

The British Society for Matrix Biology Annual General Meeting took place in the picturesque surroundings of Queen's College, Cambridge on 10th/11th April, 2006. The theme of the meeting was 'Proteases - the cutting edge of cell biology' and was organised by Graham Riley and Gill Murphy. The meeting was preceded by a satellite symposium on April 9<sup>th</sup>, entitled 'Tendon and ligament remodelling and regeneration', organised by Graham Riley and Helen Birch.

Sixty delegates registered for the satellite symposium, comprising 23 full members of the BSMB and 6 student members. There were 31 non-members of the society, which included 8 students. Sponsorship for the satellite meeting was gratefully received from the Logres Trust, Smith & Nephew, The Biochemical Society and Dunn Laborotechnik. We hope that the proceedings of this meeting will be published in a special edition of IJEP.

One hundred and fifty eight delegates were registered for the AGM, comprising 61 full members of the society and 19 student members. There were 78 non-members of the society, which included 16 students. 31 delegates also attended the satellite symposium. Sponsorship for the main meeting was gratefully received from GlaxoSmithKline, Medivir, AstraZeneca, The Company of Biologists, British Heart Foundation, Triple Point Biologics and Invitrogen. There were also trade stands by company representatives from Helena Biosciences, Merck, R&D Systems, BMG Labtech, GE Healthcare and Dunn Laborotechnik. There were five recipients of BSMB bursaries (£125 each) to attend the meeting. Conference presentation bursaries were given to Natalie Robinson and Julie Huxley-Jones (both of the University of Manchester). Conference reporter bursaries were given to Martin Reid (Sheffield Hallam University), Matthew Jefferson (University of Newcastle) and Natalie Thompson (University of Liverpool). IJEP sponsored poster prizes of £150 each were awarded to Julie Huxley-Jones (University of Manchester), Sylvia Jordans (International University Bremen) and Charlotte Selvais (Universite catholique de Louvain, Brussels).

The first session on 'Matrix-degrading proteases', chaired by Tim Cawston (Newcastle), was opened by Professor William (Bill) Parks from the University of Washington, who presented a talk entitled 'Matrix Metalloproteinases - Old Family, New Tricks'. He suggested that the major misconception with MMPs was just looking at them as ECM turnover and degrading enzymes. ECM degradation and turnover is often not their sole or main function. MMPs can act on a wide range of targets such as cytokines, chemokines and receptors. Therefore MMPs can be described as extracellular processing enzymes, which can regulate cell-cell signalling in the homeostatic processes. Dr Parks, using gene targeted mice, has identified distinct and non-overlapping functions of different epithelial-derived MMPs. MMPs can have both beneficial and pathological effects, and targeting specific enzyme substrate interactions could provide a way to prevent pathological effects. MMP7 is a good example of how MMPs can control a range of functions in one cell type. In damaged mucosal epithelium MMP7 is induced by migrating cells. It is required for cells to shed their cell-cell attachments. In MMP7 null mice, cells cannot shed their cell-cell attachments and cannot repopulate a wound in the gut. In addition, MMP7 controls the transepithelial migration of neutrophils. Epithelial cells express a neutrophil chemokine attractant, which attaches to a transmembrane proteoglycan. MMP7 cleaves a portion of the glycoprotein releasing the chemokine. This creates a chemotactic gradient of bound chemokine, directing neutrophils to the luminal space. In MMP7 null mice the chemokine is not shed and neutrophils do not migrate. Epithelial repair is a desired outcome, but neutrophil influx can lead to inflammation. Therefore, if detrimental

interactions could be targeted, undesirable side-effects could be minimised when targeting MMPs for therapeutic benefits.

Julie Huxley-Jones (University of Manchester) gave a presentation of her work on the molecular evolution of vertebrate metalloproteinases. The transition from the relatively simple invertebrates to the much more complex vertebrates involved genome duplication events. A particular gene could then be said to have evolved at one of three levels depending on whether it was retained after duplication, not retained after duplication or duplicated later along the vertebrate lineage. The study looked at how the extracellular matrix of the vertebrate has evolved by examining the genome of *Ciona intestinalis*, an invertebrate chordate. Using BLAST analysis, sequences of genes present in vertebrates were compared to those present in *Ciona*. From this research it is thought that TIMP and ADAMTS genes arose after vertebrates diverged from *Ciona* and are therefore vertebrate specific. Some MMPs, for example some membrane-bound MMPs, have an invertebrate pattern, whereas others such as collagenases and gelatinases appear to have evolved later in vertebrate evolution. The ADAM genes do not seem to show a consistent pattern. The differences in evolution between different metalloproteinase genes give an insight into the increased complexity that accompanied vertebrate evolution.

Dr. Suneel Apte (Cleveland, USA) opened his talk with an interesting overview of the ADAMTS family before moving on to discuss some of their general regulatory principles, including coordination with substrate gene expression, post-translational modifications of enzymes and substrates, cofactor binding (e.g. fibronectin and fibulin) and inhibitors. Interestingly, Dr. Apte believes that ADAMTSs have co-evolved with their specific substrates. He backs this up with the fact that *Ciona intestinalis* has no ADAMTS-13 due to the lack of blood-type circulation. ADAMTSs have similar activities but different functions due to the expression of these enzymes in different locations throughout the body. Dr. Apte proceeded to discuss some of his recently published work on ADAMTS-9 regulation. Using a pulse-chase technique and an RP4 antibody against the prodomain (TriplePoint) it was shown that ADAMTS-9 is processed by furin at the cell surface (not in the trans-Golgi network) in a variety of cell types before being released into the media. This shows there is likely to be a variety of mechanisms for processing ADAMTSs which need to be investigated. Two inherited connective tissue disorders involving ADAMTSs were discussed by Dr. Apte to highlight their critical physiological roles. Ehlers-Danlos syndrome is characterised by fragile skin due to improper assembly of collagen fibrils. The disorder is caused by mutations of ADAMTS-2, leading to a loss of skin procollagen I processing, but no defects in procollagen II processing. Recessive Weill-Marchesani syndrome (WMS) results in a very muscular, stocky phenotype involving lens dislocation and short hands. WMS is caused by a loss of ADAMTS-10. Interestingly, Marfan syndrome has the opposite phenotype of WMS and is caused by fibrillin-1 mutations. Dr. Apte's laboratory has shown that fibrillin-1 and ADAMTS-10 co-localise thus indicating that the enzyme is a component of the fibulin network. Dr. Apte concluded his talk by emphasising the need for more extensive biochemical studies into ADAMTSs to further elucidate the roles they play in certain extracellular molecular networks.

Dr. Fraser Rogerson (Parkville, Victoria, Australia) concluded the first session by delivering a presentation on work performed with a mice strain lacking both ADAMTS-4 and -5 activities (TS-4/5  $\Delta$ -cat mice). Femoral head explant cultures were stimulated with either interleukin-1 $\alpha$  (IL-1 $\alpha$ ) or retinoic acid (RA) and aggrecanase activity measured by glycosaminoglycan (GAG) release and western blotting against aggrecan cleavage sites.

IL-1 $\alpha$  and RA both increased aggrecan cleavage in wild-type mouse femoral head explant cultures whereas in TS-4/-5  $\Delta$ -cat mice, IL-1 $\alpha$  did not increase cleavage at any cleavage site. However, RA did increase cleavage at some cleavage sites, in particular those with Gly-Gly bonds. The results presented using the TS-4/5  $\Delta$ -cat mice indicated that ADAMTS-4 and -5 are not the only aggrecanases involved in mouse cartilage aggrecan breakdown. Another aggrecanase enzyme(s) is up-regulated by RA but not IL-1 $\alpha$  with a different specificity to ADAMTS-4 and -5. It will be interesting to see the results of further studies aimed at identifying this enzyme(s).

The final session of the day, 'Structure, function and regulation of metalloproteinases' was chaired by Ian Clark. The first speaker was Professor Hideaki Nagase (London, UK) who presented his work comparing the regulation of aggrecanase activities of ADAMTS-4 and -5. Professor Nagase's group expressed recombinant ADAMTS-4 and -5 and investigated the function of the non-catalytic domains in aggrecan breakdown using a series of domain-deleted mutants. His results revealed some interesting differences and similarities between these 2 important aggrecanases. Professor Nagase showed that the full-length form of ADAMTS-4 had a higher aggrecanase activity (100%) as opposed to when the spacer region is deleted (90% activity). Deletion of the spacer and cysteine-rich region led to only 30% of the aggrecanase activity being retained. However, the full-length variant lacked general proteolytic activity on other substrates whereas removal of the spacer region led to the ability of ADAMTS-4 to cleave decorin, biglycan, fibromodulin and fibronectin. Full-length ADAMTS-5 on the other hand was shown by Professor Nagase to possess both aggrecanase and general proteolytic activities. When the 2 enzymes were compared in full-length form, ADAMTS-5 was found to have 1000 times greater aggrecanase activity than ADAMTS-4. He also showed that ADAMTS-5 activity is affected by its non-catalytic domains. The aggrecanase activity is reduced by 99% by deletion of the spacer and C-terminal thrombospondin domain. It was shown that the spacer region is responsible for the binding of ADAMTS-4 to the ECM. In contrast, ADAMTS-5 requires the cysteine-rich and spacer regions for anchorage. Professor Nagase proceeded to discuss the effect of sodium chloride (NaCl) concentration and pH on ADAMTS-4 and -5 activities. ADAMTS-4 activity decreases at 200mM NaCl whereas ADAMTS-5 shows maximal activity at 250mM NaCl. The optimal pH range over which ADAMTS-5 cleaves aggrecan is between 5.5 and 9.5 whereas ADAMTS-4 has a more specific pH range of 7.5-8.5. His group indicated that post-translational modification of ADAMTS-5 occurred at a low pH at physiological salt concentration. Professor Nagase's talk concluded with data on TIMP-3, a known inhibitor of ADAMTS-4 and -5. His group has generated TIMP-3 mutants which inhibit ADAM-17 (TACE), ADAMTS-4 and -5 but not MMPs. These inhibitors block IL-1 stimulated aggrecan breakdown. This shows that a selective inhibitor of ADAMTSs is possible in the future, based on these mutants.

Dr. David Young (Newcastle), a late replacement for Dr. Norman McKie who was unable to attend the meeting, delivered an interesting insight into metalloproteinase expression and regulation during cartilage resorption. He opened the talk by emphasizing the key role that chondrocytes have to play in osteoarthritis in that they express MMPs and ADAMTSs, the main enzymes involved in cartilage destruction. The control of these enzymes takes place at many levels including transcription, translation, activation and through inhibitors. Dr. Young utilised information generated by the Bovine Genome Project to design primer pairs for the enzymes prior to performing real-time RT-PCR on RNA extracted from bovine cartilage following stimulation with IL-1 and oncostatin M. It was shown that IL-1 increased GAG release from cartilage indicating metalloproteinase activity. Real-time PCR

analysis showed that ADAMTS-4 and -5 expression increased 100 fold following stimulation whereas ADAMTS-9 expression increased 10 fold. MMP-1 expression was up-regulated approximately 1000 fold as was MMP-13; however, only a slight up-regulation of MMP-14 was observed. By day 5 of the stimulation period there was a decrease in TIMPs, the resultant excess of metalloproteinases over inhibitors may have led to the collagenolysis observed after 10 days. The data emphasises the fine balance between TIMPs and metalloproteinases and that activation is a critical step in ECM turnover regulation. Dr. Young went on to discuss some of his work on how genes expressing metalloproteinases and their inhibitors are transcriptionally regulated by chromatin modifications. Histone deacetylases (HDACs) remove the acetyl groups from histones, resulting in euchromatin becoming heterochromatin with concomitant gene silencing. Inhibitors of HDACs (HDACi) have proved efficacious for inflammatory arthritis by prevention of cartilage destruction by suppression of metalloproteinase gene expression. Dr. Young's group has been investigating which signalling pathways HDACi interferes with. To date the NF $\kappa$ B and MAPK pathways have been looked at. NF $\kappa$ B appears to be intact whereas the MAPK is interesting as TGF- $\beta$  induction of ADAM-12 and TIMP-1 are MAPK dependent.

Rama Khokha (Ontario Cancer Institute, Toronto, Canada) gave a fascinating talk about the involvement of TIMPs in inflammation and cancer. She began by describing the events involved in the onset of cancer, and how it is connected with inflammation. An increase in NF $\kappa$ B associated with inflammation promotes survival of epithelial tumour cells. Apoptosis, angiogenesis and metastasis, are also features of early cancer. Dr. Khokha's main interest lies in the role of TIMPs in mediating or promoting inflammation. The main TIMP of interest is TIMP3, which is bound to the extracellular matrix. In order to explore its activity, TIMP3 knockout mice have been created. While the TIMP3 knockout mice were viable, the *timp3<sup>-/-</sup>/ timp2<sup>-/-</sup>*, was lethal, suggesting that TIMP2 may compensate for TIMP3. Analysis of the TIMP3 promoter and transcription factor binding sites has revealed that it contains NF $\kappa$ B and c-myc binding motifs. Increases in TIMP3 levels are also associated with apoptosis. To study the role of TIMP3 in cancer, Dr. Khokha's group have been using the mammary gland model in the mouse. Regarding the role of the TIMPs in cell proliferation and ductal elongation, it was found that TIMP1, 2 and 4 were inhibitory, whereas TIMP3 had the opposite effect. TIMP3 has been found to be a negative regulator of inflammation by regulating TNF $\alpha$ . In *timp3<sup>-/-</sup>* mice, mammary glands showed decreased lumen progression and increased adipogenesis, as well as accelerated involution. In addition, degradation of E-cadherin and also of  $\beta$ -catenin, its binding partner, was increased. TNF $\alpha$  converting enzyme (TACE) was no longer inhibited, resulting in an increase in TNF $\alpha$ . When treated with recombinant TIMP3, the observed effects on E-cadherin and TACE could be reversed. It has already been shown that some organs, for example the liver, have a deficiency of TIMP3. This can be seen by the baseline TNF $\alpha$  shedding in the liver, due to a lack of TACE inhibition caused by low TIMP3 levels. Next, the contribution of TIMP3 in inflammation was discussed. Loss of TIMP3 leads to increased inflammation in 3 ways: increased MMP activity leading to loss of cell-ECM adhesion and cytoskeletal rearrangement; increased ADAMs-mediated shedding of TNF $\alpha$  and triggering TNF-induced cell death by the caspase cascade; and an influx of inflammatory cells. In the next set of experiments, *timp3<sup>-/-</sup>* mice were given mouse mammary tumour virus (MMTV) along with the polyoma MT virus to induce a particularly aggressive form of mammary gland tumourigenesis. It was found that the number of tumours that developed in these *timp3<sup>-/-</sup>/ MMTV-PyMT* mice was significantly reduced compared to wild-type *MMTV-PyMT* mice, although PyMT expression was not

affected. To investigate whether tumour suppression applied to the epithelium or the stroma, wild-type epithelium was transplanted into both a wild-type and knockout stroma, and knockout epithelium was transplanted into both a wild-type and knockout stroma. Where both the epithelium and stroma were wild-type, the tumours developed at a normal rate. Where both were from knock-out mice, tumours did not grow. In both cases of mismatch between the epithelium and stroma, tumour development occurred but was suppressed, indicating that TIMP3 is equally suppressive of tumour formation regardless of where the tissue originated. However, this model was criticised because tumour growth is so rapid. To address this, the next experiments were carried out on mice which were given MMTV-Neu, a form of the virus which causes tumours to develop more slowly. In *timp3<sup>-/-</sup>/MMTV-Neu*, there was 50% tumour suppression compared to wild-type *MMTV-Neu* mice; in heterozygous *timp3<sup>+/-</sup>/MMTV-Neu* mice there was still some suppression. In *timp1<sup>-/-</sup>/MMTV-Neu* mice there was no suppression, which suggests this effect is specific to TIMP3. The suppressive effect of TIMP3 deficiency on tumour development is interesting compared to the results of the group's earlier work because it suggests that TIMP3 suppresses inflammation but also reduces resistance to MMTV induced mammary tumourigenesis. The mechanism of tumour suppression was also looked at, looking at epithelial to stromal swaps, bone marrow transfers and apoptosis in MEC cells. Another experiment will involve breeding of *rag<sup>-/-</sup>* mice, which are severely deficient in T and B cells; if tumour suppression occurs in these mice, then it will indicate that T and B cells are not important in this process. Further work will be to look at whether the effects seen are specific to TIMP3, if the effect is seen in other site or in human breast cancers, and whether TIMP3 haploinsufficiency exists in every phenotype.

The second day of the meeting opened with a session entitled 'Proteases and the regulation of matrix turnover', chaired by David Buttle (Sheffield). The first speaker was Vince Everts (Universiteit van Amsterdam and Vrije Universiteit, Amsterdam) who discussed intracellular remodelling of the matrix. He began by describing how the extracellular matrix (ECM) is continuously remodelled by both synthesis and resorption. Resorption and digestion of fibrillar collagen can be carried out by cells from different connective tissues: fibroblasts, chondrocytes and osteoblasts/bone lining cells. Fibroblast function was studied using periodontal ligaments in the continually erupting mouse incisor. Here there is shear stress within the ligament, which leads to eruption of the ligament and intracellular collagen release. There is also collagen degradation and apoptosis in the junctional epithelium, and collagen-containing phagolysosomes in the ligament. The rapid turnover and short half life (7 days) make this ligament the ideal model to look at ECM remodelling. To examine whether the intracellular collagen present is newly synthesised or the result of phagocytosis, Professor Everts' group looked at the effects of blocking these processes. Inhibition of phagocytosis resulted in a lack of intracellular collagen, whilst the inhibition of collagen synthesis had no effect, suggesting that the collagen detected was the result of phagocytosis. Prior to uptake of collagen by fibroblasts, both fibrillar and non-fibrillar collagen and collagen-associated non-collagenous proteins (NCPs) are partially digested by enzymes active at the plasma membrane, mainly MMPs. Following internalisation, further digestion is performed by lysosomal enzymes. It has been shown that these enzymes are mainly cysteine proteinases, since an increase in cross-linked collagen fibrils due to lack of digestion was seen when this class of enzyme, but not serine or aspartic proteinases, were inhibited. In order to elucidate which cysteine proteinase was involved, Professor Everts' group in turn inhibited cathepsins B, K and L, and created knockout mice deficient in these proteinases. Inhibition of cathepsin B led to reduced collagen degradation, but the knockout mouse showed no accumulation of collagen fibrils,

suggesting that collagen digestion is still taking place. The same pattern was found for cathepsin L. However, in mice where cathepsin K was inhibited or deficient, an accumulation of non-digested collagen was found. This accumulation is seen in pycnodysostosis, an osteosclerotic disease in which areas of non-digested bone are found adjacent to osteoclasts. These results suggest that a cathepsin K deficiency could be responsible in this condition. Professor Everts then talked about which MMPs are involved in digestion of the collagens and NCPs prior to uptake by fibroblasts. It has been shown that MMP-1 is not involved in this process, but MMP-2 and MMP-14 (MT1-MMP) have been implicated. MMP-2 levels have shown a positive correlation with the amount of phagocytosed collagen detected, and inhibition of MMP-2 has been seen to block phagocytosis. It was thought that collagen is denatured before uptake by fibroblasts, due to the presence of H<sup>+</sup>-ATPase, which lowers the pH. This has been confirmed by blocking the action of H<sup>+</sup>-ATPase, which resulted in the inhibition of hydroxyproline release and complete blockage of collagen phagocytosis. To investigate the extent to which MT1-MMP influences collagen resorption, knockout mice were created. It was found that there was huge phagocytic activity in the *MT1MMP*<sup>-/-</sup> mice, and the number of phagosomes in the intracellular space increased significantly. This suggests that MT1-MMP is not involved in segregation and uptake of collagen fibrils. It can be concluded from these experiments that there are two pathways of collagen digestion: MT1-MMP is involved in pericellular cell remodelling, whilst MMP-2 mediated collagen digestion occurs intracellularly. It is thought that the extracellular pathway is increased in inflammation, as IL-1 is increased and TGFβ is decreased, and that the intracellular pathway is involved in normal turnover, when there is a decrease in IL1 and an increase in TGFβ. Bone lining cells (BLCs) are also capable of resorbing collagen during ECM remodelling. These cells cover the inactive bone surface and clean it by engulfing and resorbing collagen fibrils before bone resorption by osteoclasts. By culturing bone with either cysteine proteinase inhibitors or MMP inhibitors then looking at the effects on the bone matrix, it could be ascertained that BLC cleaning of resorption pits is dependent on MMP activity. After cleaning, the BLCs deposit a cement layer. Professor Everts concluded his excellent presentation by giving a brief overview: how different cell types can internalise and digest ECM components; how intracellular digestion is essential for normal matrix turnover; how the ECM is internalised during digestion and remodelling, and how MMPs and cysteine proteinases are involved in these processes.

Klaudia Brix (International University, Bremen) talked about lysosomal proteases in extracellular matrix remodelling. In order to understand the significance of proteolytic cleavage in disease, we must first understand the biological functions of proteases. The main focus of work in Professor Brix's laboratory is analysis of the lysosomal cysteine cathepsins and their roles in remodelling in the intestines, skin and thyroid. In the skin, the cathepsins are involved in rapid migration of keratinocytes during wound healing. They are expressed in epidermal keratinocytes, with cathepsin B being expressed in the keratinocytes of all layers of human skin. During wound healing, cathepsin B is increased. Five days after wounding, a hyperproliferation zone is created between the dermal and epidermal layers. An experiment was carried out using a human keratinocyte cell line (HaCaT) monolayer. The monolayer was scratch-wounded, and it was found that cathepsin B was secreted, whilst cathepsins L and V are not. Mature cathepsin B is secreted into the extracellular space of wounded cells, and further studies show that inhibition of this extracellular cathepsin B results in an impaired migration capability of wounded keratinocytes and only partial cell regeneration. This suggests that it is this cysteine protease which mediates extracellular matrix remodelling in healing. In order to determine

the source of cathepsin B, a biotinylated probe was used to label the active site. This showed that when HaCaT cells wounding occurs, cathepsin B is secreted from the cell surface. Further work showed that this cell-surface cathepsin is proteolytically active. Professor Brix went on to discuss experiments that had been performed to investigate the theory that annexin II tetramer and LRP1 are binding partners of cathepsin B. This was ruled out in the case of annexin, as the p11 light chain of the tetramer did not co-localise with cathepsin B. However, it was found that LRP1 does co-localise with cathepsin B during repair of permeabilised cells. Inhibition of binding between cathepsin B and LRP1 resulted in an increase in mature cathepsin B release from lysosomes, therefore it can be concluded that HaCaT keratinocyte LRP1 is a receptor for secreted cathepsin B. The roles of the other cathepsins have also been examined. Cathepsin V is localised in sub-nuclear structures and is thought to regulate cell proliferation. Cathepsin L is expressed in the vesicles of keratinocytes. Both are thought to be involved in intra-epidermal migration. Of the cathepsins, only B is involved in extracellular matrix remodelling. The next part of this interesting lecture concerned the role of cathepsins B and L in the intestine. L is mainly found in the intestinal epithelium, whereas B is localised in the endosomes and lysosomes, and also in association with the apical plasma membrane. In order to look at the effect of surgical trauma to the intestinal epithelium, an *in vivo* rat model was used. In this model, cathepsin B was increased, whereas L was decreased, suggesting that each of these proteases has its own specific function in the mucosa. Cathepsin D also decreased and it is possible that it could be a diagnostic marker for local damage to the intestinal mucosa. Even though cathepsin B release increased, there was no associated change in its mRNA. Possible targets for cathepsin B have been suggested as APN/CD13,  $\beta$ 4 integrin, and E-cadherin. When cathepsin B is released, there is damage to the extracellular matrix, which suggests an impaired integrity of the mucosal barrier. This hypothesis was supported by a lack of collagen IV and laminin in the basal mucosa of affected areas, and the fact that cathepsin B deficient mice show an overexpression of both of these proteins. The final topic of Professor Brix's talk was lysosomal cysteine proteases in the thyroid. Thyroglobulin, the precursor of thyroid hormones, is a substrate of these proteases. By looking at mice deficient in each cathepsin, it could be elucidated that cathepsins B, K and L are involved in proteolysis of thyroglobulin. Thyroid function is essential for growth, brain development, metabolism and thermoregulation, and prehormone processing by cysteine proteases is vital for maintaining this function. In order to look at protease function under various conditions of different sites in the thyrocyte, an *in vitro* degradation assay, which mimics neutral, oxidizing conditions of the extracellular space and the acidic, reducing environment of the endocytic vesicles, was used. It was then possible to illustrate that the cathepsins are capable of cleaving the substrate under a variety of conditions, but the result depends on the environment, suggesting that specific proteases are required in exact locations to maintain thyroid function. The presentation was brought to a close with an overview and conclusion that analysis of lysosomal cysteine proteases and interaction with their substrates provides a valuable understanding of the physiology of epithelial cells in both health and disease.

Vince Ellis (Norwich) introduced the subject of plasminogen activation by uPA in the context of its role in several pathological disorders including, cardiovascular disease, arthritis and inflammation. The importance of the plasminogen system in these conditions is based on it being a versatile and powerful source of proteolytic activity able to dynamically alter the interaction between cells and their extracellular matrix. Because of this potential, the plasminogen system is subjected to tight regulation through several factors including the natural receptor for uPA (uPAR) which is known to increase the

efficiency of plasmin generation. Earlier work by Vince showed that the tetraspanin family member CD82 caused a large decrease in uPAR dependant activity which was not due to either altered uPAR expression or uPA activity. Instead it was concluded that in the presence of CD82, the receptor was rendered “cryptic,” causing its relocation to focal adhesions where it was sequestered by the integrin  $\alpha_5\beta_1$ , which offered dynamic regulation of pericellular proteolytic activity. The next part of the talk focussed on the activation of uPA as the process initiating the activation of plasminogen. Several proteases have been implicated in the initiation of the cascade including the recently discovered type II transmembrane serine protease (TTSP) matriptase. Dr Ellis stated that although little evidence exists for a role in uPA activity, they had discovered a role in the plasmin related growth factor, hepatocyte growth factor/scatter factor (HGF/SF) activation. In addition to matriptase activation, hepsin was also shown to activate HGF/SF. In conclusion, Dr Ellis stated that the membrane association of serine proteases allows their localisation and subsequent modulation of activity with specific substrates.

Session 4 was the BSMB Society Session, in which speakers were chosen from the submitted abstracts giving a range of topics within the field of matrix biology. This session was chaired by Bruce Caterson and Dylan Edwards. Silvia Jordans (Bremen, Germany) opened the session with a talk focussed around the production of the thyroid hormones  $T_3$  and  $T_4$  from their common pre cursor thyroglobulin (Tg) as a model to investigate the complexity and interaction of the cysteine cathepsin protease network. Human thyroid tissue was used to carry out a range of investigations that looked at the localisation and transport of cathepsins within tissue whilst an in vitro assay was used to determine the proteolytic activity of several family members under differing conditions. The first major conclusion was that cathepsins B, K and L were mainly distributed within endo-lysosomal compartments. Importantly however, double staining showed that cathepsin L-positive vesicles lacked cathepsin S thereby indicating that endo-lysosomal vesicles contain distinct sets of proteases. The trafficking of active and inactive cathepsins was then investigated through a combination GFP labelling and activity based probes, with the conclusion drawn that transport is not dependent on the enzyme activity. The final part of the talk described how there is good evidence that cathepsins were subjected to secretion into the extracellular follicle lumen and how they had shown that in these non favourable conditions, all cathepsins were still able to degrade their substrate. Subsequent comparison of cleavage patterns then suggested that the location of individual cathepsins and the subsequent proteolysis they induce might be a key regulator of subsequent trafficking and secretion within the same network.

Natalie Robinson (University of Manchester) discussed her research on disease mechanisms of mutations within the C-terminal domain (CTD) of cartilage oligomeric matrix protein (COMP). COMP is a cartilage extracellular matrix (ECM) structural protein of the thrombospondin family. If there is dysfunction in the ECM proteins, chondrodysplasia can arise. Two skeletal dysplasias caused by COMP mutations are pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED). COMP is composed of 5 identical subunits, each of which consists of an N-terminal coiled-coil domain (which is unique to this protein), 4 EGF-like (type II) repeats, 8 calmodulin-like (type III) repeats and a large globular C-terminal domain. Previously it was thought that the *COMP* mutations that cause PSACH and MED only occurred in the type III repeats, which contain calcium binding loops. Patients with *COMP* mutations in this domain retain mutant COMP and other ECM proteins in the chondrocytes, resulting in chondrocytes with enlarged rER. However, PSACH and MED patients who have mutations in the CTD of

COMP have been identified. Since this domain acts as a binding site for type IX collagen and other matrix proteins, it is possible that mutations in this area could prevent these interactions. The purpose of this study is to therefore investigate how mutations in the CTD can affect the structure and function of COMP. Four CTD mutations were chosen to be introduced into *COMP* cDNA, as well as a wild-type COMP and a common type III mutation. Transfection of a mouse chondrocyte cell line (ATDC5) followed, and the cells were then cultured in monolayer or alginate beads for 8 weeks. Western blotting of media from monolayers and immunohistochemistry of alginate beads showed that CTD mutant COMP was secreted into the matrix rather than being retained by the cell, as it would be in the type III mutation. Staining of beads revealed that matrix disposition, nodule formation and collagen secretion were similar to wild-type. These results suggest that there are differences in disease mechanisms resulting from mutations at each site. It seems that CTD mutations produce more heterogeneous effects than type III mutations, so the resulting chondrodysplasia could be due to disruption of interactions with other matrix proteins, thus resulting in abnormal composition of the cartilage matrix.

Charlotte Selvais (Brussels, Belgium) continued the high standard of talks in the BSMB Society Session by discussing the binding of MMP-7 (matrilysin-1) to human epithelial cell membranes and its resistance to TIMP-2. MMP-7 has a broad spectrum of pericellular activities and is therefore recruited to the cell surface. MMP-7 has been shown to bind to the human endometrium, therefore the group looked at potential receptors that facilitate this binding. Charlotte showed that MMP-7 and CD44v3 colocalised, indicating that CD44v3 is a putative important binding site for active MMP-7. Furthermore, the latent form of MMP-7 was shown to colocalise to receptor CD151, which is localised in lipid rafts. Charlotte continued by discussing the binding mechanism of MMP-7. Only the active form of MMP-7 bound to the human endometrial carcinoma derived Ishikawa cell line. Furthermore, the binding of <sup>125</sup>I-labelled MMP-7 is specific to Ishikawa cells (ICs). Further work is ongoing to characterise the membrane binding site of MMP-7. Cholesterol sulphate is one such proposed site which is being investigated. Interestingly the catalytic properties of MMP-7 appear to be retained during cell-surface binding through its active domain. Charlotte showed that MMP-7 is resistant to TIMP-2 when it is bound to ICs. The data presented supports the theory that membrane localisation and docking of metalloproteinases represents an important activational step.

Gareth Wayne (Stevenage, UK) gave a presentation entitled: 'TIMP-3 inhibition of ADAMTS-4 (aggrecanase-1) is regulated by interactions between aggrecan and the C-terminal domain of ADAMTS-4'. Using a method involving a FAM-TAMRA (FITC) labelled FRET peptide previously identified by phage display, aggrecan peptide hydrolysis by ADAMTS-4 was monitored. It was noted that TIMP-3 was a potent inhibitor of the ADAMTS-4 FRET assay whereas TIMP-1 was a poor inhibitor. Interestingly, TIMP-3 inhibition increased in the absence of NaCl. Gareth observed that aggrecan increased TIMP-3 potency when it was perhaps expected to compete. In contrast, aggrecan abolished TIMP-1 inhibition. Furthermore, he showed that aggrecan modulation of TIMP-3 potency was dependent on the C-terminal domains of ADAMTS-4, in particular the TSP-1 motif (through GAG side chain interaction). Further data showed that in the absence of NaCl, the increased potency of TIMP-3 required the cysteine-rich region of ADAMTS-4. However, when NaCl was present, it was found that the spacer region of ADAMTS-4 was required to increase TIMP-3 potency. The talk was concluded by discussing the mechanism by which aggrecan affects TIMP-3 potency. It was shown that aggrecan does not alter the conformation of the catalytic cleft but acts as a competitive inhibitor of ADAMTS-4 with

respect to the FRET assay. Interestingly, another potential inhibitor of ADAMTSs, CGS27023, was unaffected by aggrecan or NaCl.

John Wardale (Macclesfield, UK) presented his groups data on the role of the 95kDa, cell surface serine protease fibroblast activation protein (FAP $\alpha$ ) in the chronic disease of osteoarthritis (OA). With a 15 fold up regulation in OA compared to normal cartilage, it is logical to assume that FAP $\alpha$  is important in the chronic disease of OA. This concept was investigated through the over expression of wild type and mutant versions in human chondrocytes which were then subjected to microfluidic, western blot and enzymatic activity assays. The microfluidic cards were described as an OA fingerprint, and included 10 genes from individual processes implicated in OA including angiogenesis and inflammation. Disappointingly, no significant differences were recorded between the WT and mutant forms of FAP $\alpha$ , except for decreased cell adhesion. The second major conclusion was that FAP $\alpha$  acts not as a collagenase but as a gelatinase, which is consistent with its inability to release proteoglycan from cartilage. In conclusion, because of the negative results, FAP $\alpha$  was not pursued as a drug target but it may still have a protective role in OA and be able to reduce aberrant matrix production and down regulate catabolic genes.

Kaisa Lehti (Helsinki, Finland) described her work on the mouse aortic vascular smooth muscle model. The talk was focussed around the loss of a typical smooth muscle phenotype at P<sub>0</sub> through de-differentiation over subsequent passages characterised by a reduction in the levels of contractile proteins such as actin and myosin. As a way to investigate this, the expression of MT1-MMP at the cell surface was investigated. MT1-MMP is known to be expressed in vascular smooth muscle cells (VSMC) at sites of vessel wall development and vascular injury. The presence of MT1-MMP at these sites is consistent with Kaisa's recent publication that MT1-MMP associates with PDGFR $\beta$  causing the efficient induction of mitogenic and chemotactic signalling by PDGF-BB. The exact role of MT1-MMP was investigated by the comparison of cells extracted from WT and mutant MT1-MMP cell lines. Several major findings were made, including unstable PDGF $\beta$  receptor formation and a loss of de-differentiation in mutant compared to wild type cells. Interestingly, the loss of de-differentiation could be mimicked by MAPK inhibition in wild type cells. Kaisa then summarised the implications of these data by suggesting important roles for MT1-MMP in PDGFR $\beta$  clustering and stabilisation in caveolae, PDGFR signal transduction following PDGF BB stimulation and controlling the contractile machinery in VSMC.

The final session of the meeting entitled 'Proteases and the regulation of cell activities' (chaired by Gill Murphy, Cambridge) was opened by Dr. Ulla Wewer (Copenhagen), who discussed the effects of ADAM12 on cell signalling. ADAM12 exists in 2 forms, one possessing a transmembrane domain and cytoplasmic tail (ADAM12-L) and the other lacking both these domains (ADAM12-S). The enzyme has been shown to promote tumour progression and urine levels of ADAM12 correlate with breast cancer progression. Dr. Wewer has focused on the role of ADAM12 in cell adhesion and signalling by using purified, full-length ADAM12 as an adhesion substrate. Her group showed that  $\alpha 9\beta 1$  is the preferred integrin receptor for full-length ADAM12 and in cell types that do not express this receptor other members of the  $\beta 1$  integrin family can replace its ligand-binding activity. Cells deficient of  $\alpha 9\beta 1$  bound to full-length ADAM12 by  $\beta 3$  integrin and a blocking antibody to  $\beta 3$  inhibits this interaction. Interestingly, the morphology of cells expressing  $\alpha 9\beta 1$  in response to ADAM12 is more rounded. Further studies have shown

that phosphoinositide-3 (PI3)-kinase activity inhibits the spreading of such cells in response to full-length ADAM12 binding. For the next part of her talk, Dr. Wewer discussed Rho GTPase activation downstream of ADAM12 binding. Her data showed that such signalling contributed to cell spreading and stress fibre formation. Dr. Wewer concluded her talk with a fascinating insight into the structure and morphology of ADAM12. Electron microscopy showed that ADAM12 has a four-leafed clover structure and that the excised prodomain remains bound to the mature enzyme. The prodomain is thought to be involved in folding of ADAM12 and its trafficking to the cell surface. Without it ADAM12 remains in the cell.

Agnes Noël (University of Liege) spoke about her work on membrane-associated proteases and angiogenesis. She began by discussing how MMPs play a significant role in angiogenesis, and therefore cancer progression, by mediating extracellular matrix degradation and activating or inactivating a variety of factors such as growth factors, chemokines and cytokines. Attempts to prevent this MMP activity by using broad spectrum MMP inhibitors in clinical trials have failed, so Dr. Noël's team have looked at the contribution of specific MMPs in cancer progression by using MMP-deficient mice. Malignant keratinocytes were cultured on a collagen gel then reintroduced to a host mouse, which would normally lead to tumour formation. In the wild-type mouse, a vascularised invasive tumour developed. However, in the wild-type mouse treated with galardin, a broad-spectrum MMP inhibitor, tumour growth and vascularisation was blocked, supporting the role of MMPs in tumour formation. The same experiment was performed on knockout mice deficient in MMP-2, -3, -8, -9 or -11; all showed invasion and angiogenesis similar to those in the wild-type. Next, double knockout mice were produced: those deficient in both MMP-3 and MMP-9 again had normal tumour growth; however, those deficient in MMP-2 and MMP-9 displayed a complete lack of angiogenesis and invasion. This suggests that these two MMPs are most important in these processes. Further work attempted to recreate this result *in vitro*; however, it was found that in this case angiogenesis still occurred. This gives an idea of the importance of the involvement of the host environment and host cell type in tumour progression. Dr. Noël continued by reviewing the investigations that her group has carried out on other MMPs, especially MMP-19. MMP-19 is not produced by the tumours themselves, but instead by the host cells, especially mesenchymal cells. These cells also produce MMP-2, whereas MMP-9 is mainly produced by neutrophils. It was found that MMP-19 deficient mice gave opposite results to MMP-2/MMP-9 double knockout mice in terms of angiogenesis. In the wild-type, there were spaces between the malignant keratinocytes and endothelial cells *in vitro*. However, in the *MMP19<sup>-/-</sup>* mice, the endothelial cells infiltrated the collagen layer and migrated towards the keratinocytes, indicating acceleration of angiogenesis. A further assay using Matrigel plugs showed that vascularisation of *MMP19<sup>-/-</sup>* mice was greatly increased compared to wild-type. These results suggest that whilst MMP-2 and MMP-9 are positive regulators of angiogenesis and invasion, MMP-19 is a negative regulator and should therefore not be inhibited. This may explain the failure of broad spectrum MMP inhibitors in clinical trials. The next part of the presentation concerned the MT-MMPs. MT1-MMP is an activator of other MMPs, including pro-MMP-2, and it has been shown that deficiency of MT1-MMP is lethal. MT1-MMP is also a regulator of vascular endothelial growth factor A (VEGF-A) expression. An experiment was carried out in which human breast carcinoma MCF7 cells were transfected with MT1-MMP DNA. The overexpression of MT1-MMP in these cells resulted in upregulation of VEGF-A expression, increased invasion (*in vitro*), increased tumourigenicity and increased vascularisation. Previous studies have shown a positive correlation between MT1-MMP

levels and VEGF-A expression in human breast carcinomas and human gliomas. These studies suggest that full activity of MT1-MMP is required for upregulation of VEGF-A, specifically through the Src-tyrosine kinase pathways. The penultimate part of Dr. Noël's talk continued the MT-MMP theme, this time with reference to the role of MT4-MMP in cancer. MT4-MMP is a glycosylphosphatidyl-inositol (GPI) anchored protease, which has been implicated in breast cancer progression. It is an activator of ADAMTS4, but not of pro-MMP-2. It has been shown by staining that MT4-MMP is produced by tumour cells as opposed to stromal cells. It can also be produced by metastatic tumour cells in the lymph node. Research showed that cells which overexpressed MT4-MMP developed tumours, whereas those with normal expression did not. Histological studies found that tumour cells with overexpression of MT4-MMP were larger than those with normal expression, but there was no difference in the number or coverage of the cells. It is clear from looking at the results of investigations into both of these MT-MMPs that MT4-MMP does not affect angiogenic factors such as VEGF-A in the way that MT1-MMP does. It can therefore be deduced that MT1-MMP and MT4-MMP control angiogenesis through different mechanisms. The final part of this intriguing presentation was brought to a close with an overview of the research covered and the conclusion that MMP-2, MMP-9, MT1-MMP and MT4-MMP are possible targets for anti-cancer treatments.

And finally Dr Yoshiumi Itoh, from the Kennedy Institute of Rheumatology, Imperial College, gave an entertaining talk entitled: 'Cellular interactions with the extracellular matrix: regulation and dysregulation of the pericellular microenvironment'. Dr Itoh described the role of the ECM as much more than a space-filler. The ECM communicates a vast amount of information to the cells such as cell differentiation and cell survival signals. This property of the ECM places each cell in a unique microenvironment. Cells can affect their microenvironment by modifying the ECM with ECM-degrading enzymes. Dr Itoh is interested in one such enzyme called MT1-MMP. The enzyme is a type I transmembrane proteinase and is essential for skeletal development. MT1-MMP also promotes cell invasion and motility by pericellular ECM degradation. Dr Itoh continued his talk by showing that the cytoplasmic domain can interact with the subunit of adaptor protein 2, which is responsible for guiding proteins into clathrin cages. He also highlighted that palmitoylation was essential for MT1-MMP to function. The highlight of the talk was the Fluorescence resonance energy transfer work. MT1-MMP1 GFP and MT1-MMP RFP were transfected into cells. The FRET suggested that MT-MMP activation was localised to the leading and trailing edge of migrating cells. He concluded by presenting some data based on MDCK epithelial cells. HGF causes these cells in a collagen matrix to migrate. MT1-MMP was shown to be vital for this. Moreover, MT1-MMP was localised to the basal side of the epithelial cells. Dr Itoh provided evidence for the importance of MT1-MMP in cellular regulation of the pericellular microenvironment, as well as its key function in cell migration.