COST

Domain Committee "BMBS"

COST Action (BM1002*)*

Start Date (*start date of the Action)*

Nanomechanics of intermediate filament networks - NANONET

MONITORING PROGRESS REPORT

Reporting Period: *from (April 2011-April 2012)*

This Report is presented to the relevant Domain Committee.
It contains three parts:

I. Management Report *prepared by the Grant Holder*
II. Scientific Report *prepared by the Chair of the Management Committee of the Action*
III. Previous versions of the Scientific Report*; i.e., part II of past reporting periods*

The report is a "cumulative" report, i.e. it is updated annually and covers the entire period of the Action.

Confidentiality: the documents will be made available to the public via the COST Action web page except for chapter *II.D. Self evaluation*.

Based on the monitoring results, the COST Office will decide on the following year's budget allocation.

Executive summary (max.250 words):

NANONET is dedicated to integrate and increase the scientific knowledge on intermediate filaments. These filaments are a key component of the cytoskeleton and they integrate a full spectrum of cellular responses to biochemical, biomechanical and cellular stresses. NANONET started with 14 participating parties and now 18 countries have signed the MoU. In the first year of NANONET we have established four working groups which all are dedicated to specific scientific aspects of the intermediate filament network. All WGs are growing in number of participants. Currently, WG1 "Mechanotransduction and cell signalling" consists of 39 members; WG2 " Nanomechanical properties and impact on cell mechanics" of 34 members; WG3 " Translation to bio-inspired materials" of 5 members; and WG4 "Intermediate filaments in health and disease" of 64 members. A website was set up, five working group meetings were organized, 4 STSMs were granted and 1 conference was organized, in conjunction with the MC meeting of 2011. Each WG has planned at least one meeting in 2012 and we have planned a training school. Furthermore, we have decided to increase the number of STSMs in 2012 to 6. NANONET has made a start with building a strong network of established researchers, early stage researchers and students to stimulate, to formalize, and broaden the existing and developing collaborations between the different research groups, to promote interdisciplinary interactions and to train young scientists for this multi-disciplinary field of research.

I. Management Report prepared by the Grant Holder

I.A. COST Action Fact Sheet

- **COST Action** *BM1002-Nanomechanics of intermediated filament networks - NANONET*
- **Domain** *BMBS*

- **Action details:**

CSO Approval: *(25/07/2010)* **End date:** *(08/11/2014)*
Entry into force: *(09/11/2010)* **Extension:** *(not applicable)*

Objectives *(from DB as in About COST)*
The aim of the Action is to establish a scientific platform to take full advantage of the emerging knowledge on intermediate filaments and novel developments in cell biology, molecular biology, (bio)chemistry, engineering, mathematics and physics in appropriate commercial and medical applications.

- **Parties:** *list of countries and date of acceptance*

Austria *(07/10/2010)*	Greece *(30/07/2010)*	Poland *(18/06/2011)*
Belgium *(02/09/2011)*	Hungary *(date)*	Portugal *(date)*
Bulgaria *(date)*	Iceland *(date)*	Romania *(date)*
Croatia *(date)*	Ireland *(08/09/2010)*	Serbia *(date)*
Cyprus *(date)*	Israel *(28/10/2010)*	Slovakia *(08/09/2010)*
Czech Rep. *(22/06/2011)*	Italy *(02/09/2011)*	Slovenia *(30/07/2010)*
Denmark *(08/12/2010)*	Latvia *(date)*	Spain *(30/07/2010)*
Estonia *(date)*	Lithuania *(date)*	Sweden *(14/12/2010)*
Finland *(04/11/2010)*	Luxembourg *(date)*	Switzerland *(date)*
FYR of Macedonia *(date)*	Malta *(date)*	Turkey *(date)*
France *(20/09/2010)*	Netherlands *(30/07/2010)*	United Kingdom*(30/07/2010)*
Germany *(14/09/2010)*	Norway *(date)*	

- **Intentions to accept:** *list of countries and date*
Turkey - no date yet, although MC has voted in favour to accept Dr. B. Erdem Alaca as a MC member.

- **Other participants:**
None

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- **Action Web site:** *http://www.nanofilament.eu*
- **Grant Holder Representative:** Ms. Ria Grimbergen, bm1002-nanonet@nin.knaw.nl

• **Working Groups** *(list of WGs and names and affiliations of participants)*

WG 1 - Mechanotransduction and cell signalling
Workgroup leader - Dr. Sandrine Etienne-Manneville, Institut Pasteur, Paris, France

Title	First Name	Last Name	Organisation	Country
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Dr	Yolanda	De Pablo	University of Gothenburg (Göteborg, Sweden)	SE
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Dr	Leopold	Eckhart	Medical University of Vienna (Vienna, Austria)	AT
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Dr	Catherine	Even	Université Paris Sud (Orsay, France)	FR
Dr	Roland	Foisner	Medical University Vienna (Vienna, Austria)	AT
Mr	Florian	Geisler	Institute of molecular and cellular anatomy (Aachen, Germany)	DE
Mr	Kevin	Gesson	Medical Univ. of Vienna, Max F. Perutz Lab., Dept. of Biochemistry (Vienna, Austria)	AT
Ms	SYLVIE	HENON	University Paris Diderot (PARIS, France)	FR
Prof.	Elly	Hol	Netherlands Institute for Neuroscience (Amsterdam, Netherlands)	NL
Prof.	Pavel	Hozák	Institute of Molecular genetics ASCR, v.v.i. (Prague, Czech Republic)	CZ
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Ms	Karin	Jaeger	Medical University of Vienna (Vienna, Austria)	AT
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Dr	Cecile	Leduc	Institut Pasteur (Paris, France)	FR
Prof.	Rudolf	Leube	RWTH Aachen University (Aachen, Germany)	DE
Dr	Alain	LILIENBAUM	Univeristé Paris 7 Denis Diderot (Paris, France)	FR
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Prof.	Daniel	Navajas	Institute for Bioengineering of Catalonia (Barcelona, Spain)	ES
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Dr	Nicole	Schwarz	MOCA (Aachen, Germany)	DE
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Mr	Oscar	Stassen	Netherlands Institute for Neuroscience (Amsterdam, Netherlands)	NL
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WG 2 - Nanomechanical properties and impact on cell mechanics
Workgroup leader - Prof. Dr. Sarah Köster - Georg-August-University, Göttingen, Germany

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Mr	Jannick	Langfahl	Georg-August-Universität Göttingen (Göttingen, Germany)	DE
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WG 3 - Translation to bio-inspired materials
Workgroup leader - Prof. Dr. Roy Quinlan - University of Durham, Biophysical Sciences Institute, School of Biological and Biomedical Sciences, Durham, United Kingdom

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WG 4 - Intermediate filaments in health and disease
Workgroup leader - Prof. Dr. Milos Pekny - University of Gothenburg, Institute for Neuroscience and Physiology, Dept. Clinical Neuroscience and Rehabilitation, Gothenburg Sweden
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Dr	Ulrika	Wilhelmsson	University of Gothenburg (Gothenburg, Sweden)	SE
Dr	Reinhard	Windoffer	MOCA, RWTH University (Aachen, Germany)	DE
Dr	Lilli	Winter	University of Vienna, Max F. Perutz Laboratories (Vienna, Austria)	AT
Dr	Norbert	Zilka	Institute of Neuroimmunology (Bratislava, Slovak Republic)	SK
Prof.	Robert	Zorec	Laboratory of Neuroendocrinology-Molecular Cell PhysiologyMedical FacultyUniversity of Ljubljana (Ljubljana, Slovenia)	SI
Ms	Petra	Zugschwerdt	DKFZ (Heidelberg, Germany)	DE

I.B. Management Committee member list

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I.C. Overview activities and expenditure

Meetings

Meeting Type	Date		Place	Country		Cost	Total
	Start	End					**37963.83**
WG	7-2-2011	8-2-2011	Säröhus	Sweden		2872.00	
WG	18-5-2011	20-5-2011	Bonn	Germany		2257.25	
MC / conference	16-6-2011	19-6-2011	Mykonos	Greece		15545.82	
Conference	16-6-2011	19-6-2011	Mykonos	Greece		11715.00	
WG	10-10-2011	10-10-2011	Paris	France		2707.19	
WG	18-11-2011	18-11-2011	Heidelberg	Germany		945.77	
WG	20-2-2012	20-2-2012	Göttingen	Germany		1920.80	

STSM

Beneficiary	Date		Place	Country		Cost	Total
	Start	End					**4943**
Dechat	25-9-2011	1-10-2011	Heidelberg	Germany		750	
Ortolani	17-10-2011	11-11-2011	Santiago de Compostela	Spain		2000	
Elliott	5-12-2011	16-12-2011	Amsterdam	Netherlands		648	
Sobol	11-12-2011	23-11-2011	Utrecht	Netherlands		1545	

Workshops

Title	Date		Place			Cost	Total
	From	To	From	To			
							0

General Support Grants

Beneficiary	Date					Cost	Total
Grant holder		Secretarial support				6372.90	**6372.90**

Schools

Title	Date	Place				Cost	Total
							0

Dissemination

Title	Date	Place				Cost	Total
Website						1500	**1500**

Others

€50779.73

II. Scientific Report

II.A. Innovative networking

- *Innovative knowledge resulting from COST networking through the Action.*

  The activities planned for the first year of NANONET have almost all been executed. We established 4 WGs, we organized 5 WG meetings (up to April 2012), the NANONET website has been setup, 4 early stage researchers visited laboratories in our network and were financially supported by an STSM grant.

  Innovative knowledge requires often more than 1 year of research, therefore it is hard at this stage of our COST Action to already report on this topic. In the first year there has been extensive exchange of knowledge during the 5 work group meetings and at a European conference, which was dedicated to intermediate filaments. Furthermore, the four STSMs have revealed initial data containing innovative knowledge:

  [1] STSM Thomas Dechat, Max F. Perutz Laboratories, Department of Medical Biochemistry, Medical University of Vienna, AT. Host: Prof. Harald Herrmann, German Cancer Research, Center (DKFZ), DE. See annex 1 for scientific report.

  Objective: Investigate the role of a lamin interacting protein, the lamina-associated polypeptide (LAP) 2alpha, in the assembly process of lamin A.

  Results: Preliminary data point to an inhibitory effect of LAP2 on the assembly of lamin A.

  [2] STSM Michela Ortolani, Istituto di Gentica Molecolare CNR Unita Operativa di Bologna, IT (seen annex 2). Host: Prof. David Araujo-Vilar,Departamento de Medicina. Universidade de Santiago de Compostela,Santiago de Compostela (ES). See annex 2 for scientific report.

  Objective: Evaluating of pathological changes associated with senescence in laminopathic fibroblasts and adipocytes, with a focus on genes mutated in laminopathies, such as Dunnigan-type familial partial lipodystrophy (FPLD).

  Results: The preliminary data suggests that a group of genes is differentially expressed in senescent versus young FPLD adipocytes, which could explain their abnormally increased proliferation and differentiation in the neck, as well as the complete loss of adipose tissue in other body districts.

  [3] STSM Jayne Elliott, Durham University,Durham(UK). Host: Prof. Gijsje Koenderink, AMOLF,Amsterdam (NL). See annex 3 for scientific report.

  Objective: Assess how the viscosity of the solutions of filaments are altered when interacting with AlphaB-Crystallin (CryAB).

  Results: This is the first time that passive microrheology has been used to investigate the properties of intermediate filament networks. The first experiments have been set up in this short 2 week STSM.

  [4] STSM Margaryta Sobol, Institute of Molecular Genetics of the ASCR, v.v.i./ Department of Biology of the Cell Nucleus, CZ. Host: Prof. Judith Klumperman,Cell Microscopy Centre, University Medical Centre Utrecht, Utrecht(NL). See annex 4 for scientific report.

  Objective: Learning correlative light and electron microscopy combined with the Tokuyasu method and implementing this technique in the home laboratory. Colocalization of the lamin A and B intermediate filaments with other proteins is investigated.

  Results: Subcellular localization of lamin A and B was established and no colocalization with PIP2 was observed.

- *Significant scientific breakthroughs as part of the COST Action. (Specific examples)*

  We anticipate that sharing knowledge and tools will facilitate significant breakthroughs. However, one year after the start of the network it is too early to report on scientific breakthroughs resulting from NANONET.

- *Tangible medium term socio-economic impacts achieved or expected.*

  This is not easy to answer for our network, as it is purely fundamental science. The STSMs and WG meetings will help young researchers from the participating partners to establish their careers in science and to learn from the different disciplines. Novel developments in understanding the biology, biochemistry and biophysics of intermediate filaments will potentially aid the development of novel therapies for intermediate filament based diseases.

- *Spin off of new EC RTD Framework Programme proposals/projects.*

  See below.

- *Spin off of new National Programme proposals/projects.*

  Major national, international and EU-funded programs of NANONET participants launched after NANONET went into operation. However, no new EC RTD Framework programme proposals / projects have yet been started that have directly evolved from NANONET.

II.B. Inter-disciplinary networking

- *Additional knowledge obtained from working with other disciplines within the COST framework.*

  The STSMs are perfect examples of new and additional knowledge obtained from working with other disciplines in the COST framework. The intermediate filament field is truly interdisciplinary and the WG meetings and STSMs show excellent examples of interactions between the different disciplines including mathematics, physics, biology and medicine. For example Jayne Elliott, trained as a biomedial scientist, visited the physics lab of Gijsje Koenderink lab to learn rheology.

- *Evaluation of whether the level of inter-disciplinarity is sufficient to potentially provide scientific impacts.*

  The level of inter-disciplinarity is sufficient to provide scientific impact. It is clear from the STSM applications and the WG programs that the researchers involved in NANONET highly value interdisciplinarity. For instance, the WG1 meeting in Paris and the WG2 meeting in Heidelberg (as can be read in the meeting reports, see annex 6) show a broad multi-disciplinary program.

- *Evaluation of whether the level of inter-disciplinarity is sufficient to potentially provide socio-economic impacts.*

Not applicable to our network.

II.C. New networking

- *Additional new members joining the Action during its life.*

  At the kick-off meeting in 2010 we started with 12 parties that accepted the MoU and 2 that had intentions to accept. Up to April 2012 in total 18 parties have accepted the MoU and joined NANONET. Switzerland has indicated to be interested to join, however no further actions have been taken by the Swiss scientists. Turkey has asked to join and the MC has approved this, although the involved scientist and the COST office has been informed about the MC decision the joining of Turkey has not occurred yet. We will actively invite other intermediate filament scientists from non participating countries to join NANONET.

- *Total number of individual participants involved in the Action work.*

  There are 109 participants registered in eCOST.

	Total	ESR	Female	%ESR	%female
	41	12	16	29.27	39.02
MC members	31	8	14	25.81	45.16
MC substitutes	9	3	2	33.33	22.22
Other participants	109	66	54	60.55	49.54

  Not all participants of the Conference and WG meetings were registered in eCOST, as we have the policy to mainly register the participants that will be reimbursed by NANONET or that are a student in the labs of the MC members and substitute members. Thus the number of scientists attending NANONET meetings is substantially larger than the number of participant registered in eCOST.

- *Involvement of Early Stage Researchers in the Action, in particular with respect to STSMs, networking activities, and Training Schools. In addition, justification should be provided if less than 4 STSMs were carried out during the year.*

  Four STSMs were granted, all four grantees were Early Stage Researchers (ESRs).
  We have not organized a training school yet, such an event is scheduled for the summer of 2012.

  Five WG meetings and one conference were organized up to April 2012: (see for a selection of scientific meeting reports annex 6).

  WG4 meeting in Säröhus, Sweden - The role of notch signaling, intermediate filaments and cell vesicle trafficking in cell differentiation, and beyond. Meeting participants:21, ESRs: 15. NANONET reimbursed travel costs and accommodation for 8 ESRs (FI and DE). This was a meeting organized by a Swedish and Finnish group, and since the meeting was in Sweden, mainly Finnish students got a reimbursement.

  WG4 meeting in Bonn, Germany - Cross-striated Muscle in Health and Disease. The number participants at this meeting was 112, they were mainly from Germany and Austria. Total number of ESRs at the meeting has not been communicated with the grant holder and the chair, and cannot be deduced from the list of participants, which is available. NANONET reimbursed travel costs and accommodation for 5 ESRs and 2 established researchers (DE, AT).

  Combined conference and MC meeting in Mykonos, Greece - 7th European Conference on Intermediate Filaments in Health and Disease. Total number of meeting participants was about 100. NANONET reimbursed travel costs and accommodation of 20 MC members, of which 3 were ESRs. Furthermore, NANONET reimbursed the travel costs and accommodation of 15 ESRs, who were participating in the conference (from IL, DE, UK, FI, AT, NL, EL, SE, SI, FR). Two external experts (IL, SG) gave a lecture and their travel costs were reimbursed.

  WG1 meeting in Paris, France - Role of intermediate filaments in mechanotransduction. For this meeting 17 scientists from several participating countries came together in Paris (AT, CZ, FR, EL, NL, SE). NANONET reimbursed travel costs and accommodation for 3 ESRs and 3 established researchers (SE, NL, CZ, AT, EL, NL).

  WG 2&3 meeting in Heidelberg, Germany - Mechanical properties of intermediate filaments. Almost 30 scientists from all over Germany, Netherlands and Belgium came together in Heidelberg. NANONET reimbursed travel costs and accommodation for 7 ESRs and 1 established researcher (DE, NL, BE).

WG2 meeting in Göttingen, Germany. Mechanical properties of intermediate filaments. About 20 scientists from different European countries came together for this meeting. 11 young scientists gave short presentations. NANONET reimbursed travel costs and accommodation for 3 ESRs and 1 established researchers (DE, NL, HE).

- *Involvement of researchers from outside of COST Countries -*
  Prof Birgit Lane, is an expert on intermediate filaments from Singapore, she has been invited as an expert at our Intermediate filament meeting in Mykonos. Sofar, however, there is no official involvement of researchers from outside COST countries.

- *Advancement and promotion of scientific knowledge through publications and other outreach activities.*

  Numerous papers have been published in 2011 by the MC members and substitutes (see list in annex 7). There is no publication yet which is a direct result of the COST networking activity. The preliminary results obtained during the lab exchanges funded by the four STSM grants, will be studied in more depth and might evolve in a publication in which COST will be acknowledged.

- *Activities and projects with COST network colleagues.*

  COST network colleagues meet at NANONET WG meetings, at the intermediate filaments meetings in Europe and in the United States. Many of the NANONET members will participate in the Gordon Conference on intermediate filaments which will be held in June 2012. There are several collaborations and exchanges between the NANONET colleagues:
  For example a new collaboration between Pekny (SE) and Hol (NL) on the role of GFAP in brain diseases; ongoing exchange and colloaboration between Koenderink (NL) and Herrmann (DE) on biophysical properties of intermediate filaments. There are interactions between NANONET and the FP7 program EduGlia.

- *The capacity of the Action members to raise research funds.*
  Major national, international and EU-funded programs of NANONET participants launched after NANONET went into operation.

III. Previous scientific report(s)

This is the first report, therefore there are no previous scientific reports.

Annex 1 - STSM Scientific Report Thomas Dechat

The Role of LAP2alpha in the Assembly of Lamin A

Scientific Report

The main goal of the work done at the DKFZ in Heidelberg in the scope of this fellowship was to preliminarily study the effects of LAP2α on the assembly properties of lamin A (LA). We used purified recombinant proteins expressed in E.coli for this purpose, which were stored in a buffer containing 8 M urea.

Two different LA assembly strategies were pursued in this study. First, LA was assembled into filaments. Therefore, LA and LAP2α were mixed already in urea buffer to a final concentration of 0.2 µg/µl each or 0.2 µg/µl LA and 0.5 µg/µl LAP2α, respectively. The samples were dialyzed at 37°C against a Tri-buffer, pH=8.0 containing 250 mM NaCL and subsequently against a MES-buffer, pH=6.5 containing 250 mM NaCl. After the dialysis an aliquot was fixed with glutaraldehyde and processed for electron microscopy by uranyl acetate staining. The rest of the samples were centrifuged for 15 min at 13,000 rpm at 20°C and total, supernatant and pellet fractions were analyzed by SDS-PAGE. This experiment clearly revealed an influence of LAP2α on the filament assembly properties (Fig. 1A) and solubility (Fig. 2A) of LA. While LA only assembled into quiet homogenous filaments (Fig. 1A, left panel), the addition of LAP2α leads to the disruption of the filament homogeneity and to the formation of thinner filamentous structures, which appear to be interconnected with each other and also with the thicker filaments still present (Fig. 1A, middle panel). An excess of LAP2α leads to a stronger disruption of the LA filaments and to the formation of thinner and shorter filamentous structures (Fig. 1A, right panel). The pelleting assays revealed an increase in the solubility of LA upon addition of LAP2α, as best seen by the reduction of LA in the pellet fraction (Fig. 2A).

Secondly, the influence of LAP2α on the paracrystal formation of LA was investigated. Therefore, LA and LAP2α were initially dialyzed from the urea buffer into a Tri-buffer, pH=7.4 containing 300 mM NaCl. The proteins were then mixed in the Tris-buffer to a final concentration of 0.09 µg/µl each or 0.09 µg/µl LA and 0.15 µg/µl LAP2α, respectively and subsequently dialyzed against the same buffer containing 50 mM NaCl instead of 300 mM. After the completion of the dialysis the samples were analyzed in the same way as the filament assembly samples with the only exception that the aliquots processed for electron microscopy were not fixed with glutaraldehyde. Similar to the results obtained in the first experiment, the addition of LA impaired the assembly properties of LA. In the absence of LAP2α LA assembled into regular paracrystalline structures (Fig. 1B, left panel), while the addition of LAP2α leads to an opening and “unwinding” of the paracrystals (Fig. 1B, middle panel) and in an excess even to the formation of filamentous structures (Fig. 1B, right panel). The pelleting assay again revealed an increase in the solubility of LA upon the addition of LAP2α. While LA alone is not soluble (Fig. 2B, left panel), increasing amounts of LAP2a leads to an increasing solubility of LA (Fig. 2B, two right panels).

Take together these preliminary studies point to an inhibitory effect of LAP2α on the assembly of lamin A. Further studies based on our initial data will be undertaken in the near future both in Vienna and in Heidelberg to investigate the role of LAP2α in the assembly of lamin A structures in more detail.

Figure 1:

LA was assembled into filaments (A) or paracrystals (B) in the absence or presence of various amounts of LAP2α and analyzed negative staining transmission electron microscopy.

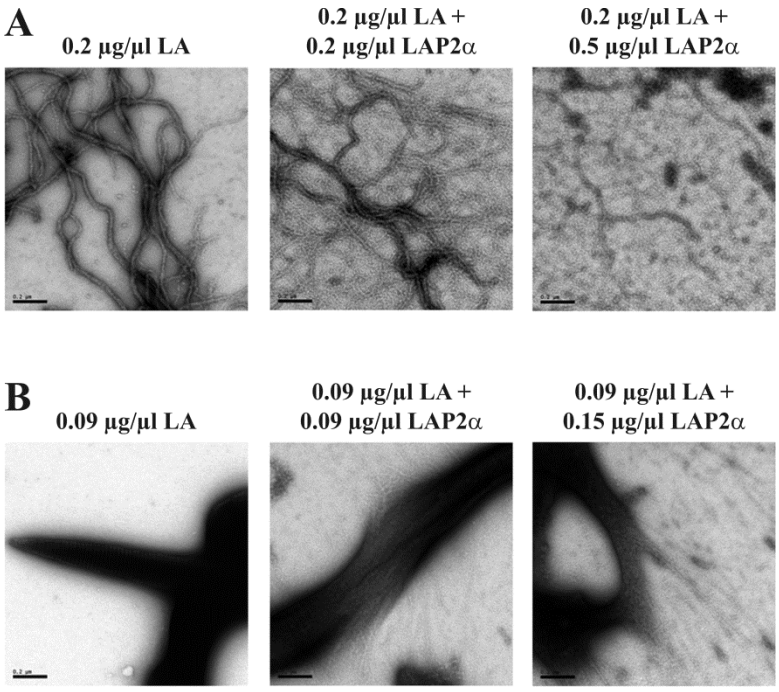
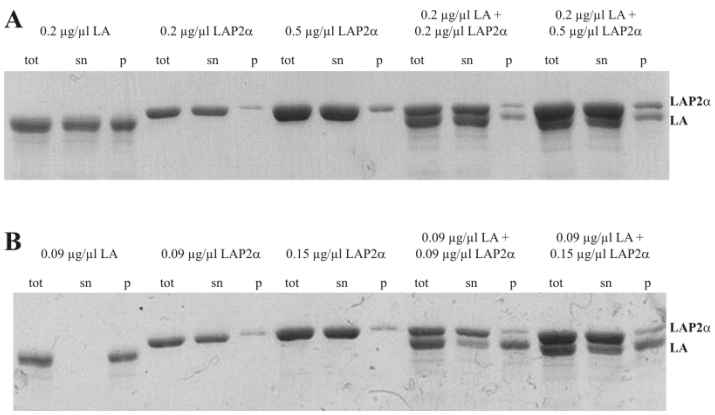


Figure 2:

LA was assembled into filaments (A) or paracrystals (B) in the absence or presence of various amounts of LAP2α and total (tot), supernatant (sn) and pellet (p) fractions obtained after centrifugation were analyzed by SDS-PAGE.



Annex 2 - STSM Scientific Report Michela Ortolani

Evaluation of mRNAs in lipodystrophy and senescent cells: LMNA, FACE1, PPARG and related genes

BACKGROUND

Lipodystrophies are a heterogeneous group of human disorders characterized by the anomalous distribution of body fat or generalized loss of adipose tissue.
Several types of lipodystrophy have been characterized at the molecular genetic level, including Dunnigan-type familial partial lipodystrophy (FPLD), partial lipodystrophy with mandibuloacral dysplasia (MAD), syndromes of partial lipodystrophy with cardiomyopathy. In FPLD and MAD, lamin A/C mutations have been linked to disease, whereas a form of partial lipodystrophy associated with PPARg mutations has also been described. Other lipodystrophies are acquired or drug-induced, such as the lipodystrophy syndrome that is associated with the use of highly active antiretroviral treatment (HAART). In addition, progeroid syndromes such as Hutchinson–Gilford progeria (HGPS) and atypical-Werner syndrome (a-WS) show generalized lipodystrophy, often combined with insulin resistant diabetes mellitus. In recent years, two major players have emerged as being possibly involved in the pathogenesis of lipodystrophies. The first is lamin A/C, the nuclear lamina constituent mutated in FPLD and MAD cells as well as in progeroid syndromes with lipodystrophy. Moreover, mutations of ZMPSTE 24, the metalloprotease involved in lamin A processing, cause diseases featuring a lipodystrophy phenotype such as MAD in humans. The second emerging protein in the pathogenesis of lipodystrophy is the sterol regulatory element binding protein1 (SREBP1), a transcription factor whose localization and transactivation
ability appear to be altered in acquired lipodystrophy. In this context, it is noteworthy that mutations of PPARg transcription factor, which is transcribed downstream of SREBP1 activation and mediates adipocyte differentiation, are responsible for other forms of partial lipodystrophy. Moreover, a single point mutation in the PPARg promoter has been associated with FPLD. Lamins A and C are nuclear lamina proteins obtained by alternative splicing of the *LMNA* gene and are almost ubiquitously expressed in differentiated tissues. Before being assembled in the nuclear lamina, lamin A undergoes complex post-translational modifications including farnesylation of the C-terminus and protease cleavage. Farnesylation of pre-lamin A is necessary for the following
steps of protein cleavage, as the prelamin A endoprotease fails to bind non-farnesylated lamin A sequence. Mature lamin A forms a heterodimeric complex with lamin C, which appears to play structural and functional roles, not completely elucidated.

EXPERIMENTAL PLAN

In this project we will evaluate the expression of genes as *LMNA*, *FACE1*, *PPRG*, GH-receptor and related genes in MAD fibroblasts and adipocytes and in senescent adipocytes. We will use human cell cultures derived from consenting lipodystrophic patients and established in our laboratory or in the laboratory of Prof. Araujo-Vilar at University of Santiago de Compostela.

METHODS

Cells at low passage number and senescent cells from both control and FPLD will be used for the gene expression analysis and results will be compared to identify genes involved in adipocyte senescence. Cellular senescence will be monitored by morphological and biochemical parameters (nuclear size, cell shape, presence of autophagic vacuoles, beta-Galactosidase staining, IL6 expression) in adipocytes and fibroblasts at passages 12-22.
We will examine gene expression by Real-Time PCR and Universal ProbeLibrary (UPL) assay that use 165 pre-validated, real-time PCR probes that will allow to quantify any transcript of our interest. PL is based on only short hydrolysis probes, they are labeled at the 5' end with fluorescein (FAM) and at the 3' end with a dark quencher dye. The sequences of the 165 UPL probes ensuring optimal coverage of all transcripts in a given transcriptome. Within the human transcriptome, each probe binds to approximately 7000 transcripts, while each transcript is detected by approximately 16 different probes. Only one specific transcript is detected at a time in a given PCR assay, as defined by the set of interest PCR primers.
Improvement of statistical evaluation of results will be obtained by dedicated software. All the RT-PCR study and the statistical evaluation of results will be performed at the University of Santiago de Compostela.
Further studies, to be performed in our laboratory using both Western blot and immunofluorescence analysis, will allow us to validate data obtained by RT-PCR.

PRELIMINARY DATA

Fibroblast and adipocyte cultures have been established in our laboratories from consenting lipodystrophic patients and healthy subjects and examined for prelamin A levels, lamin A/C levels and nuclear distribution of prelamin A and heterochromatin markers. Futher, analysis of adipose tissue from neck, an area of abnormal accumulation of fat in lipodystrophic patients, has been performed. We have determined a significant increase in LMNA expression in adipose tissue from FPLD patients, corresponding to elevated levels of prelamin A, lamin A and lamin C proteins. Normal levels of LMNA expression, lamin A/C protein, but increased amount of prelamin A have been detected in cultured

adipocytes. These findings strongly suggest that some circulating factor induced in vivo in lipodystrophic patients might upregulate LMNA itself. Moreover, we have examined PPARgamma expression in FPLD adipocytes and we have identified a defect in expression levels and isoform proportion. PPARgamma 1 appears normally expressed in FPLD adipocytes, while PPARgamma 2 is almost undetectable. The phenotype of FPLD adipocytes is different from control adipocytes from the same body district in that FPLD adipocytes show high proliferative and differentiation ability at low passage, while they do not differentiate at late passage, even in the presence of adipocyte differentiation medium. The whole evaluation of these data suggests that a group of genes is differentially expressed in senescent versus young FPLD adipocytes, which could explain their abnormally increased proliferation and differentiation in the neck, as well as the complete loss of adipose tissue in other body districts. The proposed research is aimed at the identification of this set of genes, as well at the technical optimization of RT-PCR analysis in human adipocytes and adipose tissue.

EXPECTED RESULTS

The whole evaluation of the expected results will be aimed at defining an expression profile of young versus senescent adipocytes. Genes that will show significant changes during ageing and/or in FPLD versus senescent cells, will be considered as biomarkers and tested as potential pathogenetic factors for *LMNA*-linked lipodystrophy.

Annex 3 - STSM Scientific Report Jayne Elliott

STSM Scientific Report

COST Action: BM1002

STSM Topic: The Rheological Study of AlphaB-Crystallin Interactions with Type III Intermediate Filaments

STSM Dates: 5/12/11-16/12/11

STSM Applicant: Ms Jayne Elliott,Durham University,Durham(UK) , j.l.elliott@durham.ac.uk

Host: Gijsje Koenderink,AMOLF,Amsterdam(NL), gkoenderink@amolf.nl

A short scientific mission was undertaken for 2 weeks at the FOM institute AMOLF in the lab of Gjisje Koenderink to investigate the G' and G'' of wild-type (WT) desmin solutions and what effect WT and the cardiomyopathy-causing mutant R120G alphaB-crystallin (CryAB) has on these networks. This is the first time that passive microrheology has been used to investigate the properties of intermediate filament networks. Double-particle passive trapping was used to gain information on the bulk properties of the solutions, using 1064 and 808 nm lasers. Previous single particle trapping was carried out with beads added after assembly was completed and thus an initial experiment was carried out to compare the elasticity profile of the networks in the presence of beads added before and after the final assembly stage and this is shown in figure 1.

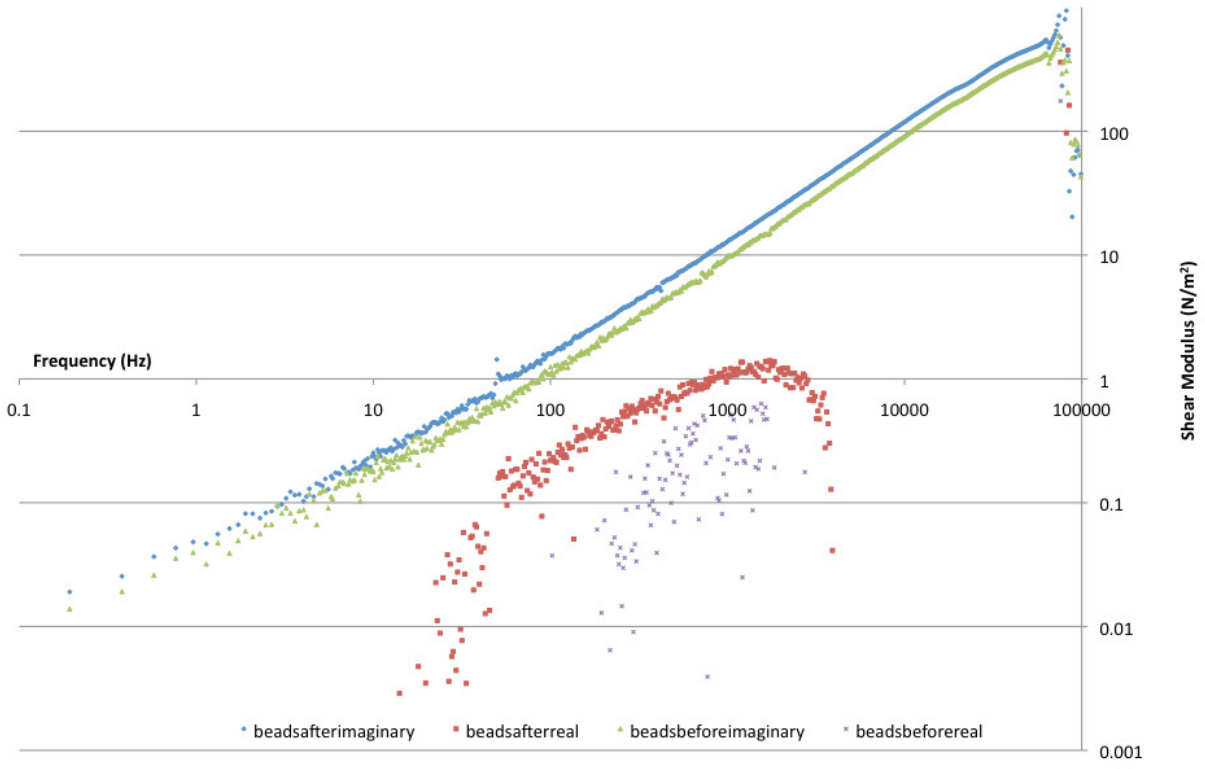


Figure 1; The shear modulus of the filament networks isn't very different when the beads are added before or after the final compaction and elongation stage of assembly.

Analysis of WT desmin solutions showed they are predominantly viscous with a small elastic profile compared to fine fibrin clots, which are predominantly elastic.

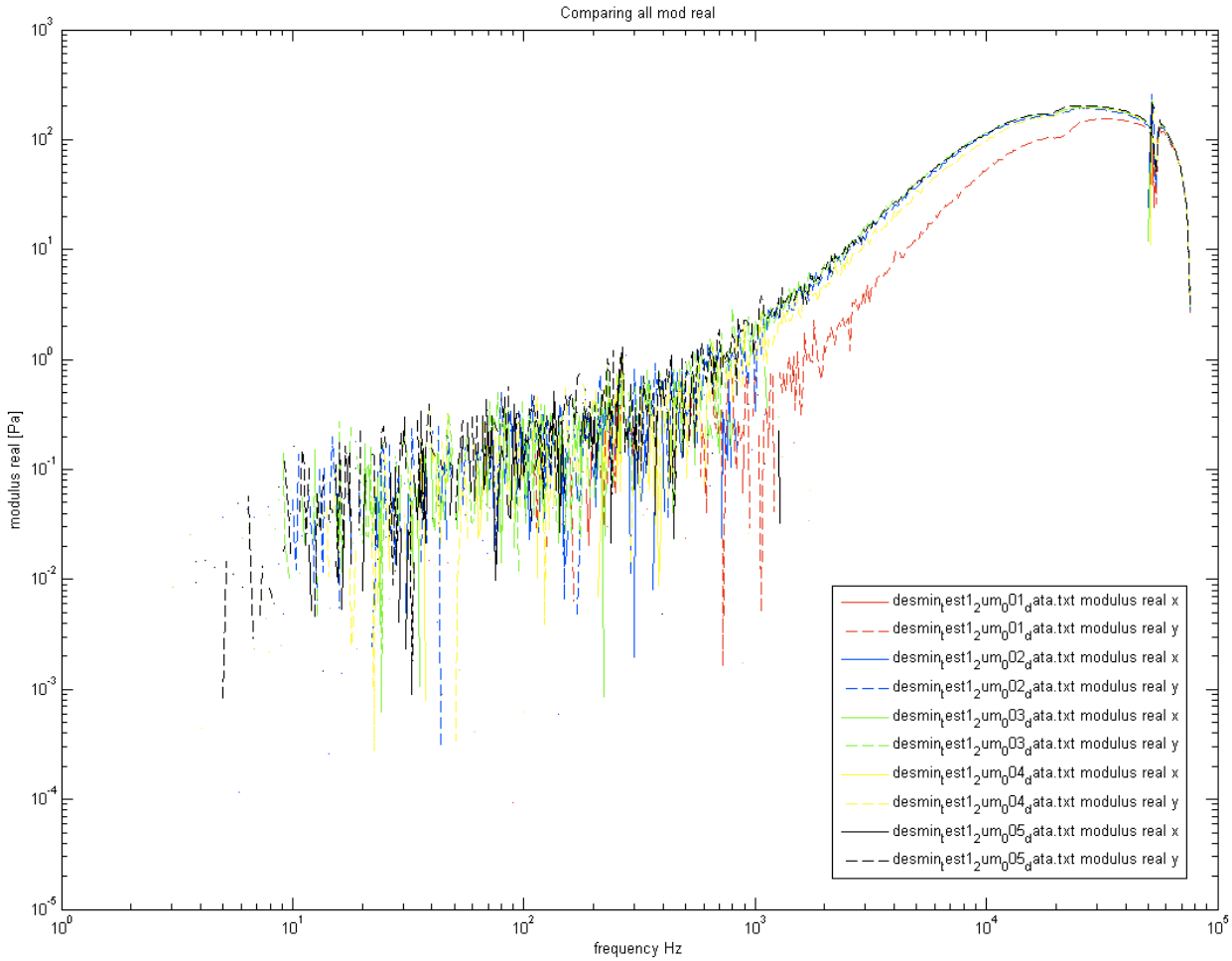


Figure 2;The real shear modulus of five independently trapped beads in the desmin networks show that it is predominantly viscous with a small elastic profile.

The viscosities of the networks were also different comparing desmin alone and those with the R120G mutant CryAB, at the lower frequencies as shown in figures 3 and 4. Analysis of the data collected with the other ratios of R120G:desmin is required to see which ratio is optimum for the set-up used to enable the beads to be trapped within the aggregated networks as opposed to being trapped around the networks and thus give a higher viscosity than desmin alone.

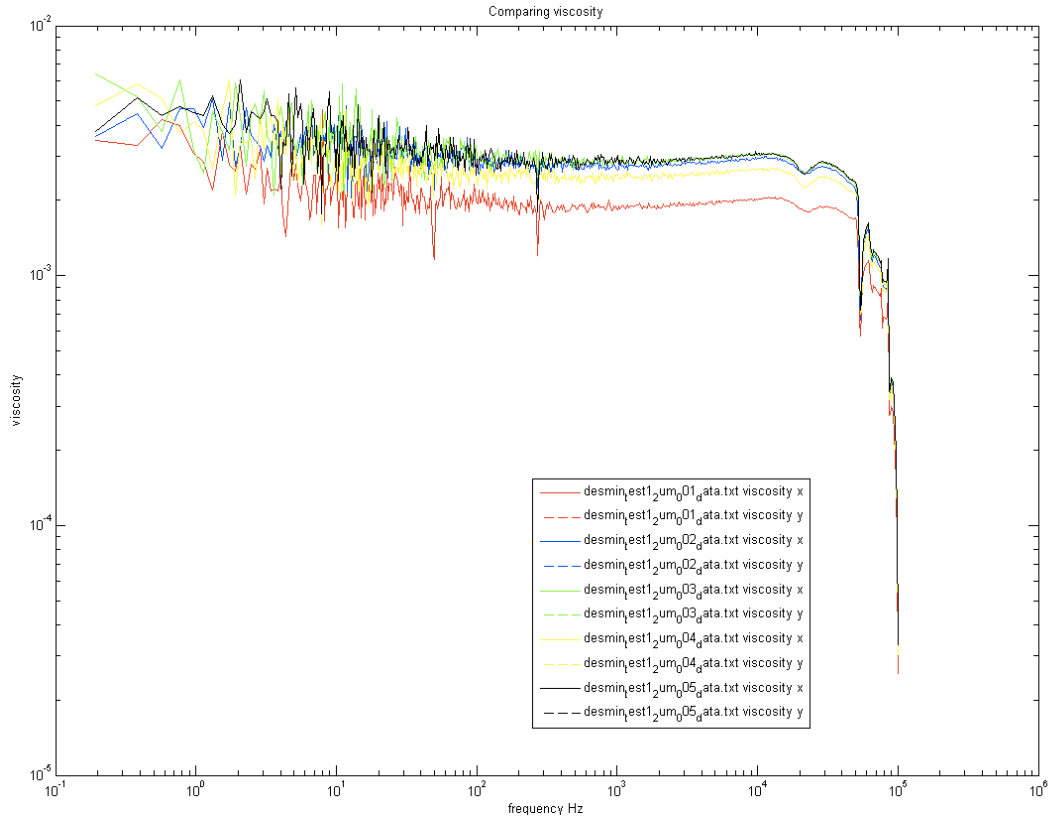


Figure 3; Frequency-dependent viscosity of desmin networks from 5 trapped beads

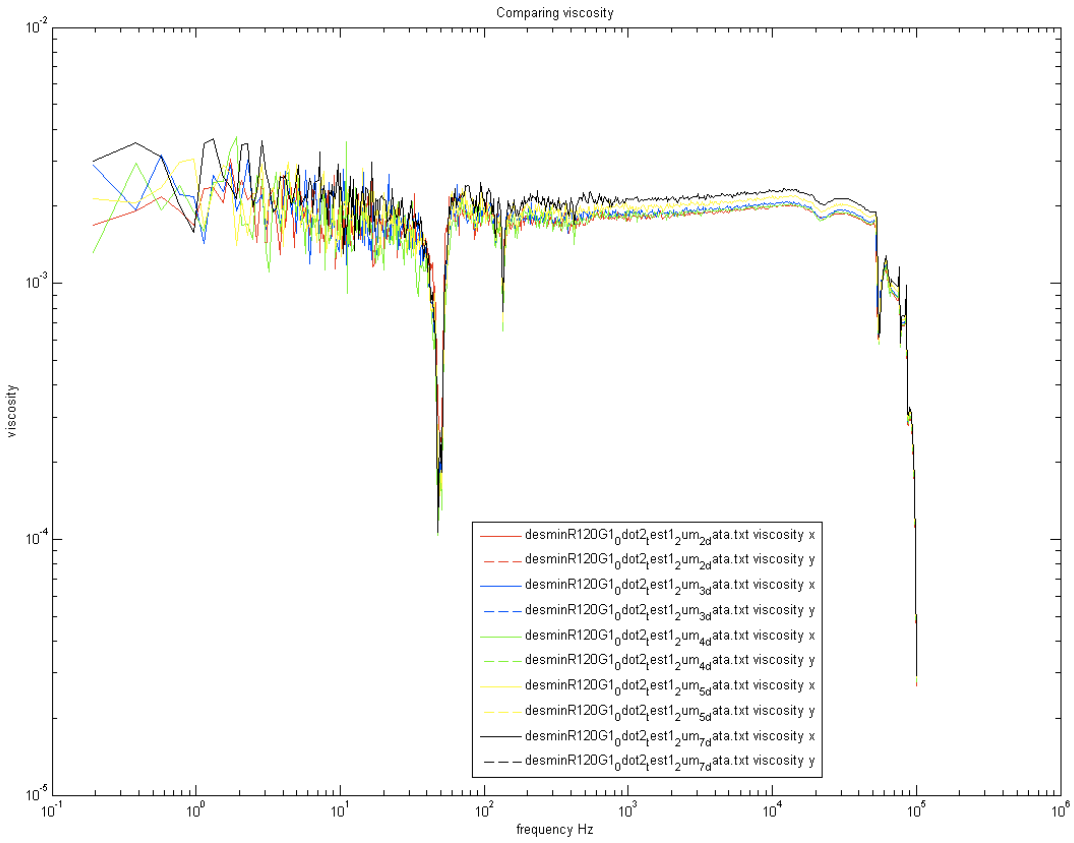


Figure 4; Frequency-dependent viscosity of a 1:2 molar ratio of desmin: R120G mutant CryAB from 5 trapped beads

As a comparison, beads were trapped in WT vimentin networks also in the presence and absence of WT CryAB and this will be compared to fine fibrin clots with CryAB. Hopefully in the presence of WT CryAB there is a change in the G'' of the networks, showing that the filament interactions have been reduced in its presence.

Jayne Elliott (Durham University, UK)

Annex 3 - STSM Scientific Report Margaryta Sobol

STSM Scientific Report

Action Number: BM1002 - Nanomechanics of intermediate filament networks (NANONET)
STSM Title: "Intra-nuclear localization of phospholipids relative to intermediate filament network"
Beneficiary/Applicant: Dr. Margaryta Sobol, Institute of Molecular Genetics of the ASCR, v.v.i. / Department of Biology of the Cell Nucleus, Prague (Czech Republic)
Host: Prof. Judith Klumperman, Cell Microscopy Centre, University Medical Centre Utrecht, Utrecht (Netherlands)
STSM Dates: 11-23 December, 2011
STSM Place: Cell Microscopy Centre (CMC), University Medical Centre Utrecht, Heidelberglaan 100, 3584 CX Utrecht, Netherlands

Purpose of the visit:
1. Mastering correlative light and electron microscopy (CLEM) combined with the Tokuyasu method, because optimized for immunoreactions on fast-frozen sections, it can provide excellent labeling of antigens, mostly not achievable on resin section. Following experiments are to be performed:

1) (Co-)localization of (pre-)lamin A and PIP 2
2) (Co-)localization of lamin B and PIP2
3) (Co-)localization of myosin I and lamins
4) (Co-)localization of PIP2 and farnesylated proteins (anti-farnesyl antibody)

Description of the work carried out during the visit:
Dr. Sobol visited CMC during December 11-23 (10 working days). Meetings, experiments, microscopy observations and discussions were held in colaboration with following researchers at the CMC:
- Prof. Judith Klumperman
- Viola Oorschot
- Suzanne van Dijk
- Corlinda ten Brink

Dr. Sobol provided brief presentation about the preliminary studies carried out in Department of Biology of the Cell Nucleus suggesting that nuclear myosin I, PIP2, and some lamins might be the

main architectural elements involved in the maintenance of chromatin, transcriptional machineries and processing factories as well as playing a role in the regulation of gene expression. Detailed CLEM experiments were designed and worked out on the human cultured cell lines (HeLa and U2OS) prepared by Tokuyasu method. The antibodies against pre-lamin A, PIP2, lamin A, lamin B, myosin I and farnesylated proteins were used in mono- and double-immunolabeling experiments followed by combinations of secondary antibodies conjugated with fluorescent markers, bridging antibodies and protein A-gold. Correlative light microscopy and electron microscopy observations were done to acquire as complete as possible information about the localization of PIP2 and other potential binding partners.

Description of the main results obtained:

1. In human cultured cells prepared by Tokuyasu method, PIP2-labeled structures formed roundish islets in nucleoplasm as well as were localized in different nucleolar subdomains.
2. Lamin A was localized on the nuclear membrane, in the nucleolar subcompartments, and in majority in nucleoplasm, forming distinguishable foci.
3. Lamin B was mainly concentrated on the nuclear membrane in clearly defined loci, but as well in nucleoplasm and in nucleoli.
4. The antibodies against pre-lamin A, myosin I and farnesyl revealed very low level of detection and were not used in the following experiments.
5. Co-localization experiment on human cultured cells using Tokuyasu method and CLEM with anti-PIP2 and anti-lamin A antibodies showed specific patterns for both PIP2 and lamin A, although no real co-localization or overlapping was revealed in neither nucleoplasm nor nucleoli.
6. The absence of real co-localization was also demonstrated in the experiment using anti-PIP2 and anti-lamin B antibodies, while the pattern was specific for either PIP2 or lamin B.
7. The co-localization experiments using Tokuyasu method and CLEM with the antibodies mentioned above against nuclear antigens are quite unique, and they belong to a wider project on nuclear lipids and their binding partners in the cell nucleus. Dr. Sobol will use the results for designing further experiments on nuclear lipids involvement in nuclear functions, and potentially, they will become part of publications on this subject.

Dr. Margaryta Sobol, PhD
Institute of Molecular Genetics of the ASCR, v.v.i. / Department of Biology of the Cell Nucleus, Prague, Czech Republic

Annex 5

Spin off of new National Programme proposals/projects with relevance to NANONET.

Annex 6 - Examples of WG meetings.

Meeting on « Role of intermediate filaments in mechanotransduction » COST Nanonet WG1 at Institut Pasteur, Paris on October 10, 2011.

A work group meeting was organized on October 10, 2011 around the subject of Intermediate filaments in mechanotransduction. It brought together 17 scientists from several participating countries: Austria, Czech Republic, France, Greece, Netherlands and Sweden. This meeting was a great opportunity to openly discuss new results and ideas on this subject. The program (see below) was composed of talks given by confirmed researchers as well as students and post-docs. The talks were dedicated for a part to the biophysical approaches that can be used to investigate mechano-transduction, such as AFM and mechanotransduction. In vivo models were also presented. Another part of the talks was focussed on specific intermediate filament proteins and cell systems with an emphasis on GFAP in glial cells, Desmin in muscle cells and keratin in epithelial cells. These talks were very interactive with many questions and discussion during each talk and also between talks. The discussions continued during breaks and lunch time. Technical approaches were thoroughly discussed to determine the advantages and inconvenient of each techniques. These discussions gave interesting feedback to students and post-docs on their work. They also led to the identification on the major problems and questions that need to be tackled in the near future.
This meeting was very successful and it was decided that another WG1 meeting will be held at the end of 2012.

Meeting program
Participants : Onnik Aglulut, Gisèle Bonne, Catherine Coirault, Yolanda De Pablo, Sandrine Etienne-Manneville, Catherine Even, Sylvie Hénon, Elly Hol, Pavel Hozak, Pierre Joanne, Cécile Leduc, Vineet Mahajan, Yannis Missirlis, Martina Moeton, Oscar Stassen.
Program :
9:30 Beginning of the meeting - Introduction
10:00 Catherine Coirault
Mechanotransduction in skeletal muscle: The great potentiel of 3D culture
10:30 Sylvie Hénon
Microrheology experiments for the study of mechanotransduction
11:00 Catherine Even
Elasticity of myopathic muscle cells studied by Atomic Force Microscopy
11:30 Coffee break and discussion
12:00 Sandrine Etienne-Manneville
Role and regulation of intermediate filaments during astrocyte migration
12:30 Yannis Missirlis
Stress induced tubulin rearrangements in endothelial cells.
13:00 Lunch and Discussion
14:00 Martina Moeton
Unraveling the function of GFAP delta in the specialized intermediate filament network of neurogenic astrocytes
14:30 Oscar Stassen
The role of the protein GFAP in mechanotransduction
15:00 Yolanda De Pablo
GFAP and vimentin role in ROS detoxification after hypoxia.
15:30 Coffee break and discussion
16:00 Vineet Mahajan
Cross -sheet conformation of keratin 8 is a specific feature of Mallory-Denkbodies as compared to other hepatic inclusions.
16:30 Pierre Joanne
In vivo models for desminopathies
17: 00 Pavel Hozak
17:30 End of the meeting.

Meeting on „Mechanical properties of intermediate filaments“
(COST Nanonet WG 2)
at DKFZ Heidelberg on November 18, 2011

On November 18, 2011, almost 30 scientists from all over Germany and from the Netherlands and Belgium came together in Heidelberg to discuss new results, approaches and ideas in the field of intermediate filament (IF) mechanics.
A first, fairly large portion of the program was dedicated to IF assembly since assembly conditions and parameters play an important role for the properties of the resulting filaments and superstructures like networks and bundles. The experimental methods that are employed by the different research groups to study the assembly range from electron and atomic force microscopy, analytical ultracentrifugation, static light scattering (SLS), x-ray crystallography and small angle x-ray scattering (SAXS). Combining results from these experiments, it is possible to derive assembly kinetics and intermediate steps.
Moving on “from small to large”, studies on individual filaments and entangled networks thereof were presented and discussed. The mechanical properties of individual filaments need so be thoroughly known in order to understand more complex assemblies like networks. Here, techniques ranging from microfluidics to variations of rheology (active, passive, optical tweezers) come into play.
The meeting ended with the question how, in living cells, IF networks are formed and disassembled – a phenomenon that can be studied by looking at specially transfected cells producing fluorescent proteins thereby enabling the researcher to visualize the network.
The meeting was characterized by very clear and focused presentations, each of which was followed or even “interrupted” by very lively and fruitful discussions. All participating researchers work in closely related fields. Therefore, the presenting students and postdocs received valuable feedback on their research that will be directly useful for future work.
At the end of this very successful meeting it was decided that the next meeting would take place in Göttingen, Germany in spring 2012.

9.00-9.30	Coffee welcome
9.30-10.00	Harald Herrmann (DKFZ Heidelberg) *IF assembly mechanisms*
10.00-10.30	Norbert Mücke (DKFZ Heidelberg) *Analytical ultracentrifugation of “difficult” IF proteins*
10.30-10.50	Coffee break/Discussion
10.50-11.20	Martha Brennich (University of Göttingen) *X-ray studies of salt and protein concentration dependence of assembly processes*
11.20-11.50	Stefan Winheim (DKFZ Heidelberg) *Early assembly of vimentin and keratin 8/18 by stopped flow*
11.50-12.20	Bernd Nöding (University of Göttingen) *Vimentin filaments confined in microchannels*
12.20-13.10	Lunch/Discussion
13:10-13:40	Gijsje Koenderink (AMOLF) *tba*
13.40-14.10	Ines Martin (University of Ulm) *Diameter-measurements of keratin 8/18*
14.10-14.40	Paul Pawelzyk (KIT, Karlsruhe) *Impact of IF- and MgCl2-concentration on in vitro keratin 8 and 18 networks*
14.40-15.00	Coffee break/Discussion
15.00-15.30	Tobias Paust (University of Ulm) *In vitro assembly keratin 8/18 and panc1 networks - active rheological measurements*
15:30-16:00	Nicole Schwarz (RWTH Aachen) *Phospho-mimetic mutants of keratin 5 and 8*

Annex 7 - Publications of MC members and substitute MC members on intermediate filaments in 2011 until April 2012.

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