

IB-course 2006
March 27-April 4

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A course
for
Master students BMS
newly appointed graduate students
and post-docs

at the Institute of Biomembranes

A short introduction to the Institute of Biomembranes

From the very beginning of the Institute, its scientific mission has been focused on the following three themes:

- 1) Structure, function and organization of lipids and proteins in biological membranes
 - 2) Biogenesis of membranes and the transport and sorting of lipids and proteins
 - 3) The role of biomembranes in signal transduction via membrane receptors, cell-cell, and cell-matrix interactions.
- Later the topic vascular membrane biology was added.

The Institute of Biomembranes was officially inaugurated on October 4, 1991. One year thereafter the University Board formally recognized the institute as a Research Institute; the Faculty of Biology was appointed as the corresponding faculty. The Institute of Biomembranes was recognized as a Graduate School by the Royal Netherlands Academy of Sciences (KNAW) in August 1994, a recognition that was renewed in June 1999 and 2004. The original number of eleven research groups that participated in the Institute / Graduate School of Biomembranes grew to a total of eighteen, as before from four Faculties: Biology, Chemistry, Medicine and Veterinary medicine.

In 2004 the IB graduate school has received re-accreditation for the forthcoming six years from the ECOS committee of the KNAW. At that time Prof. dr. Gerrit van Meer (Membrane Enzymology, Chemistry, UU) succeeded Prof. dr. Arie Verkleij, cofounder of the IB (Cell Biology, Biology, UU), as Scientific Director.

Mission of IB

The Institute and Graduate School of Biomembranes (IB) is a multi-disciplinary research-training institute at Utrecht University. Its mission is:

- *To perform cutting-edge research on biological membranes and thereby increase our insight into important processes of life.*
- *To apply the newly acquired knowledge for solving societal problems.*
- *To provide an optimal infrastructure and training for young researchers.*

Since 2004 the IB board is composed of:

- Prof. dr. Flip de Groot (Faculty of Medicine)
- Prof. dr. Bernd Helmd (Faculty of Veterinary Medicine)
- Prof. dr. Antoinette Killian (Dept. of Chemistry, Faculty of Science)
- Prof. dr. Judith Klumperman, chair (Faculty of Medicine)
- Prof. dr. Han Wösten (Dept. of Biology, Faculty of Science)

Managing director: Dr. J.J.P.A. (Hans) de Cock, Tel. 2536616;
e-mail: hdecock@biomembranes.nl

Secretariat: A.J. (Antje) Feitsma, Tel: 2533184; e-mail:
admin@biomembranes.nl

The 2006 PhD committee

- Nico Boot (Biology) 2533355; n.w.boot@bio.uu.nl
- Pieter Rijken (Chemistry) 2534157; p.j.rijken@chem.uu.nl
- Josse van Galen (Veterinary Medicine) 253 2562;
j.vangalen@vet.uu.nl
- Marnix Wieffer (Medicine) 2506480 m.wieffer@lab.azu.nl

Goal

During the course the participants will receive theoretical and some practical training (site visits) in the area of biomembrane research by experts of the Institute of Biomembranes. Detailed information of techniques and technology present in the IB will be discussed. PhD students and Post docs receive in this way a basic training in the field of biomembrane research. Your participation will be evaluated.

Research proposal

As part of this training, the participants will work out their research proposal using, amongst others, information received in the IB course. This proposal will be presented to the other participants on tuesday, April 4.

The research proposal will be a PhD project (4 years) and need to include:

1. Your research/background
2. What can the IB add to your research?
3. Which new scientific insight did you obtain and are applicable in your research?
4. Which techniques discussed in this course can be used in your research (why, advantages/disadvantages/ where in the IB)?

The participants will work in small groups on these proposals during the course.

- The group will deliver a short summary of the proposal (points 1-5) (A4 paper)
- The group will present and discuss the proposal (20 min/10 min discussion)

Recommended textbooks:

Stryer's "Biochemistry"

Alberts, *et al.* "The Molecular Biology of the Cell"

Coordinators of the course and research proposals

BTS (Biogenesis, Translocation & Sorting)

Han Wösten en Judith Klumperman

MP (Membrane Proteins)

Gerrit van Meer en Antoinette Killian

VMB (Vascular Membrane Biology)

Flip de Groot

ST (Signal Transduction)

Bernd Helms

General coordination:

Hans de Cock 030-253 6616/ hdecock@biomembranes.nl

Program

- The participants will have lunch together in the restaurant of de Kruytbuilding (first floor) and which is served between 12.00 and 13.15 h.
- Between lectures, coffee and tea is served
- Nearly all activities are organised in the H. Kruytbuilding, Padualaan 8, Utrecht (Exceptions are indicated)

Program IB course, March 27-31

Date	Theme	Name	Activity	Location
Mo, March 27 <u>Morning</u> 10.15-11.00 11.15-12.00 <i>Afternoon</i> 13.15-14.00 14.15-15.00 15.00-16.00	BTS	Van Meer/de Cock Van Meer J. Tommassen H. Wösten W. Stoorvogel	Welcome Introduction Lecture " "	W105 " " "
Tu, March 28 <u>Morning</u> 9.15-10.00 10.15-11.00 11.15-12.00 <i>Afternoon</i> 13.15-14.00 14.15-15.00 15.00-16.00	BTS IB seminar	J. Klumperman S. Rüdiger P. Rottier G. Strous Dan Cutler <i>Meeting with speaker</i>	Lecture " " Lecture IB seminar	O103 " " O103 N017, Wentbuilding
We, March 29 <u>Morning</u> 9.15-10.00 10.15-11.00 11.15-12.00 <i>Afternoon</i> 13.15-14.00 14.15-15.00	MP Site visit	A. Killian E. Breukink L. Rutten A. Killian G. Van den Ackerveken	Lecture " " CD/NMR Micro array .	W105 " " W105 "
Thu, March 30 <u>Morning</u> 9.15-10.00 10.15-11.00 11.15-12.00 <i>Afternoon</i> 13.15-14.00 14.15-15.00	MP Site visit	M. Rook J. Holthuis J. Tommassen J. Brouwer R. Wubbolts	Lecture " " MS Multiphoton micro.	O103 " " N. Gildestein "
Fi, March 31 <u>Morning</u> 9.15-10.00 10.15-11.00 11.15-12.00 <i>Afternoon</i> 13.00-14.00 14.15-15.00	Presentations IB seminar MP	Short presentations by all participants Declan Doyle <i>Meeting with speaker</i>	 IB seminar	O103 " " W105 O103

Program IB course, April 3 & 4

Date	Theme	Group	Activity	Location
Mo, April 3 <u>Morning</u> 9.15-10.00 10.15-11.00 11.15-12.00 <i>afternoon</i> 13.15-14.00 14.15-15.00	ST	P. van der Sluijs B. Gadella H. Heijnen	Introduction ST Lecture Lecture	N020 Wentbuilding
	Site visit	Paul van Bergen en Henegouwen/ Willie Geerts	Site visit EM Kruytbuilding	W105
Tu, April 4 <u>Morning</u> 9.15-10.00 10.15-11.00 11.15-12.00 <i>afternoon</i> 13.15-14.00 14.15-16.00	ST	G. Snoek B. Helms M. Gebbink	Lecture ” Introduction VMB	O103 ” ”
	VMB	IB AiO committee Participants	Introduction Presentation proposals	” ”
16.00	Drinks	All participants	Closure	O103

Locations

H.R. Kruytbuilding
F.A.F.C. Wentbuilding
Nieuw Gildestein

address

Padualaan 8, Utrecht
Sorbonnelaan 16, Utrecht
Yalelaan 2, Utrecht

Abstracts

March 27

Gerrit van Meer

Membrane Enzymology, Institute of Biomembranes, Utrecht University,
Padualaan 8, 3584 CH Utrecht, The Netherlands

The biosynthesis, transport and sorting of membrane lipids and proteins

The various cellular membranes have different protein and lipid compositions. In view of the rapid transport between these membranes via vesicles, this must be selective. The basis for the selective transport of both proteins and lipids resides in their aggregation into microdomains. For proteins this generally requires an interaction with coat proteins on the cytosolic surface of the membrane. Since lipids do not span the bilayer and since there are many more lipids than coat proteins, their aggregation must be based on their self-aggregating properties. Lipid "rafts" are thought to be membrane domains of a specific lipid composition that are enriched in specific proteins. They are thought to be involved in protein sorting and in signal transduction at the plasma membrane. An additional level of specificity is found in the transbilayer movement of the lipids by flippases.

Jan Tommassen

Dept. of Molecular Microbiology, Biology

Biogenesis of the bacterial outer membrane

The cell envelope of Gram-negative bacteria consists of two membranes, the inner membrane and the outer membrane, which are separated by the periplasm containing the peptidoglycan layer. The outer membrane is an asymmetrical bilayer with phospholipids and lipopolysaccharides (LPS) in the inner and outer monolayer, respectively. Additionally, the membrane contains integral membrane proteins as well as lipoproteins, which are peripheral membrane proteins attached to the membrane via their lipid moiety. All the components of the outer membrane are synthesized in the cytoplasm or at the cytoplasmic face of the inner membrane and have to be transported across the inner membrane and the periplasm to assemble into the outer membrane. In this lecture, the current knowledge in the field of outer membrane biogenesis will be summarized.

Han Wosten

Dept. of Molecular Microbiology, Biology

Protein secretion by fungi

Mycelial fungi play a central role in element cycling in nature by degrading dead organic material such as wood. Fungal colonization of a substrate starts with the invasion of exploring hyphae. These hyphae secrete enzymes that convert the organic material into small molecules that can be taken up by the fungus to serve as nutrients. Using GFP as a reporter, we show for the first time that exploring hyphae of *Aspergillus niger* differentiate with respect to enzyme secretion; some strongly express the glucoamylase gene *glaA*, while others hardly express it at all. When a

cytoplasmic GFP was used, 27% of the exploring hyphae of a 5-day-old colony belonged to the low expressing hyphae. By fusing GFP to glucoamylase and by introducing an ER retention signal, this number increased to 50%. This difference is due to cytoplasmic streaming of the reporter in the former case, as was shown by using a photoactivatable GFP. Our findings indicate that a fungal mycelium is highly differentiated, especially when taking into account that hyphae in the exploration zone were exposed to the same nutritional conditions.

Reference

Vinck, A., Terlouw, M., Pestman, W.R., Martens, E.P., Ram, A.F., van den Hondel, C.A.M.J.J. & Wösten, H.A.B. (2005). Hyphal differentiation in the exploring mycelium of *Aspergillus niger*. *Mol. Microbiol* **58**:693-699.

Willem Stoorvogel

Biochemistry and Cell Biology, Vet. Medicine

Membrane Transport by Specialised Mammalian Cells

March 28

Judith Klumperman

Head Cell Microscopy Center (CMC), Department of Cell Biology, University Medical Center Utrecht

Membrane traffic in development and disease

The Cell Microscopy Center is an internationally renowned expertise center for the application of advanced microscopy studies in cutting edge bio-medical research. The main microscopy techniques available are: immuno-electron microscopy, live cell imaging, correlative live cell – immuno electron microscopy. The CMC presently houses a Zeiss LSM510-Meta confocal and a Zeiss Axiovert 200MBP epifluorescence system. The CMC further houses a Leica confocal microscope for fixed samples, microtomes to prepare ultrathin (cryo)sections, 3 JEOL transmission EMs and Leica high pressure freezing equipment for the fastest way to fix specimen for microscopical assays.

The CMC is also equipped with a Drosophila lab to exploit the power of genetics and developmental biology and combine it with the microscopy facilities described above.

The general research theme in the CMC concerns intra- and extracellular communication events in relation to disease and development. Presently, there are three main research lines:

- Lysosome biogenesis in healthy and diseased cells (PI: Judith Klumperman)
- The exocytic pathway in relation to Drosophila development (PI: Catherine Rabouille)
- Cellular autophagy from yeast to mammals (PI: Fulvio Reggiori)

Stefan Rüdiger

Cellular Protein Chemistry, Chemistry

Protein folding and protein sorting in the cell

The Central Dogma states that DNA makes RNA makes protein. The least understood step in this basis sequence is how the folding of proteins into their three-dimensional structure is controlled inside the cell. This fundamental question is the focal point in the section Cellular Protein Chemistry, which consists of three research groups. The work of the group of Ineke Braakman focuses on protein folding in the endoplasmic reticulum, where the maturation of secretory and membrane proteins such as the CFTR or influenza haemagglutinine is controlled. The group of Henk Tabak investigates the origin of peroxysomes, for which it emerged only recently that the text book assumption that they are self-replicating organelles was wrong. The group of Stefan Rüdiger analyses protein folding assisted by the molecular chaperone Hsp90, which has a mysterious preference to assist folding of oncogenes.

We address our questions by using various methods to study protein folding and sorting in vivo and in vitro. Our methods range includes cell culture, pulse chase experiments, semi-permeable cell systems, fluorescence microscopy, yeast genetics, protein purification, in vitro stability analysis of proteins and fluorescence spectroscopy. In collaboration with other groups in the IB or the Bijvoet Center, we use proteomics approaches, mass spectroscopy, EM and NMR spectroscopy

Peter J.M. Rottier

Virology Division, Faculty of Veterinary Sciences.

The biology of coronaviruses, with emphasis on how these viruses enter their target cells.

The presentation will have 2 parts. In the first part a general overview will be given about coronaviruses. Coronavirus infections will be discussed at three levels: the level of the cell, which involves the complete infection cycle going from the penetration of these viruses into cells to the stage where new viral particles are massively being produced; the level of the organism, where aspects of virulence and pathogenesis will be discussed; the level of the population, with emphasis on the development of vaccines.

In the second part of my talk I will go into more detail regarding the process of coronavirus entry. In this part I will review our work on the viral spike protein, which is responsible for the fusion of the viral and cellular membrane that leads to the delivery of the viral genome into the cell's cytoplasm

Ger Strous,

Celbiology, UMCU

Cell biology of the growth hormone receptor

The SCF ubiquitin ligases play a pivotal role in the regulation of cell division and various signal transduction pathways which in turn are involved in cell growth, survival and transformation. SCF(TrCP) recognises the double phosphorylated DpSG-XpS destruction motif in β -catenin and I κ B. We show that the same ligase drives the endocytosis and degradation of the GH receptor in a ligand-independent fashion. The WD40 domain binds directly and specifically to a novel recognition motif, previously designated as the ubiquitin-dependent endocytosis motif. An active Neddylation system is also required for receptor degradation, implicating ubiquitin ligase activity. Overexpression of Jak2 inhibits degradation, presumably by interfering with TrCP activity. Although the underlying mechanism is likely complex, we show that local concentrations of SCF E3 components are key factors in degradation and cell surface abundance of a major cytokine receptor. These findings connect NF κ B functions with GH receptor regulation via SCF(TrCP) and Jak2

IB Seminar**Dan Cutler****Sorting nexins – diverse regulators of endosomal sorting and signalling.**

Selected papers:

Kaur, J., Cutler, D.F. (2002) P-selectin targeting to secretory lysosomes of Rbl-2H3 cells. *J. Biol. Chem.* 277:10498-505.

Blagoveshchenskaya, A.D., M.J. Hannah, S. Allen, and D.F. Cutler. 2002. Selective and signal-dependent recruitment of membrane proteins to secretory granules formed by heterologously expressed von Willebrand factor. *Mol. Biol. Cell* 13:1582-93.

Hannah, M., Williams, R., Kaur, J., Hewlett, L., Cutler, D. (2002) Biogenesis of Weibel-Palade bodies. *Semin. Cell Dev. Biol.* 13:313-324.

Hannah, M.J., A.N. Hume, M. Arribas, R. Williams, L.J. Hewlett, M.C. Seabra, and D.F. Cutler. 2003. Weibel-Palade bodies recruit Rab27 by a content-driven, maturation-dependent mechanism that is independent of cell type. *J Cell Sci.* 116:3939-48.

Michaux, G., L.J. Hewlett, S.L. Messenger, A.C. Goodeve, I.R. Peake, M.E. Daly, and D.F. Cutler. 2003. Analysis of intracellular storage and regulated secretion of 3 von Willebrand disease-causing variants of von Willebrand factor. *Blood.* 102:2452-8.

Michaux, G., and D.F. Cutler. 2004. How to roll an endothelial cigar: the biogenesis of Weibel-Palade bodies. *Traffic.* 5:69-78.

Williams, R., T. Schluter, M.S. Roberts, P. Knauth, R. Bohnensack, and D.F. Cutler. 2004. Sorting nexin 17 accelerates internalization yet retards degradation of P-selectin. *Mol Biol Cell.* 15:3095-105.

Lui-Roberts, W.W., L.M. Collinson, L.J. Hewlett, G. Michaux, and D.F. Cutler. 2005. An AP-1/clathrin coat plays a novel and essential role in forming the Weibel-Palade bodies of endothelial cells. *J Cell Biol.* 170:627-36.

Michaux, G., K.B. Abbitt, L.M. Collinson, S.L. Haberichter, K.E. Norman, and D.F. Cutler. 2006a. The physiological function of von Willebrand's factor depends on its tubular storage in endothelial Weibel-Palade bodies. *Dev Cell.* 10:223-32.

Michaux, G., T.J. Pullen, S.L. Haberichter, and D.F. Cutler. 2006b. P-selectin binds to the D'-D3 domains of von Willebrand factor in Weibel-Palade bodies. *Blood.*

Dan Cutler: IB seminar and discussion afterwards
Paper to be discussed after IB seminar

Theos, A.C., D. Tenza, J.A. Martina, I. Hurbain, A.A. Peden, E.V. Sviderskaya, A. Stewart, M.S. Robinson, D.C. Bennett, D.F. Cutler, J.S. Bonifacino, M.S. Marks, and G. Raposo. 2005. Functions of adaptor protein (AP)-3 and AP-1 in tyrosinase sorting from endosomes to melanosomes. *Mol Biol Cell.* 16:5356-72.

March 29

Antoinette Killian

Biochemistry of Membranes, Chemistry

Understanding membrane proteins

Topics:

- short introduction of research themes within the dept. Biochemistry of Membranes
- model membrane systems
- architecture of membrane proteins
- use of synthetic peptides

Techniques:

- solid state NMR on peptides and lipids in membranes
- circular dichroism and FT-IR as tools to determine the secondary structure of (membrane) proteins

Eefjan Breukink

Biochemistry of Membranes, Chemistry

Interaction of antibiotics with membranes

Topics:

- Antibiotics, focussed on nisin
- bacterial cell wall synthesis,
- interaction of antibiotics with membranes

Techniques:

- Use and preparation of different model membrane systems
- Binding assays: biochemical assays, isothermal titration calorimetry
- Fluorescence spectroscopy: Trp fluorescence, quenching, FRET

Lucy Rutten

Crystallography, Chemistry

Structure determination of membrane proteins by crystallography

This presentation will focus on technological aspects of structure determination by crystallography of several enzymes in the bacterial outer membrane.

March 30

Martin Rook

Medical Physiology and Sports Medicine, UMCU

Physiology of membrane protein channels

Topics:

- Short introduction of research themes in Medical Physiology
- Stem cell models for studying ion and gap junction channel regulation.
- Patch Clamp, a tool to study expression and function of ion channel proteins in embryonic and adult heart progenitor cells.

Techniques:

- Stem cell differentiation
- Ectopic ion channel expression
- Gene silencing
- Patch Clamp / Whole Cell Voltage Clamp
- (PCR, immuno-cytochemistry / Western blot)

Joost Holthuis

Membrane Enzymology, Chemistry

The emerging role of lipid flippases in membrane trafficking

Cells display asymmetric lipid distributions across their plasma membranes with the aminophospholipids concentrated in the inner leaflet. How this asymmetry is established and what purpose it serves for the functioning of cells is not well understood. We identified two P-type ATPases required for aminophospholipid transport across the plasma membrane in yeast and uncovered a functional link between ATPase-dependent lipid pumping and endocytic vesicle formation. Recently, we found that the yeast Golgi contains similar ATPases with a critical role in secretory vesicle budding. Our current work aims to unravel the mechanism of ATPase-dependent lipid transport in relation to membrane deformation and vesicular trafficking.

Techniques:

yeast genetics; subcellular fractionation; lipid transport assays; flow cytometry; membrane protein purification and reconstitution.

Jan Tommassen

Dept. of Molecular Microbiology

Transport proteins in the bacterial outer membrane

The outer membrane of Gram-negative bacteria is not energized by a proton gradient and energy-rich compounds, such as ATP, are not available in the periplasm. An intriguing question then is how transport processes, such as the uptake of nutrients and the secretion of proteins, are energized. The bacteria have found many different solutions to this problem. In this lecture, several membrane proteins involved in these processes will be described, ranging from simple diffusion porins, which allow for the

influx of nutrients by passive diffusion, to highly complex, multi-component machineries, which inject proteins directly from the bacterial cytoplasm into the cytoplasm of eukaryotic target cells.

March 31

IB seminar
Declan Doyle

Structural changes involved in Kir channel opening and closing

The combination of two processes define the function of any ion channel: selectivity and gating. The crystal structures of several K⁺ potassium channels has revealed at the molecular level how K⁺ channels perform these function. This talk will explore the structural features of K⁺ channels that allow the channel to first select for K⁺ ions over the smaller monovalent cations such as Na⁺ (selectivity) and for Kir channels control the opening and closing of the ion conduction pathway (gating).

References:

Kuo A, Gulbis JM, Antcliff JF, Rahman T, Lowe ED, Zimmer J, Cuthbertson J, Ashcroft FM, Ezaki T, Doyle DA. Crystal structure of the potassium channel KirBac1.1 in the closed state. *Science* 2003, 300,1922-6.

Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R. The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity. *Science* 1998, 280, 69-77.

Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. Crystal structure and mechanism of a calcium-gated potassium channel. *Nature*. 2002, 417, 515-22.

Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. The open pore conformation of potassium channels. *Nature* 2002, 417, 523-6.

April 3

Peter van der Sluijs

Dept. Cell Biology UMC Utrecht

Regulation of secretory lysosome function in haematopoietic cells

Melanocytes and haematopoietic cells combine the functions of lysosomes and secretory granules into a hybrid organelle, the melanosome and secretory lysosome, respectively. Secretory lysosomes are thought to be distinct from conventional lysosomes. For instance stimulation of the T cell receptor complex on CD8-positive T cells, or the high affinity Fc γ receptor on mast cells triggers the release of biological effectors like granzymes in CTLs, and of histamine in mast cells. The molecular mechanisms underlying secretion from lysosomes remain largely to be defined. In this lecture I will discuss our strategies to find and functionally characterize proteins important for this pathway.

Techniques

Proteomic screens for identification novel proteins involved in membrane transport

Genetic biochemical and light microscopy-based protein interaction assays

Recombinant protein expression and purification

Creation of transfected cell lines by various methods

Fluorescence techniques including live cell imaging and FACS analysis

Transport assays in endocytic and exocytic pathways in higher eukaryotes

Contact information

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B.M.

Veterinary Medicine

Gadella

Signal transduction pathways involved in sperm activation and mammalian fertilization

Mammalian sperm are not able to fertilize eggs immediately after ejaculation. They acquire fertilization capacity after residing in the female tract for a finite period of time that varies depending on the species and such a period of time in the female tract is required for the sperm to acquire their fertilizing capacity. Freshly obtained rabbit sperm introduced into the Fallopian tubes shortly after ovulation were not able to penetrate the eggs; instead if sperm were introduced a few hours before ovulation, the majority of the eggs were later observed to be fertilized. This observation led to the conclusion that freshly ejaculated sperm are incapable of penetrating the zona pellucida immediately, and that sperm must remain within the female tract for a period before they are able to penetrate the eggs. Following these original observations, many studies confirmed that the environment of the female tract induces a series of physiological changes in the sperm; these changes are collectively called "capacitation". Inherent to these first observations was that capacitation-state became defined using fertilization as end-point. However, a variety of evidences suggest that the functional changes occurring in the sperm during capacitation are not one event, but a combination of concomitant processes; mainly, the sperm acquisition of the

ability to undergo an agonist (e.g. zona pellucida, progesterone) induced acrosome reaction and the modification in the motility pattern known as hyperactivated motility (both enabling efficient zona drilling so that the sperm can reach the oolemma). The acquired ability of the capacitated sperm to drill the zona enables them to reach the oolemma.

Although more than 50 years have passed since sperm capacitation was first reported and conditions for *in vitro* capacitation has been established in a variety of mammalian species, it is noteworthy that the molecular basis of this process is still today not well understood. Nevertheless, recent work is beginning to point to a unified model of how this event is controlled at the molecular level. To dissect the molecular mechanisms involved in sperm capacitation, most authors have used *in vitro* capacitation systems incubating the sperm in chemically defined buffers that mimic the glucose and electrolyte content of the oviduct and are enriched with serum albumin components. It is important though to keep in mind that capacitation occurs in the female tract and sooner or later, *in vitro* capacitation models will need to be validated *in vivo* taking into consideration the physiology of the female track that is under hormonal control. The purposes of this lecture are to consider some recent contributions towards our understanding of capacitation and fertilization, to summarize open questions in this field, and to discuss future avenues of research.

Harrie Heijen

Haematology, UMCU

Morphology of megakaryocyte and blood platelet

Blood contains ~ 150,000-350.000 platelets/mm³. Platelets are non-nucleated discoid cells, 2-5 um diameter large, that derive from the megakaryocyte in the bone marrow. The life span of a circulating blood platelet is 8-11 days.

In this introduction we will discuss MK differentiation and maturation, the ultimate formation of platelets, and their function. Issues that will be addressed: morphology of MK and platelet, biosynthesis, endocytosis, organelle biogenesis, platelet birth, adhesion and cell dynamics.

Functional aspects: Adhesion to damaged endothelium and exposed collagen induces cytoskeletal rearrangement, platelet shape change, and release of storage granules. These actions lead to the promotion of the coagulation cascade and the formation of a stable clot and the arrest of bleeding. Cellular responses upon adhesion and activation, cytoskeletal rearrangements, microtubule organization

Electron microscopy. Ultrastructure, (trans) location of macromolecules, EM-Tomography.

Light microscopic imaging: Visualization of platelet dynamics in real time (i.e. adhesion under flow conditions. Different microscopical methods will be shown (confocal microscopy, interference reflection contrast microscopy), including the localization of receptors, visualization of cytoskeletal dynamics.

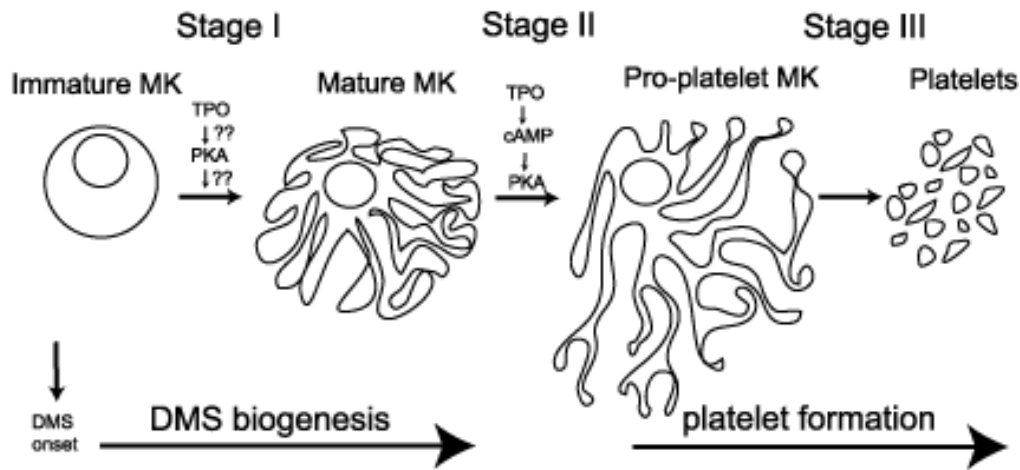
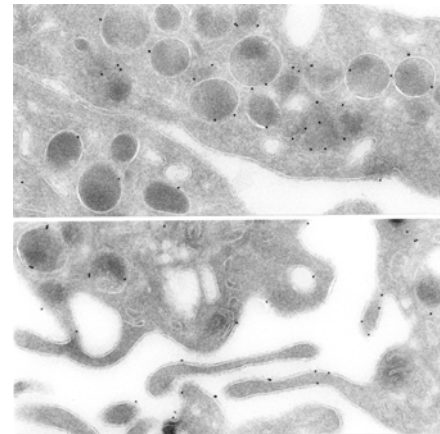
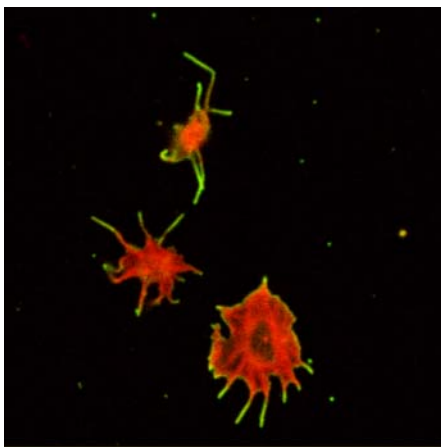


Fig.1: Model of DMS biogenesis and platelet formation



Paul van Bergen en Henegouwen
 Dept of Biology, Celbiology, UU

How to stop signal transduction?

Stimulation of cells with growth factors initiates a sequence of signaling events that leads to cell proliferation. Many details of this process are known for the receptor of the epidermal growth factor mainly because this receptor was the first growth factor receptor that was sequenced (1984). Binding of EGF to this receptor induces the trans- or cross-phosphorylation of the intracellular domain by receptor dimerization. The signaling process is further continued by binding of signaling proteins via their SH2- or PTB-domains to the activated receptors finally resulting in the onset of the cell cycle. Of crucial importance for the regulation of cell proliferation is the negative feedback. For EGF-signaling at least four different mechanisms exists, among which are affinity control and receptor down regulation. Details of these mechanisms will be presented in this lecture.

Contact information

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April 4

Gerry T. Snoek

Biochemistry of Lipids, Chemistry

Phosphatidylinositol Transfer Proteins: Emerging roles in cell proliferation, cell death and survival.

The phosphatidylinositol transfer proteins (PI-TP's) are characterized by their ability to transfer phosphatidylinositol, PI (PI-TP α) or PI and sphingomyelin, SM (PI-TP β) between membranes *in vitro*. Recent studies in our laboratory have shown that PI-TP α and β are involved in the regulation of cell proliferation and cell death (apoptosis) and therefore probably play a significant role in processes involved in carcinogenesis. In addition, data are available that PI-TP α plays an important role in the prevention of neurodegeneration.

The techniques we use in this study will be discussed: fluorescence techniques to determine *in vitro* lipid transfer activity, cellular localization of proteins and apoptotic events, radioactive labeling techniques to study lipid metabolism, analysis of (oxidized) proteins by 2-D-electrophoresis, analysis of amino acid sequence of proteins, 2-D-phosphopeptide mapping and 2-D-phospho-aminoacid analysis.

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Bernd Helms

Biochemistry and Cell Biology, Veterinary Medicine

Signaling during Host-Pathogen Interactions

Successful establishment of infection by bacterial pathogens requires adhesion to host cells and cellular invasion, followed by intracellular multiplication, dissemination to other tissues, or persistence. Bacteria use sophisticated strategies to establish a complex host/pathogen molecular crosstalk that leads to subversion of cellular functions and establishment of disease. In many cases, pathogens divert existing intracellular membrane transport pathways to escape the immune response. We will focus on strategies of some intracellular pathogens and discuss how they target specific host cell lipids and proteins. Especially the modification of signaling pathways appears effective in changing their temporal and spatial distribution, making them ideal modulators of local and transient cellular mechanisms.

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Martijn Gebbink

Vascular membrane biology, UMCU

Endothelial cells line blood vessels. Besides a physical function as a barrier between tissue and blood to allow blood flow these cells have important physiological functions. Under normal conditions endothelial cells are anti-thrombogenic to prevent thrombotic events. During injury endothelial cells become pro-thrombogenic to prevent blood loss (hemostasis). Subsequently endothelial cells regulate wound

healing by initiating blood clot lysis. In addition endothelial cells mediate inflammatory processes to conquer (potential) hazardous infection. Finally endothelial cells help to control blood pressure. Taken together endothelial cells play an important role in homeostasis. Hence, dysfunction of endothelial cells is implicated in a wide variety of (inflammatory) diseases, including atherosclerosis, hypertension and thrombosis

"Biogenesis, Translocation & Sorting" (BTS)

- Mechanisms of protein- and lipid-insertion in-, and translocation through membranes in prokaryotic and eukaryotic cells (included are cytoplasmic- and outer-membranes, ER, mitochondria, or chloroplasts).
- Biogenesis of membrane-, secretion- and organelle-proteins (included are aspects like folding, co- and post-translational modifications, and the assembly in macro molecular complexes).
- Mechanisms of addressing, sorting, transport and translocation of proteins and lipids in the cell.

"Membrane proteins" (MP)

- Structural and topographic analysis of membrane-proteins/enzymes, by way of Röntgen-analysis, EM, and chemical- and genetic techniques;
- How do these proteins work (biochemical characterisation, ligand-binding sites, specificity);
- Regulation of membrane protein functions by way of membrane structure/-components (effects of membrane lipids/lipid-organisation on the activity of membrane enzymes and / or the characteristics of membrane proteins).

"Signal transduction" (ST)

Localisation and function of:

- Membrane-associated proteins like phospholipases, kinases, receptors and pore- and transport proteins.
- Signal transduction components, like ras, MAP-kinase, etc.
- Phospholipid transport proteins.
- Cell cycle regulation: cdk's, cyclines, etc.

"Vascular Membrane Biology" (VMB)

- Cardiovascular risk factors (hypertension, uremia) and endothelial cell function and the role of progenitor cells.
- Aging of the vessel wall and endothelial cells by way of genomics, proteomics, electron microscopy and cell biological techniques.
- Interaction between haemostatic proteins and cell surface receptors on platelets, blood cells and endothelial cells, implications for haemostasis and thrombosis.

Participants (14)

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