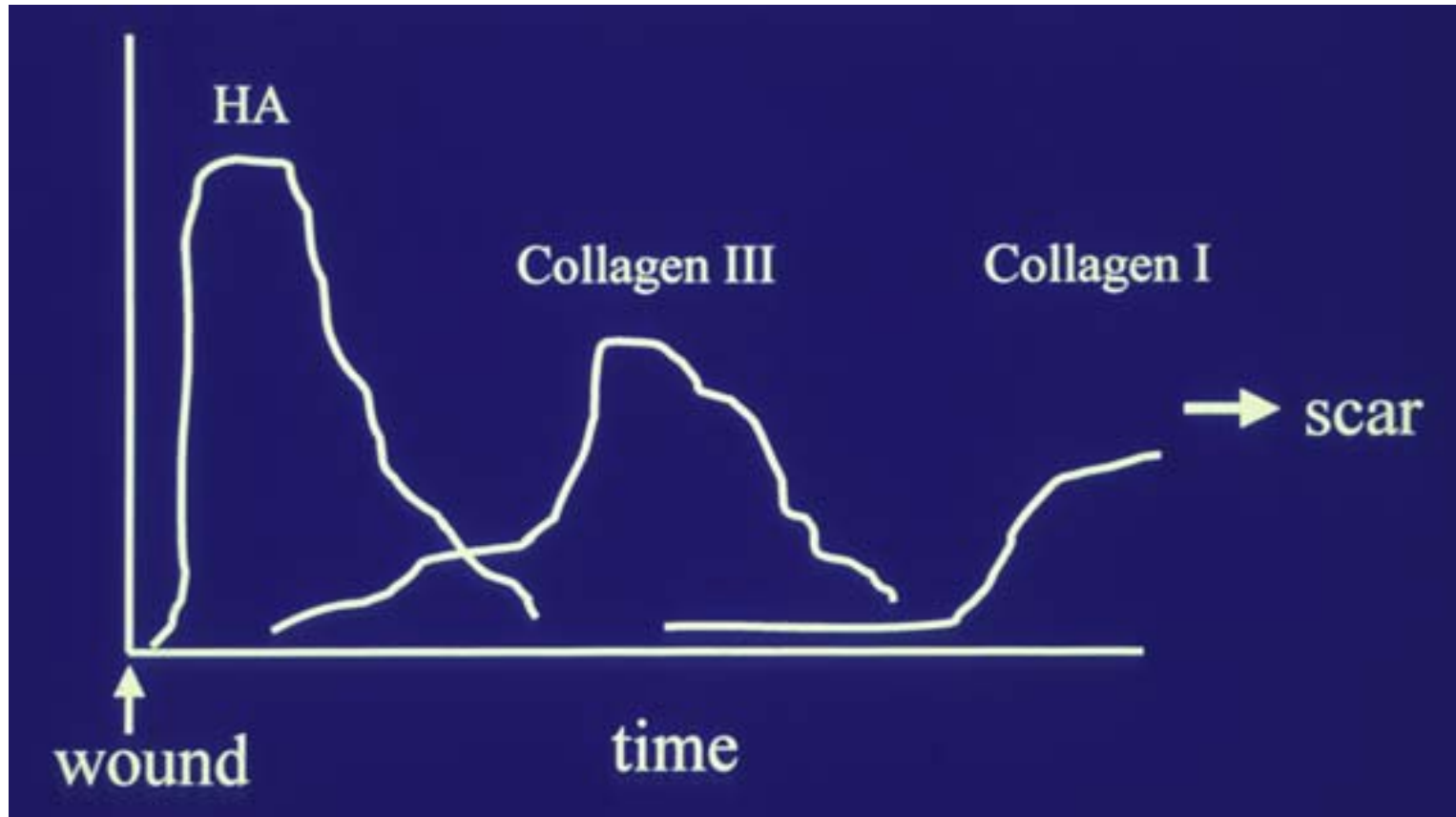
Halozyme seems to be in the enviable position of having no direct competition. ¶

Zoom [1d](#) [5d](#) [1m](#) [3m](#) [6m](#) [YTD](#) [1y](#) [5y](#) [10y](#) [Max](#)

Jun 12, 2006 - Jun 11, 2007: +7.93 (325%)

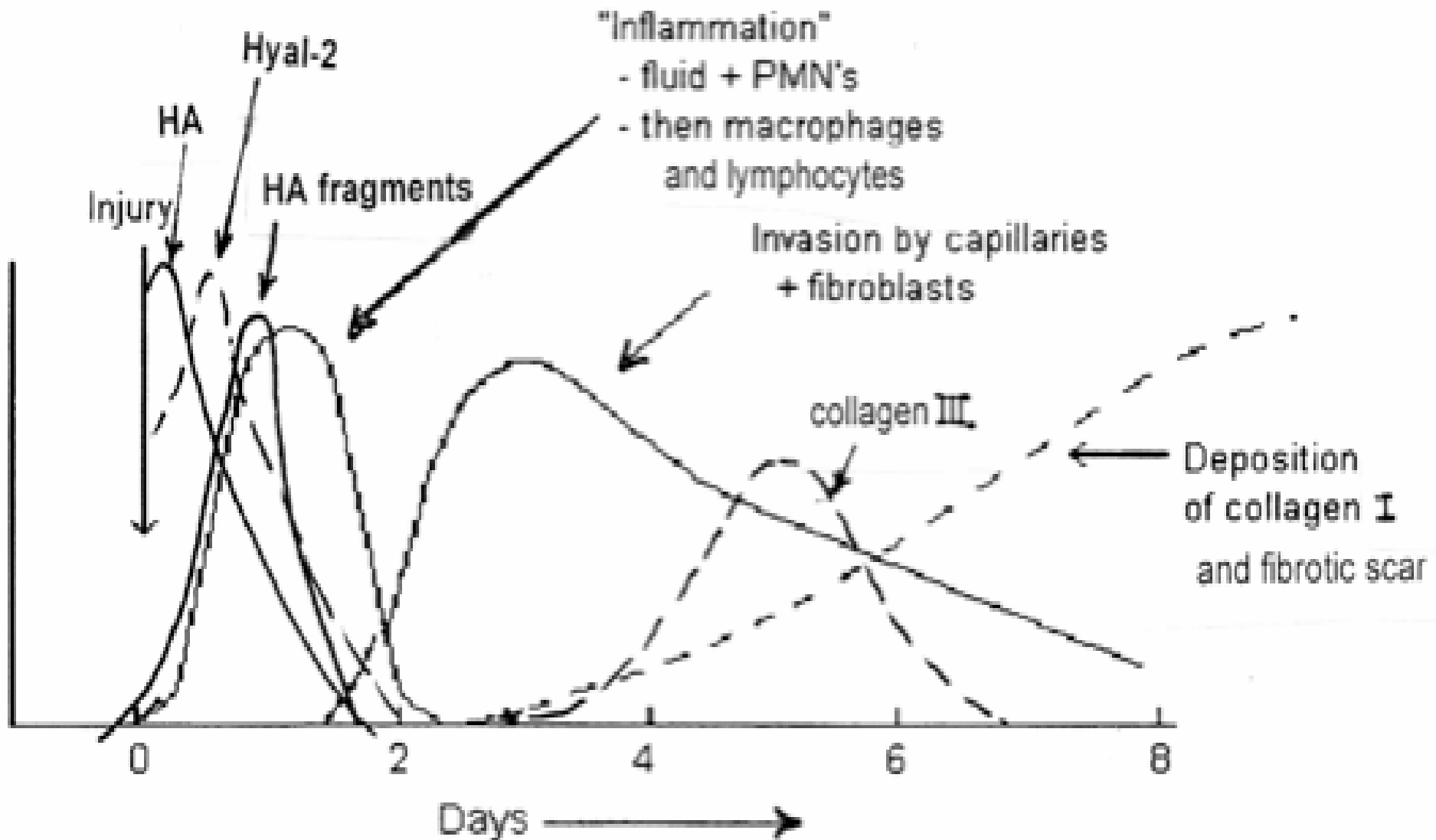


Wound Healing



Time frame for events in the wound healing cascade

SCHEMATIC OF WOUND HEALING :



Mechanism of platelet Hyal2 generation of signalling fragments of hyaluronan

