International Society for Matrix Biology Newsletter

No. 3 - April 2006

Contents

From the President's Desk

Nominations ISMB Council

Hopefully you will have received an email from Peter Bruckner asking for nominations for ISMB Council. The closing date for nominations is May 15, and these should be emailed to Peter [peter.bruckner@uni-muenster.de]. Although the bylaws allow for a Council of up to eighteen members, we are calling for nominations for six new positions, bringing the Council size to nine members, more in keeping with the membership base of the Society. These new Council Members will join the three most recently elected and ongoing members, Kathy Cheah, Lynn Sakai and Klaus von der Mark. A written ballot will take place and appointments will be made by June 15, in time for the Council to be convened at the 2006 AGM of ISMB to be held at the FECTS-ISMB meeting in Oulu.

Please give some creative thought to possible candidates who will help contribute to the growth and development of ISMB. Current Council Members may be re-nominated. The position of Vice-President is currently vacant, so both the Vice-Presidency and Presidency will also be elected by members from a slate of candidates being developed by Council.

FECTS-ISMB Meeting and Travel Fellowships

A reminder that the deadline for "early-bird" registration for the FECTS-ISMB meeting has been extended until April 15. Visit www.fects-ismb.org for conference registration and program details. ISMB is supporting this meeting by providing Travel Fellowships for matrix biologists outside of the usual FECTS 'catchment", such those in the USA and the Asia/Pacific region. These travel awards will be competitively awarded to early career scientists from all countries outside of Europe who are ISMB members and who have registered for the meeting Application is by brief letter of application outlining your case for the award of the fellowship along with your cv details and abstract(s) for presentation at the FECTS-ISMB meeting. The letter of application should be sent by April 15 to the chairman of the meeting, Taina Pihlajaniemi [taina.pihlajaniemi@oulu.fi].

Rupert Timpl Award

The "Rupert Timpl Award" will be made in conjuction with the 2006 FECTS-ISMB meeting. The award is made every two years and is based on the "best paper" on a subject in matrix biology published by a young scientist in the preceding two years. The closing date for nominations for the 2006 award is April 30 and papers published in 2004 and 2005 will be considered. Submissions should be made to Peter Bruckner [peter.bruckner@uni-muenster.de].

Regards,

John Bateman

Meeting Announcements

12th Annual Canadian Connective Tissue Conference

University of Ottawa, Canada, May 25-27, 2006

Gordon Research Conference "Basement Membranes"

This meeting will be held in Il Ciocco, Italy from June 18-23, 2006. Conference website: http://www.grc.org/programs/2006/basement.htm

The XXth meeting of the Federation of European Connective Tissue Societies

We are happy to inform that almost 400 participants have registered to the XXth FECTS meeting - joint with ISMB, in Oulu, Finland, July 1-5, 2006, www.fects-ismb.org

However, we are happy to host more and therefore we have EXTENDED THE FOLLOWING DEADLINES:

ABSTRACT SUBMISSION: April 15th, 2006
EARLY REGISTRATION WITH A REDUCED FEE: April 15th, 2006

Welcome to Oulu!,
On behalf of the Organizing Committee
Johanna Myllyharju

Gordon Research Conference "Signal Transduction by Engineered Extracellular Matrices"

Connecticut College, New London, CT, July 2-7, 2006
http://www.grc.org/programs/2006/sigtrans.htm

4th European Meeting on Elastin

This conference will be held in Lyon, France from July 9-12, 2006. Conference website: http://www.ujf-grenoble.fr/BIO/elastin2006/.

Gordon Research Conference "Proteoglycans"

This conference will be held in Andover, NH, USA from July 9-14, 2006. Conference website: http://www.grc.org/programs/2006/protglyc.htm.

American Society for Matrix Biology Biennial Meeting

To be held in Nashville, Tennessee from November 1-4, 2006. Meeting website: http://www.asmb.net/nationalmeeting/.
Note that the call for papers deadline: August 1st 2006

Matrix Research Update

In Press Publications

Sensing extracellular matrix: An update on discoidin domain receptor function
W. F. Vogel, R. Abdulhussein , C. E. Ford
Cellular Signalling
Discoidin Domain Receptors (DDRs) have recently emerged as non-integrin-type receptors for collagen. The two mammalian gene products Discoidin Domain Receptor 1 and -2 constitute a subfamily of tyrosine kinase receptors that are selectively expressed in a number of different cell types and organs. Upon collagen activation, DDRs regulate cell adhesion, proliferation and extracellular matrix remodeling. Here we review the various signaling pathways and cellular responses evoked by activated DDRs. Additionally, we give an overview of the more recent advances in understanding the role of DDRs in various human diseases, in particular during tumor progression, atherosclerosis, inflammation and tissue fibrosis. Furthermore, we discuss potential roles of genes homologous to mammalian DDRs identified in flies, worms and sponges. We show that the structural organization of these DDR-related genes is highly conserved throughout evolution suggesting that invertebrate DDRs may also function as receptors for collagen. By highlighting current questions about these unusual collagen receptors, we hope to attract new research on DDRs from a variety of different fields.

Cell surface processing of pro-ADAMTS9 by furin.
B.-H. Koo, J.-M. Longpre, R. P. T. Somerville, J. P. Alexander, R. Leduc, S. S. Apte
The Journal of Biological Chemistry
Processing of polypeptide precursors by proprotein convertases (PCs) such as furin typically occurs within the trans-Golgi network. Here, we show in a variety of cell types that the propeptide of ADAMTS9 is not excised intracellularly. Pulse-chase analysis in HEK293F cells indicated that the intact zymogen was secreted to the cell-surface and was subsequently processed there before release into the medium. The processing occurred *via* a furin-dependent mechanism as shown using PC inhibitors, lack of processing in furin-deficient cells, and rescue by furin in these cells. Moreover, down-regulation of furin by siRNA reduced ADAMTS9 processing in HEK293F cells. PC5A could also process proADAMTS9, but similarly to furin, processed forms were absent intracellularly. Cell-surface, furin-dependent processing of proADAMTS9 creates a precedent for extracellular maturation of endogenously produced secreted proproteins. It also indicates the existence of a variety of mechanisms for processing of ADAMTS proteases.

Regulation of procollagen amino-propeptide processing during mouse embryogenesis by specialization of homologous ADAMTS proteases; Insights on collagen biosynthesis and dermatosparaxis.

C. LeGoff, R. P. T. Somerville, F. Kesteloot, K. Powell, D. E. Birk, A. Colige, S. S. Apte

Development

Mutations in ADAMTS2, a procollagen amino-propeptidase, cause severe skin fragility, designated as dermatosparaxis in animals, and a subtype of the Ehlers-Danlos syndrome (dermatosparactic type or VIIC) in humans. Not all collagen-rich tissues are affected to the same degree, arguing for compensation by its homologs, ADAMTS3 and ADAMTS14. In situ hybridization of Adamts2, Adamts3 and Adamts14 and the genes encoding the major fibrillar collagens, Col1a1, Col2a1 and Col3a1 during mouse embryogenesis, demonstrated distinct tissue-specific, overlapping expression patterns of protease and substrate genes. Adamts3 , but not Adamts2 or Adamts14, was co-expressed with Col2a1 in cartilage throughout development and with Col1a1 in bone and musculotendinous tissues. ADAMTS3 induced procollagen I processing in dermatosparactic fibroblasts, suggesting a role in procollagen I processing during musculoskeletal development. Adamts2, but not Adamts3 and Adamts14, was co-expressed with Col3a1 in many tissues including the lungs and aorta, and Adamts2-/- mice showed widespread defects in procollagen III processing. Adamts2-/- mice had abnormal lungs characterized by decreased parenchymal density. However, the aorta and collagen fibrils in the aortic wall appeared normal. Although Adamts14 lacked developmental tissue-specific expression, it was co-expressed with Adamts2 in mature dermis, possibly explaining the presence of some processed skin procollagen in dermatosparaxis. The data show how evolutionarily related proteases with similar substrate preferences may have distinct biological roles owing to tissue-specific gene expression, and provides insights on collagen biosynthesis and the pathobiology of dermatosparaxis.

Crystal structure of the biglycan dimer and evidence that dimerisation is essential for folding and stability of class I small leucine rich repeat proteoglycans.

P. G. Scott [scottp@ualberta.ca], C. M. Dodd, E. M. Bergmann, J. K. Sheehan, P. N. Bishop

The Journal of Biological Chemistry

Biglycan and decorin are two closely related proteoglycans whose protein cores contain leucine-rich repeats flanked by disulfides. We have previously shown that decorin is dimeric both in solution and in crystal structures. In this study we determined whether biglycan dimerizes and investigated the role of dimerisation in the folding and stability of these proteoglycans. We used light scattering to show that biglycan is dimeric in solution and solved the crystal structure of the glycoprotein core of biglycan at 3.40 A resolution. This structure reveals that biglycan dimerizes in the same way as decorin, i.e. by apposition of the concave inner surfaces of the leucine-rich repeat domains. We demonstrate that low concentrations of guanidinium chloride denature biglycan and decorin, but that the denaturation is completely reversible following removal of the guanidinium chloride, as assessed by circular dichroism spectroscopy. Furthermore, the rate of refolding is dependent on protein concentration, demonstrating that it is not a unimolecular process. Upon heating decorin shows a single structural transition at a Tm of 45-46^{o}C, but refolds completely on cooling to 25^{o}C. This property of decorin enabled us to show both by calorimetry and light scattering that dimer to monomer transition coincided with unfolding and monomer to dimer transition coincided with refolding; thus these processes are inextricably linked. We further conclude that folded monomeric biglycan or decorin cannot exist in solution. This implies novel interrelated functions for the parallel b sheet faces of these leucine-rich-repeat proteoglycans including dimerisation and stabilization of protein folding.

Recently published

Altered integration of matrilin-3 into cartilage extracellular matrix in the absence of collagen IX

B. Budde, K. Blumbach, J. Ylöstalo, F. Zaucke, H. W. A. Ehlen, R. Wagener, L. Ala-Kokko, M. Paulsson, P. Bruckner, S. Grässel

Molecular and Cellular Biology (2005)

v25, no. 23, p10465-78

The matrilins are a family of four non-collagenous oligomeric extracellular matrix proteins with a modular structure. Matrilins can act as adapters which bridge different macromolecular networks. We therefore investigated the effect of collagen IX deficiency on matrilin-3 integration into cartilage tissues. Mice harbouring a deleted *Col9a1* gene lack synthesis of a functional protein and produce cartilage fibrils completely devoid of collagen IX. Newborn collagen IX knockout mice exhibited significantly decreased matrilin-3 and COMP (cartilage oligomeric matrix protein) signals, particularly in the cartilage primordium of vertebral bodies and ribs. In the absence of collagen IX a substantial amount of matrilin-3 is released into the medium of cultured chondrocytes instead of being integrated into the cell layer as in wild type and COMP deficient cells. Gene expression of matrilin-3 is not affected in the absence of collagen IX, but protein extraction from cartilage is greatly facilitated. Matrilin-3 interacts with collagen IX containing cartilage fibrils, while fibrils from collagen IX knock out mice lack matrilin-3 and COMP deficient fibrils exhibit an intermediate integration.

In summary, the integration of matrilin-3 into cartilage fibrils occurs by both a direct interaction with collagen IX and indirectly with COMP serving as an adapter. Matrilin-3 can be considered as an interface component, capable of interconnecting macromolecular networks and mediating interactions between cartilage fibrils and the extrafibrillar matrix.

Job Advertisements

Assistant/Associate Professor Faculty Position

The Department of Orthopaedics and Rehabilitation at Oregon Health and Science University is seeking a research scientist with expertise in biomechanical engineering related to skeletal tissues, for an Assistant/Associate Professor faculty position. This is a joint recruitment with the Bone and Mineral Unit in the Department of Medicine.

The successful applicant will be expected to establish a research program that complements basic and applied projects in bone and cartilage biology, as well as actively participating in the education and administrative programs of the Departments. S/he will direct an established biomechanics laboratory and will have opportunities for affiliation with the bioengineering programs of the OGI School of Science and Engineering.

To apply, please submit CV, a description of current research and future research interests and names and addresses of references to: Dr. Brian Johnstone, Mail code: OP31, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239 or email to johnstob@ohsu.edu. OHSU is an affirmative action and equal opportunity educator and employer.

Postdoctoral positions available in developmental Biology and Biochemistry of Metalloproteases, Extracellular Matrix and Proteoglycans

One or more post-doctoral fellowships are available to study the biology and biochemistry of the family of ADAMTS proteases and in particular, to help to unravel their roles in extracellular matrix networks. This is one of the fastest-moving and most exciting fields in matrix biology. ADAMTS proteases have been implicated in arthritis (aggrecanase), inherited connective tissue disorders, cell migration and angiogenesis.

Ongoing projects include proteomics for identification of ADAMTS substrates, determination of the role of ADAMTS proteases in cell signaling and embryogenesis, structure-function analysis of specialized regions of these enzymes, ADAMTS-proteoglycans and the role of ADAMTS10 in Marfan syndrome.

Our laboratory will suit highly motivated new (or expected in 2007) PhD or MD/PhD graduates who are interested in augmenting or developing skills in mouse genetics, embryology, cell biology/signaling, and protein chemistry. The laboratory offers a positive, stimulating, fast-moving and constructive environment. The Lerner Research Institute (www.lerner.ccf.org) has state of the art research facilities in a major clinical center, the Cleveland Clinic Foundation, and is affiliated with the adjacent Case Western Reserve University. Cleveland and its vicinity offer an affordable, high quality of life with outstanding recreational and cultural opportunities.

Contact: Suneel S. APTE, MD, PhD, Lerner Research Institute, Cleveland Clinic Foundation [**aptes@ccf.org**]

Three Post-Doctoral Positions Available Immediately

Three Post-Doctoral positions are available immediately in the laboratories of Dr. Susanna Cardell, Dept of Medical Microbiology and Immunology, Goteborg University, Sweden, Dr. Lydia Sorokin, Institute of Biochemistry and Pathobiochemistry, Muenster University, Germany, and Dr. Dan Holmberg, Dept of Medical Biosciences, Umea University, Sweden.

The successful candidates will join a multi-disciplinary, energetic team to study the role of the extracellular matrix and cell-cell interactions in the development and control of pancreatic infiltration and islet destruction underlying Type 1 diabetes, funded by the Juvenile Diabetes Research Foundation.

Successful applicants will have received a Ph.D. or M.D. no longer than three years prior to their application and a background in molecular and cellular biology, genetics and/or immunology.

Submit Resume to:
Dr Susanna Cardell, Dept Med Microb. & Immunology, Goteborg University, Box 435, S-405 30 Goteborg, Sweden [**susanna.cardell@microbio.gu.se**].

Dr Lydia Sorokin, Institute of Biochemistry and Pathobiochemistry, Muenster University, Waldeyerstr. 15, D-48149 Muenster, Germany [**sorokin@uni-muenster.de**].

Dr Dan Holmberg, Dept. Medical Biosciences, Building 6M, Umea University, S-901 85 Umea, Sweden [**dan.holmberg@medbio.umu.se**].

Please include full contact information for three references. Applications lacking references will not be considered. We thank you in advance for your interest. Only those applicants selected for an interview will be contacted.

Post-Doctoral Positions Available

Post doctoral positions are available immediately for studies on:
1) Inflammation in the central nervous system, in particular mechanisms of T-cell transmigration of blood vessels in mouse models of experimental autoimmune encephalitis. Work involves investigation of the role of extracellular matrix molecules and matrix metalloproteinases on T-cell extravasation, and T-cell signal transduction pathways. See J. Cell Biol. **153**, 933-945, 2001, for details.
2) Laminin biochemistry Project involves generation of recombinant extracellular matrix molecules using a eucaryotic system, and biochemical characterization and functional analyses of native and recombinant extracellular matrix molecules with relevance to human diseases, such as diabetic and autoimmune glomerulonephritises and congenital muscular dystrophies; analysis of knockout mice.

Candidates should submit a full CV to Dr Lydia Sorokin, Institute of Biochemistry and Pathobiochemistry, Muenster University, Waldeyerstr. 15, D-48149 Muenster, Germany [**sorokin@uni-muenster.de**].

Please include full contact information for three references. Applications lacking references will not be considered. We thank you in advance for your interest. Only those applicants selected for an interview will be contacted.

Responsible Newsletter Editors

Jamie Fitzgerald: fitzgerj@ohsu.edu
Dieter Reinhardt: dieter.reinhardt@mcgill.ca

Helen Muir – Obituary
Tribute to Professor Helen Muir 1920-2005
Given by Professor Tim Hardingham, University of Manchester, at the funeral service, St Mary's Church, Hornby-in-Bedale, N. Yorks on 7th Dec 2005

Where do I start? I first met Helen almost 40 years ago, when she interviewed me for my first job as a young research scientist. My friends who were more experienced than me and had already come across Helen told me before I travelled to the interview in London, that "she will eat you for breakfast". So the odds weren't on me being successful. However, I survived and she gave me the job, which very much impressed my colleagues and I stayed working with Helen for many years and in that time I learnt an immense amount from her and more and more as the years went by. Helen had a remarkable life and career, and for those of you who did not know her well, she was born in India in 1920 and only had formal education when she came to school in Europe aged about 10. Once at school she received little teaching in science, which she was interested in, but typical of Helen she managed to cram to gain entrance to Somerville College, Oxford. Initially this was to read Medicine, but she quickly changed to Chemistry, which, under the guidance of her tutor, Dorothy Hodgkin, she found much more interesting. Helen graduated in 1944 and stayed on at Oxford to do research and a doctorate. She was a young, beautiful and exceptionally bright scientist and she was destined for great success and achievement. Helen's first work was on the chemical synthesis of penicillin, for it was war-time (1940s) and the supply of penicillin to stop wound infection was most important, so this was a high priority project. Helen then moved on to research that was more biological when she joined Professor Albert Neuberger at the National Institute for Medical Research in London. Her work was initially on porphyrin biosynthesis and then she went on to study collagen biosynthesis and this led her into her interest in human connective tissues, where most of her later success and achievements are based.

These were Helen's formative years in research and she was finding her independent academic feet and at this stage there was only modest portent of the achievements that lay ahead. For Helen was later to become the first woman appointed to the Council of the MRC (Medical Research Council), which was leading council deciding the nations medical research priorities. She was also later appointed as a Trustee of the Wellcome Trust and this was at a time when the Trust was evolving to become the major biomedical research funding agency in the UK, indeed it became even larger than the government's funding and Helen played a major part in that expansion of the Trust. It's a measure of the high regard in which Helen was held that she was selected for these important positions. She was the subject of a Channel 4 documentary on 3 women in science entitled "Our brilliant careers" and it reminded us, that a woman, such as Helen, in the era in which she lived, had to be exceptional to succeed in this male dominated world of science, not only exceptional, but also very determined and that was certainly a trait that Helen had in abundance.

The important positions Helen held are onerous and require much hard work and endeavour, however, they do not come entirely without some perks. The MRC Council is chaired by a nonscientist and when Helen was on Council, the Chair was the Duke of Northumberland. Coincidently he was a keen hunting man and Helen was also very keen on hunting, so much to her delight she was on occasions a guest of the Duke of Northumberland out hunting at Alnwick Castle. To

say that Helen was keen on hunting is perhaps an understatement, indeed at one point it almost cost her her life. She had a bad riding accident in the late 50s, but typical of Helen, it didn't stop her hunting and she continued until about 10 years or so ago. Much of her riding was done here in Bedale, but she does have the dubious honour of being cautioned by the police for speeding in Richmond Park in London, ….this was the park police … and Helen was on her horse and she was galloping when she shouldn't. She did, however like travelling fast and it was known that a green Mark 2 Jaguar was regularly seen as a blur on the A1 heading north from London, for here in Bedale was the main home of her riding exploits and this is where she escaped to leave behind the mess and confusion of London and to sample the clear air of Yorkshire. Helen also liked ballet and when I first knew her she regularly went to ballet classes, as she put it, to keep fit. She admitted that she wasn't so agile, poised or elegant as the young ballerinas also in the class, who incidentally were less than half her age, but this far from fazed Helen and as far as we were concerned she had all the poise and elegance of any ballerina, she was certainly our prima donna.

Much of Helen's career was spent at The Kennedy Institute of Rheumatology in Hammersmith, which was the world's first specialist Rheumatology Institute and it was funded by the Arthritis Research Campaign. Helen was recruited to the Kennedy to Head a Research Division in 1966 and she went on to become Director in 1977. The major achievement of Helen' s was in moving research in joint diseases from an era of observational descriptive pathology to the molecular and cellular analysis of the processes underlying these diseases. Basically, this was bringing better science to tackle these tough chronic medical problems. Her achievements here can be measured by the plethora of awards that flooded her way. Helen won the Bunim Prize, the Steindler Award, the Feldberg Prize, the Neil Hamilton Fairley Medal, the Ciba Medal and she was awarded the CBE for her contribution to medical research. She was also awarded many honorary degrees including from the University of Edinburgh, where her great grandfather had been Principle. She was also elected to become a Fellow of The Royal Society, the most prestigious scientific society in the UK. Not only an FRS, Helen was also elected a Fellow of the Swedish Academy of Sciences. Its one thing to be so highly recognised in ones own country, but it's a true measure of the esteem in which Helen was held that she was so honoured in another country. Helen also had a great influence on people, particularly the many national and international scientists who spent time at the Kennedy Institute.

She had a great influence on many, including myself, and I'm pleased that others, similarly influenced, are here with us today. I've also been getting e-mails all week from Helen's many friends and progeny from around the world. Indeed there is a league of friends of Helen that straddles the globe, …. we call it "Helen Muir's bag carriers", HMBC, not least for the reason that you didn't have to be long at the Kennedy without travelling with Helen to some meeting, or other, and being required to carry her rather old battered blue suitcase with a strap around it and sort out the travel arrangements - so if you were allowed to carry the bag it was like a badge of honour. We've talked about having a club tie and maybe a dinner and I'm sure we will one day.

I have organised more retirement parties and meetings for Helen than I care to remember. Frequently these were scientific meetings in her honour. There was one when she was 65, one when she was 67, one when she was 70, another at 75 and a very special one in 2000, when she was 80. We organised a 2-day meeting in Manchester with 65 scientists from around the world talking science in Helen's honour. Basically, it should have been clear from the start that one thing that Helen would never do is retire. The last gathering we had was just 2 months or so ago to celebrate Helen's 85th birthday, again with a few members of the bag carriers present, this time at her house, a small gathering, but very much in Helen's honour. Now, it's not just anyone who can generate such respect and devotion. Helen was many things, but she was certainly never dull. She couldn't stand fools and often let them know it. She couldn't stand pomposity and although she was a grand lady she had a wry sense of humour and liked a good laugh. She was very much without prejudice and judged people on their merits.

Helen's last few years have not been without difficulty, there was pain discomfort and increasing personal frailty and she typically bore it with stoic resolve. Here I must pay tribute to the massive contribution that Andrea and the local team have made in providing Helen with the care and attention she has required, basically making her life bearable. Its hasn't been an easy task and I know that increasingly in recent years and certainly in the last few months, it has just about taken over Andrea's life. As I've seen in visiting Helen, they have done an outstanding job in organising it all and providing it all and I know Helen was immensely grateful and reassured by the love and care she received from Andrea, Anita and Jane, and from the trust she had in John and the way he managed the other affairs around Langlands House. My view of Helen can be summed up in an e-mail I received from the Chairman's Office of The Wellcome Trust, which said that Helen was held "in very high esteem" and she was known as "a very special lady". I'm pleased that we are all here today to say a sad farewell to Helen and to celebrate the life and achievements of this very special lady.

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From the Editor's Desk

Dear readers of Matrix Biology,

I am pleased that some readers are responding to my recent invitation (see Editorial, Matrix Biology 24, 509, 2005) to submit contributions to these Editorial pages, and below you will find the first (and, I hope, not the last) of these Guest Editorials. Stimulated by a recent article in Matrix Biology, Paul Bornstein presents some very interesting ideas on the mechanisms by which fibrillar collagen mutations may cause phenotypic abnormalities.

Bjorn R. Olsen

In a recent paper in Matrix Biology, Pfeiffer et al. (2005) provide new information relating to the phenotype of *oim* mice, which lack a functional α2(I) collagen chain. However, I believe that this phenotype, which resembles moderately severe osteogenesis imperfecta in humans, may be of interest to matrix biologists for reasons that extend beyond the additional deficits described by the authors. The o*im* mouse carries a naturally occurring mutation that causes the deletion of a single nucleotide near the 3′ terminus of the translatable proα2(I) mRNA. This deletion alters the reading frame for the final 48 amino acids in the α2(I) chain, and leads to the use of a new stop codon that adds additional amino acids to the protein. Since *oim* mice appear to have normal levels of proα2(I) mRNA, reasons for the lack of a translated chain are still unclear.

A variety of mutations in the human *COL1A2* gene, mostly splice-site mutations, are also associated with the absence of an α2(I) chain. These disorders represent a recessively inherited form of the Ehlers-Danlos syndrome (EDS), and are characterized by joint hypermobility, skin hyperextensibility, and cardiac valvular defects (Schwarze et al., 2004). Surprisingly, many of these patients lack bone involvement and, in contrast to *oim* mice, the α2(I) mRNA in these individuals is generally unstable.

While initial studies of *oim* mice focused on the skeletal defects in these animals, subsequent investigations have uncovered abnormalities in tendons, in myocardium, and in the aorta (see Pfeiffer et al., 2005 for references). In these tissues, as well as in bone, collagen content is significantly reduced. What might be the reason for the reduced collagen content of tissues in mice that lack an α2(I) chain? One possibility is that a collagen molecule composed of three α1 chains is inherently less stable than a heterotrimeric molecule. There are no structural data to support this possibility, and indeed the Pro+Hyp contents of mouse and human α1 chains, properties that promote a stable triple helix, exceed those of α2 chains. It should also be noted that homotrimeric $(\alpha 1)_3$ molecules, known as α1 trimer collagen, account for a significant proportion of the collagen in tissues during normal mammalian embryonic development. However, a decrease in the order of axial packing and a loss of crystalline lateral packing of collagen fibrils was observed in *oim* tail tendon, and collagen synthesized by *oim* fibroblasts in culture was found to be more susceptible to limited proteolysis than heterotrimeric collagen (McBride et al., 1997). On the other hand, lysyl-derived cross-links were increased in *oim* aortas (Pfeiffer et al., 2005), suggesting a relatively normal packing of collagen molecules. It is possible that alterations in fibril formation in the *oim* mouse reflect changes in the association of fibril-associated macromolecules, such as decorin and type V collagen, with nascent fibrils.

I would like to propose an additional explanation, which has not been considered in the literature, for the reduced collagen content in tissues of the *oim* mouse, and in patients who lack the α2(I) collagen chain. Types I, II, and III procollagens contain N- and C-terminal domains that are released proteolytically during or following secretion of the biosynthetic precursors. While the functions of the C-terminal sequences, which include chain association and initiation of triple helix formation, are well established, those of the N-terminal propeptide (N-propeptide) are poorly understood (Bornstein, 2002).

The N-propeptide of type I procollagen, as released physiologically by procollagen N-protease (ADAMTS 2), contains a globular domain largely encoded by exon 2 in the α1(I) procollagen gene, and a short triple helix that terminates in a non-triple-helical 'telopeptide' sequence, which separates this helix from the major collagen helix. More than 25 years ago, Wiestner et al. (1979) provided evidence for a feedback inhibitory effect of monomeric N-propeptide on synthesis of types I and III collagens by fibroblasts in culture. However, the mechanism of this inhibition remained elusive despite attempts of several groups, including our own, to elucidate it. A significant advance occurred when it was realized that the cysteine-rich domain (CRD), encoded by exon 2 in type II

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procollagen from mammals and *Xenopus*, was homologous to the CRDs in proteins in both vertebrates (chordins) and invertebrates (sog) that played important morphogenetic roles in these animals (Zhu et al., 1999; Larrain et al., 2000). Furthermore, *Xenopus* chordin was shown to bind to BMP4, and the N-propeptide of type II procollagen was found to bind to BMP2 and TGFβ1. The latter finding was of particular interest since, in contrast to procollagens I and III, procollagen II was shown to undergo alternative splicing. During embryonic development type IIA procollagen, containing the exon 2 sequence, is expressed in multiple tissues, whereas type IIB procollagen, lacking the exon 2 sequence, is expressed postnatally, predominantly in differentiated chondrocytes. These findings provided indirect support for a role of the globular domain of the type II procollagen in developmental processes.

Because of the interest of our laboratory in the function of the type I N-propeptide, we generated a mouse with a targeted deletion of exon 2 in the *Col1A1* gene, thus replicating the type IIB splice form of type II procollagen in type I procollagen (Bornstein et al., 2002). To our surprise, homozygous mutant mice were essentially normal. In particular, none of the steps in collagen biogenesis thought to be dependent on the N-propeptide were defective. However, there was a significant, but background-dependent, fetal mortality, which suggested a role for the type I collagen N-propeptide in developmental processes. Convincing evidence for an important role of the type II N-propeptide in development of the heart is provided by the very recent evidence that mice with a targeted deletion of exon 2 in the *Col2A1* gene die in utero with cardiac malformations (Cheah et al., 2005). Interestingly, this phenotype is also highly dependent on the genetic background of the mice.

The substitution of a third α1 chain for an α2 chain, as occurs in the *oim* mouse and in some patients with EDS, has two consequences for the N-propeptide of the resulting homotrimeric type I procollagen. Since the proα2 chain lacks the CRD sequence encoded by exon 2 in proα1, the N-propeptide released from homotrimeric type I procollagen will have three rather than two potential binding sites for cytokines. That difference could be important since, as shown by Larrain et al. (2000), more than one CRD is required for effective binding of BMP4 by chordin. Furthermore, since there are no interchain covalent bonds in type I N-propeptide, the multimeric character of the propeptide depends entirely on the stability of the short triple helix at its C-terminus. The amino acid compositions of the mouse and human repeating Gly-X-Y sequences in the short triple helix predict that an N-propeptide composed of three proα1 chains will have greater thermal stability than one composed of two proα1 and one proα2 chains. I therefore suggest that another consequence of a lack of the α2 chain in mice and humans, in addition to those resulting from differences in the collagen molecules themselves, might be the enhanced interaction of the N-propeptide released during processing of the homotrimeric procollagen with members of the TGFβ superfamily. The effects of such interactions should be considered in studying the phenotypes of *oim* mice and patients with this subset of EDS, since the very high degree of identity in amino acid sequences in the CRDs of types I, II, and III procollagens makes it likely that they share similar intrinsic properties. The marked differences in the phenotypes of mice with a targeted deletion of exon 2 in types I and II procollagens can be attributed to the very different spatial and temporal patterns of expression of the two procollagens, and to the possibility of partial compensation by type III procollagen, which is often co-expressed with type I, in mice that lack exon 2 in type I procollagen.

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