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Bio-TecReport

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Organ für Life Science Kongresse

ELSO 2004
4. bis 8. September 2004 in Nizza

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ELSO IN NIZZA

Wissenschaft und mehr

Bereits zum zweiten Mal war Nizza im letzten Monat gut gelaunte Gastgeberin für das Jahrestreffen der European Life Scientist Organization (ELSO). Das sommerliche Nizza präsentierte sich von seiner besten Seite als 1500 Wissenschaftler zum 4. Jahrestreffen ins großzügige Acropolis Conference Centre kamen, um 250 Vorträge, 650 Posterpräsentationen und eine Industrieausstellung mit mehr als 40 Ausstellern zu genießen. Das Programm war dicht gepackt mit Vorträgen hochrangiger Wissenschaftler aus Europa und der ganzen Welt und die vollen Hörsäle und übervollen Postersäle haben anschaulich bewiesen: Wissenschaft ist und bleibt des Wissenschaftlers Lieblingsthema.

ELSO 2004 fand gemeinsam mit dem von der International Federation of Cell Biology und der Französischen Gesellschaft für Zellbiologie organisierten 8. Weltkongress für Zellbiologie statt und demnach war die Zellbiologie auch in diesem Jahr wieder ein zentrales Thema des ELSO Treffens. Darüber hinaus repräsentierte das Meeting jedoch alle Aspekte der molekularen Lebenswissenschaften. Immunologie, Genetik, Pflanzenbiologie, Mikrobiologie, Biochemie, Strukturbiologie, Entwicklungsbiologie und Molekulare Medizin fanden ihren Platz. ELSO möchte Grenzen durchlässig machen und zwar nicht nur zwischen den verschiedenen Fachdisziplinen. Auch zwischen den verschiedenen europäischen Forschungsnationen. Dazu unterstützt ELSO die Idee einen Europäischen Forschungsrat einzurichten und mit der Einladung von Octavi Quintana Trias den Eröffnungsvortrag des diesjährigen Meetings zum Thema Forschungsförderung durch die EU zu halten, wurde ein weiteres Signal in Richtung einer Europäischen Forschungsgemeinschaft gesetzt.

Forscher aus 13 europäischen Nationen gingen in Nizza an den Start und es sind in jedem Jahr vor allem junge Nachwuchswissenschaftler, die der Tagung ihr besonderes Gesicht geben. Es ist inzwischen eine gute Tradition, dass sich die junge Forschergeneration auf den ELSO Meetings trifft, um sich zu den Top-Themen der Wissenschaft auszutauschen und darüber hinaus Informationen und Hilfe für einen erfolgreichen Fortgang ihrer Karriere zu erhalten. Die Kongressgebühren für Studenten sind extrem gering und die Posterausstellung, stets Domäne der Doktoranden und Postdocs, hat auf jeder Jahrestagung eine zentrale Stellung – im Zeitplan der Veranstaltung und auch unter dem räumlichen Aspekt, nämlich viel beachtet mitten in der Industrieausstellung. Als zusätzliches Novum wurde in diesem Jahr der Career Mentoring Lunch veranstaltet. Eine seltene und gern genutzte Gelegenheit für das leibliche Wohl zu sorgen und gleichzeitig mit einem arrivierten Wissenschaftler in der Funktion eines Mentors über alle brennenden Fragen der beruflichen Möglichkeiten und Herausforderungen zu diskutieren. Eine weitere Gelegenheit zur Karriere-Nachhilfe für Postdocs und Junior Gruppenleiter bot eine Session zum Thema Funding.

ELSO 2004: eine hochkarätige wissenschaftliche Veranstaltung und wichtiger Baustein auf dem Weg zu einem Wir-Gefühl der Forscher in Europa.

ELSO MEETINGS

ELSO 2005 will be in Dresden, Germany from 3–7 September 2005
ELSO 2006 will be in Nice, France from 9–13 September 2006

WELCOME TO NICE

All about Science

From September 4 to 8 the European Life Scientist Organization (ELSO) returned to the Cote d'Azur resort of Nice for its fourth annual congress. ELSO 2004 brought together 1500 scientists, 250 speakers, 650 poster presentations and over 40 commercial exhibitors who joined the Acropolis conference centre, enjoying excellent science, beautiful weather and an exciting city.

As in 2002, ELSO 2004 was held in collaboration with the French Cell Biology Society (SBCF), but this time it was also a joint venue of the Eighth World Congress for Cell Biology. ELSO strives to integrate all areas of the molecular life sciences that impinge on cell biology: immunology, genetics, plant biology, microbiology, biochemistry, structural biology, development and molecular medicine. As well as breaking down the boundaries between subjects, ELSO also wants to create a european research area where scientists and information may move freely. ELSO founder and president Kai Simons points out "What is really missing in Europe is this feeling of being together. The flair of the ELSO meetings and the participation of so many young scientists is helping to create this sense of unity."

The Nice meeting brought together researchers from 13 European and 12 other countries. Speakers came from as far away as Japan, Australia, the USA, Canada and Korea, as well as all points of Europe.

ELSO is very much geared towards bringing young scientists together at the annual meeting, as well as helping them in their personal development as scientists and helping to create opportunities for their future careers. To make the meeting very accessible to young researchers registration fee for the congress is kept to a strict minimum for students. The poster sessions – traditionally the domain of PhD students and postdocs – have a two-hour slot in the early afternoon when there are no symposia or minisymposia distracting. Additionaly several young scientists appreciated the Career Mentoring Lunch, which was tried out for the first time this year, discussing various career issues with older mentors.

Welcome to ELSO 2004. Bio-TecReport is presenting some of this years Highlights.

Diseases of the cell nucleus

T. MISTELI, BETHESDA

The cell nucleus was the first intracellular organelle to be discovered and has been the object of intense interest of cell biologists for many years because of its fundamental function in the cell. The recent discovery that defects in the structure of the nucleus cause human disease has identified the mechanisms that maintain nuclear architecture as novel therapeutic targets.

Bio-TecReport

Dr. Tom Misteli

Like any other 6 year old, Joey likes to play football. But he is different from his friends in that he is unusually skinny, virtually bold and has wrinkled skin. Joey has a rare pre-mature aging disorder called Hutchinson-Gilford progeria syndrome that makes him look decades beyond his true age. For many years, the cause of the disease was a mystery and most clinical research focused on defects in metabolic pathways. In 2003 the gene that causes the disease was identified as a one of the major architectural elements of the human cell nucleus. This surprising finding represents the culmination of the recent realization that defects in some of the most fundamental mechanisms involved in the cell biological organization of the genome in the nucleus are responsible for several human diseases ranging from muscular dystrophy to cancer.

The cell nucleus harbors virtually the entire human genome and is the site of some of the most essential cellular processes including gene expression, DNA replication and repair. In order to maximize the efficiency of these events, they are spatially organized and coordinated within the nucleus by structural elements. The most prominent architectural proteins of the nucleus are the lamin proteins. The A- and B-type lamins are filamentous molecules, which form an intricate meshwork underlying the nuclear membrane and are also found in the nuclear interior. Although one of the main functions of the lamina is likely to provide structural support for the nucleus, the lamina network also interacts, directly or via adaptor molecules, with chromatin and in this way anchors regions of chromatin to the nuclear periphery. This anchoring function may be functionally relevant as it might confer an active or inactive status to a particular gene or genome region.

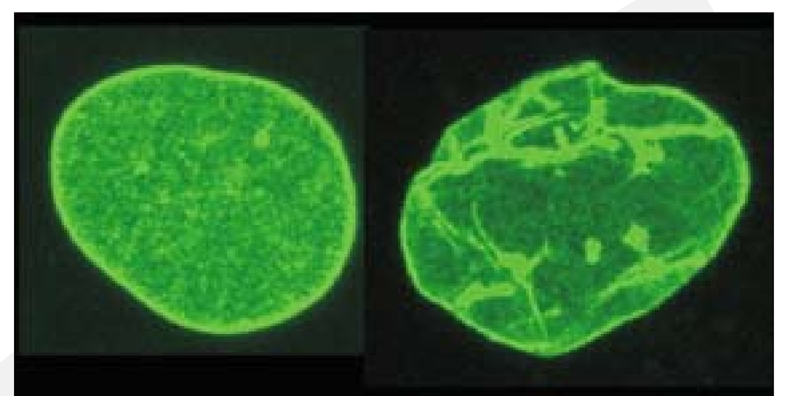
Fig.: A cell nucleus from a healthy individual and from a pre-mature aging syndrome patient.

Mutations in lamins have been identified as the genetic cause of a group of human diseases, the laminopathies. Most prominently, lamin defects cause various types of muscular dystrophies, lipodystrophies and at least two types of pre-mature aging syndromes. Most of these diseases are recessive and the mutant lamin protein generally acts in a dominant negative fashion affecting the normal behavior of endogenous lamins. The dominant negative nature of the mutant protein requires that for therapeutic purposes the mutant protein must be eliminated or at least neutralized.

How mutations in a structural protein of the cell nucleus cause disease is still a mystery. One possibility is that incorporation of mutant lamin alters the mechanical properties of the cell nucleus making it more prone to damage and cell death. This idea fits the

ADHESIONS TAKE OFF

Podosomes

S. LINDER, MÜNCHEN, GERMANY

Podosomes are dynamic, actin-rich adhesions classically described in monocytic cells such as macrophages, dendritic cells or osteoclasts. However, they are now also being discovered in an increasing number of other cell types. Together with the related invadopodia, they constitute a group of matrix-degrading organelles implicated in cell invasion. Interest in these structures is now rising considerably, owing to both their physiological relevance and attractiveness as model systems for the study of actin dynamics.

Everyone working in cell biology is at least passingly familiar with cell-substrate contacts such as focal adhesions or focal complexes. Numerous studies on these structures have resulted in a wealth of information about cell adhesion and migration. In contrast, much less attention was focused on podosomes, which share many components with focal adhesions but constitute a different – and by no means less interesting – type of adhesive structure (for reviews, see (1) and (2)). Importantly, podosomes can be found in ex vivo model systems of invasive cells, while focal adhesions are mostly restricted to cells cultured on rigid artificial substrates. Podosomes can easily be distinguished from other cellular structures by 3 criteria: i) they consist of an f-actin-rich core (not present in focal adhesions) which is surrounded by a ring structure containing proteins such as talin or vinculin **(fig 1)**, ii) they form upon contact with the substratum on the substrate-attached part of the cell, and iii) they have a diameter of ca. 0.5 μm. In addition, they are highly dynamic and show a half-life of 2–12 min.

Podosomes are typically observed in cells which are or become invasive and have to cross tissue boundaries. They are constitutively formed in cells of the monocytic lineage such as monocytes, macrophages, (immature) dendritic cells or osteoclasts. However, podosomes can also be found or induced in a variety of other cell types such as smooth muscle cells or endothelial cells. Podosome-inducing signals include activation of Src, PKC or RhoGTPases. Moreover, cellular transformation with viruses whose oncogenes code for protein tyrosine kinases leads to the formation of similar structures, originally also called podosomes, but now termed invadopodia. Indeed, the first detailed description of podosome-type adhesions was of such virally induced invadopodia (3).

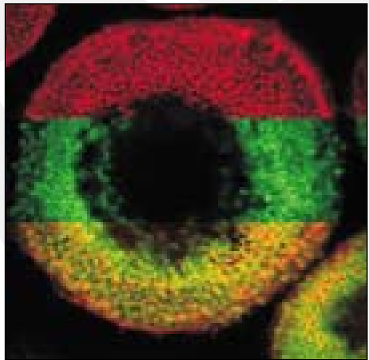

Fig 1: Podosome structure in a primary human macrophage. Confocal laser scanning micrograph of ventral part of cell. Upper third: f-actin staining (red), middle third: talin staining (green), lower third: overlay of both stainings (colocalization in yellow). Note that each podosomal f-actin-rich core is surrounded by a talin-containing ring structure. Originally published in (1).

Priv.-Doz. Dr. Stefan Linder

Podosomes are formed on the ventral part of the cell and, as shown by interference reflection microscopy, establish close contact to the substratum. Therefore, they most probably constitute adhesion structures. Their recruitment to the leading edge of migrating cells also implies a role in cell migration, possibly through localized establishment of firm contact once a cell has decided where to go. Finally, enrichment of matrix metalloproteases at podosomes and podosome-localized matrix degradation also implicate these structures in matrix remodelling and tissue invasion. In sum, podosomes probably have major roles in adhesion, migration and invasion of cells, with much of the evidence still being circumstantial and awaiting rigorous testing in ex vivo and in vivo systems.

Diseases associated with defects in podosome formation include the Wiskott-Aldrich Syndrome (WAS) and the related X-linked thrombocytopenia, both arising due to mutations in the *WASp* gene. WAS macrophages are not only unable to form podosomes but also display defects in chemotaxis. Moreover, dendritic cells from patients with chronic myeloid leukemia (CML) display both defects in podosome formation and cell migration. However, it is not clear whether absence of podosomes is at least partially causative or simply symptomatic for these diseases. At the moment, much interests is also focused on the potential role of podosomes/invadopodia in some types of cancer.

Apart from their likely physiological roles, podosomes have now also attracted attention as accessible model systems for the study of actin dynamics. Podosome cores constitute actin laboratories *en miniature*, where the concerted action of RhoGTPases, actin regulators such as WASP family proteins, Arp2/3 complex or gelsolin generate a constant localized turnover of actin filaments. Additional signals regulating podosome dynamics come from various protein kinases and the microtubule system. All this makes podosomes an attractive model for the study how integration of diverse cellular pathways is regulating localized actin dynamics.

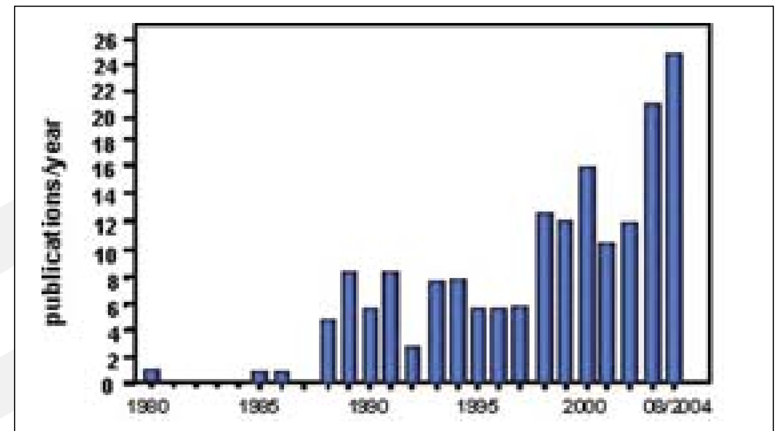

Fig 2: Statistic of publications on podosomes and invadopodia since their original observation in 1980.

Podosomes received steady, but essentially low-level coverage during the 1980's and 1990's. However, they have now captured the interest of a wider audience, and 2004 is already the most prolific year in podosome research yet **(fig 2)**. It appears that podosomes have finally left their fledgling years behind and are ready for take off.

Finally, everyone interested in adhesive structures is cordially invited to participate in the upcoming „Adhesion meeting: podosomes – invadopodia – focal adhesions", in Munich, April 28-30, 2005. For more information, please visit: http://www.adhesion-meeting.com.

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ANKÜNDIGUNG

Adhesion meeting 2005

podosomes – invadopodia – focal adhesions

Munich, April 28–30, Germany

The symposium will take place
at the Max Planck Institute of Biochemistry, Munich, Germany,

Registration deadline:
Feb. 13, 2005

Abstract deadline:
Jan. 16, 2005

Patterning and Initiation of Arabidopsis Trichome

M. HÜLSKAMP, KÖLN, GERMANY

Arabidopsis trichome patterning serves as an excellent model system to study how cell-cell interactions govern the spatially correct cell fate determination. An evolutionary conserved complex of bHLH and MYB-like transcription factors together with a WD40 protein promote trichome cell fate and are counteracted by small single-repeat MYB-like factors that are thought to move between cells. Recently it was found that most of the involved factors act in a highly redundant manner.

Arabidopsis trichomes are epidermal cells that develop on young leaves. They are spaced with an average distance of three to four cells on their first appearance and almost never develop next to each other suggesting the existence of a mechanism regulating their pattern [4]. The current model trying to explain how the trichome distribution pattern is established assumes that initially all cells produce a trichome-promoting factor [3,2]. This in turn is thought to activate factors that counteract the promoting factors and that can move into the neighboring cells. As a consequence, all cells begin to compete with each other and due to a postulated positive feedback of the activator small locations in the relative concentrations are rapidly amplified, and those cells highly expressing the trichome promoting factors develop into trichomes.

The general logic of the model is supported by genetic and molecular data, however, direct evidence for several aspects of this model is still missing. Four of the identified patterning genes act as trichome promoting factors: GLABRA1 (GL1), TRANSPARENT TESTA GLABRA1 (TTG1), GLABRA3 (GL3) and ENHANCER OF GL3 (EGL3) [7,8,15]. The gl1 and ttg1 mutants lack most trichomes, gl3 mutants have fewer trichomes and due to a redundancy with egl3, the gl3 egl3 double mutant is also glabrous. Four trichome-suppressing genes are represented by TRIPTYCHON (TRY), CAPRICE (CPC), ENHANCER OF CAPRICE TRIPTYCHON1 (ETC1), and ETC2 [12,11,5,6].

Prof. Dr. Martin Hülskamp

The trichome promoting factors form a complex in which a basic helix-loop helix factor (GL3 or EGL3) [10,15] forms a dimer that binds the MYB-related transcription factor GL1 [9] and the WD40 protein TTG1 [14,10,15]. GL1 and GL3 have transcriptional activation domains and are therefore thought to mediate the transcriptional activation. Also the negative regulators TRY, CPC, ETC1 and ETC2 encode closely homologous MYB-related transcription factors, however, they lack any obvious transcriptional activation domain [12,11,5,6]. These four genes act in a redundant manner as single mutants show either a weak patterning defect or no phenotype, but exhibit an strongly enhanced phenotype in double, triple or quadruple mutants. Yeast three-hybrid analysis has shown that TRY interferes with the interaction of GL1 and GL3 suggesting that in the presence of high concentrations of TRY a non-active TRY GL3 TTG1 complex instead of a active GL1 GL3 TTG1 complex is formed [1].

To meet the requirements of the model outlined above it would be necessary that the trichome suppressing proteins can move between cells. Intercellular movement had only been demonstrated for CPC in demonstrating that the CPC:GFP fusion protein can move into neighboring cells in the root epidermis [13]. It remains to be determined whether this is also true for TRY and the other proteins in the leaf epidermis during trichome patterning.

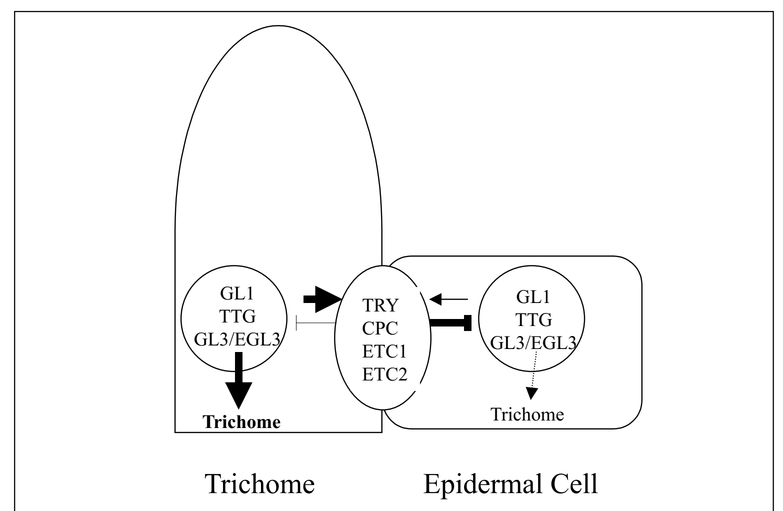

Fig: Model to explain trichome patterning.
The interactions of a developing trichome cell (left) and an epidermal cell (right) are shown. Both cells express the trichome promoting genes GL1, GL3/EGL3 and TTG1 and interact with each other through the moving transcription factors TRY, CPC, ETC1 and ETC2. In the situation shown, the expression levels of the trichome promoting factors are already higher leading to higher levels of the negative factors. This is indicated by the thickness of the respective arrows and blunted bars.

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FROM GENE EXPRESSION TO DISEASE

The mammalian circadian timing system

U. SCHIBLER, GENEVA, SWITZERLAND

In mammals, most physiological processes follow daily rhythms. These include sleep-wake cycles, blood pressure, heartbeat frequency, endocrine function, renal plasma flow and urine production, intestinal peristaltic, and hepatic metabolism. All of these rhythms are controlled directly or indirectly by a circadian timekeeper residing in the suprachiasmatic nuclei (SCN) of the hypothalamus. Wheel-running activity is a robust behavioral output of the circadian clock in laboratory rodents and provides reliable information about the intrinsic period length of the circadian oscillator under constant conditions. For example, in constant darkness, the free-running period length (tau or τ) of most laboratory mouse strains is significantly shorter than 24 hours. Hence, in order to be in resonance with geophysical time under normal conditions the clock has to be reset every day by the photoperiod. That the SCN indeed harbors the circadian master pacemaker has been demonstrated by elegant lesion and transplantation experiments in hamsters. These experiments have benefited from the availability of tau mutant animals that free-run with a dramatically shortened period length of about 20 hours. Bilateral stereotaxic lesion of the SCN resulted in immediate arrhythmic wheel-running activity in both wild-type and tau hamsters. However, SCN grafts implanted in the proximity of the lesioned hypothalamus areas rescued cyclic locomotor activity with a period that closely matched that of the donor animals[1].

Prof. Dr. Ueli Schibler

A welcome surprise came with the discovery that circadian timekeepers not only reside in SCN neurons, but in most peripheral cell types and even in cultured fibroblasts[2,3]. In the latter, circadian gene expression can be elicited by high concentrations of serum or chemicals activating various signaling pathways. In intact animals, feeding time is the dominant timing cue for peripheral clocks. It thus appears that the SCN synchronizes these clocks indirectly by driving rest-activity cycles and, as a consequence, feeding time[4,5]. However, the molecular mechanisms by which feeding/fasting cycles can phase-entrain peripheral oscillators have not yet been identified.

The discovery of circadian oscillators in abundant cell types such as hepatocytes and tissue culture cells has opened the way to biochemical experiments and has therefore greatly contributed to the elucidation of molecular mechanisms participating in circadian rhythm generation (for review see[6]). According to current believe, the circadian oscillator is composed of two interconnected feedback loops in clock gene expression that are schematically depicted in **figure 1**. This molecular clockwork circuitry generates rhythms in physiology and behavior by employing direct and indirect output pathways. In the most direct route, core components of the molecular oscillator directly drive the cyclic expression of enzymes, hormones, and other effector molecules. In less direct pathways, the molecular clock controls the cyclic accumulation of transcription factors, which in turn govern the rhythmic expression of effector genes. As illustrated in **figure 2**, the three PAR bZip proteins DBP, HLF, and TEF belong to this class of output regulators. In liver, these transcription factors accumulate with high daily amplitudes and thereby participate in the circadian regulation of metabolism and detoxification. In the brain, the amplitude of circadian PAR bZip gene expression is much lower than that observed in liver and other peripheral tissues[7]. Recent work suggests that high amplitude circadian PAR bZip gene expression would perhaps be harmful in the brain. In this organ, PAR bZip transcription factors regulate the expression of pyridoxal kinase, an enzyme involved in the production of pyridoxal phosphate (PLP) from vitamin B6. As PLP is a coenzyme of many enzymes involved in neurotransmitter metabolism, neurotransmitter homeostasis is severely compromised in PAR bZip triple knockout mice. As a consequence, a large fraction of these animals succumb to lethal epileptic seizures between 15 days and 3 months of age[7]. Animals surviving seizures nevertheless age and die prematurely. We are currently investigating the physiological causes of the accelerated aging and the reduced longevity in PAR bZip deficient mice.

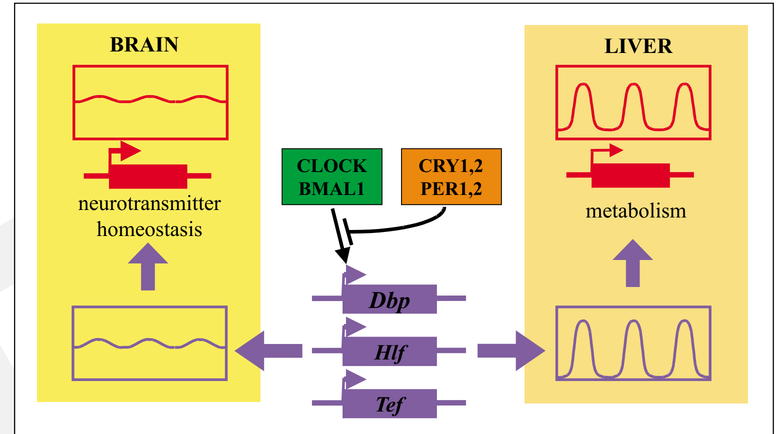

Fig 2: The clock output transcription factors DBP, HLF, and TEF regulate different processes in brain and liver.
The three PAR bZip transcription factors DBP, HLF, and TEF accumulate in a highly circadian fashion in liver, but oscillate with only small amplitudes in most brain regions. Circadian Dbp transcription is driven directly by the core components of the molecular oscillator8. The mechanisms involved in cyclic Hlf and Tef transcription have not yet been investigated. In brain, PAR bZip proteins regulate the synthesis of PLP, which in turn imparts on the metabolism of neurotransmitters. In the liver, PAR bZip proteins control the circadian transcription of enzymes involved in metabolism and detoxification.

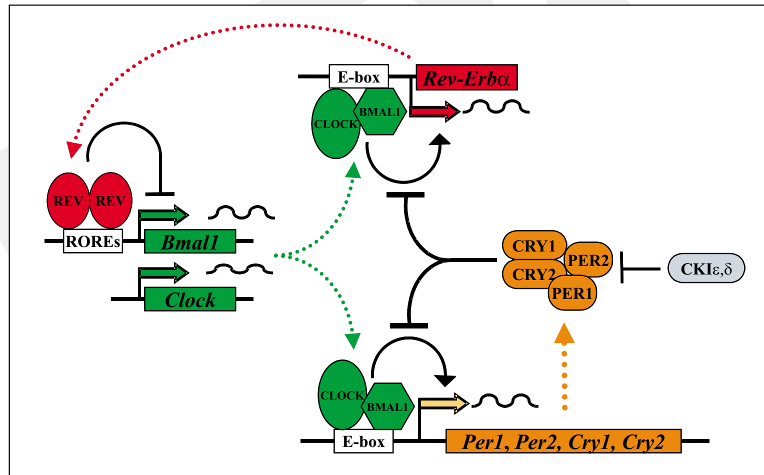

Fig 1: Model of molecular circadian oscillator.
The mammalian molecular oscillator is believed to rely on a negative feedback loop of gene expression, in which the four repressors CRY1, CRY2, PER1, and PER2 down-regulate transcription of their own genes. The four genes encoding these repressors (for simplicity shown as a single gene in the cartoon) are positively regulated by the two transcription factors BMAL1 and CLOCK, which bind as a heterodimer to E-box motifs. Once the PER-CRY repressor complexes have reached a critical concentration, they attenuate the transactivation potential of the BMAL1-CLOCK heterodimer and thereby repress their own genes. As a consequence, the concentrations of PER and CRY protein fall below the critical threshold value required for repression, and a new PER-CRY cycle can ensue. Similar positive and negative regulatory mechanisms also drive the cyclic expression of the orphan nuclear receptor REV-ERBα, which periodically represses Bmal1 (and to a lesser extent of Clock) transcription. Hence, REV-ERBα couples the circadian expression of the positive limb members BMAL1 and CLOCK to the circadian expression of the negative limb members CRYs and PERs. The protein kinases casein kinase I epsilon and I delta (CKIϵ,δ) phosphorylate PER proteins and thereby render them unstable.

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Diseases of the cell nucleus

fact that most laminopathies primarily affect tissues that are under increased mechanical stress such as muscle and adipose tissue. A second possible disease mechanism is based on the ability of lamins to interact with chromatin. Altered interactions might result in misregulation of particular sets of genes by placing them in an inappropriate nuclear environment. This scenario is in agreement with the recent realization that the position of genes within the cell nucleus might contribute to the their activity. In this case the tissue-specificity of the laminopathy symptoms might be related to the recent finding that genomes are spatially organized differently in cells from different tissues.

Apart from the relatively rare laminopathies, architectural aspects of the cell nucleus have also been realized to play a key role in cancer. One of the molecular causes of cancer is the formation of chromosome translocations. During this process, two chromosomes break and the free ends are incorrectly rejoined by the DNA repair machinery resulting in aberrant fusion of material from two chromosomes. Nuclear architecture contributes to this process since chromosomes are non-randomly arranged within the nuclear space. The non-random spatial positioning of chromosomes means that a particular chromosome will usually exist in a preferential neighborhood with a limited subset of other chromosomes. Since the re-joining event during the formation of a translocation requires the physical proximity of two chromosomes, broken chromosomes preferentially undergo translocations with their immediate neighbors. As a consequence, the position of a chromosome in the nucleus affects the choice of its translocation partners. How exactly the position of chromosomes is established and maintained and whether changes in positioning predispose cells to undergo particular translocations are areas of intense investigation and answers to these questions might provide insights into how cancer translocations form. Regardless, the non-random positioning patters of genes and chromosomes are now being explored to identify pre-malignant cells for early diagnostics.

The realization that defects in nuclear organization cause human disease has put this traditional field of cell biology squarely in the center of our efforts to understand and cure human disease. The wealth of information about basic principles of nuclear architecture accumulated since the discovery of the nucleus more than a hundred years ago will hopefully facilitate rapid progress in our understanding of the mechanisms involved in nuclear diseases. The insights we gain from these basic cell biological studies should lead to the identification of targets for drug development and, hopefully before long, to cures for children like Joey.

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Bio-TecReport INFODIENST

Gas–Generatoren von der Firma In House Gas

Die Firma In House Gas, Killearn, G63 9LE, Schottland produziert verschiedene Modelle von kompakten, zuverlässigen und effizienten Gasgeneratoren für den Laborbetrieb, die eine kontinuierliche Versorgung mit Stickstoff, Wasserstoff oder extrem sauberer Nullluft bei konstantem Druck gewährleisten.

Für Stickstoff gibt es eine Modellreihe N_2 Flow für einen extremen Reinheitsgrad von 99,9995 % und Bereitstellung von 0.4, 1, 2, 3, 5 und 10 l/min sowie eine Modellreihe N_2 Maxi Flow für 15, 20, 30, 40 und 60 l/min bei Reinheiten von 99 % und darüber. Es wird auch ein N_2Triple Flow-Modell angeboten, das Stickstoff mit drei unterschiedlichen Drücken liefern kann.

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Jan Ellenberg wins the 2004 ELSO Early Career Award

ELSO's Career Development Committee has chosen *Jan Ellenberg* of the European Molecular Biology Laboratory in Heidelberg, Germany to receive the fourth ELSO Early Career Award. The 36 year-old German has been an Interdisciplinary Group leader in the Gene Expression and Cell Biology Programmes at EMBL since 1999 where his team is studying the remodelling of the nucleus during cell division.

To study the breakdown and reformation of the nucleus as living cells divide, Ellenberg's group are using state-of-the-art microscopy methods including quantitative 4-D confocal microscopy, laser photobleaching/photoactivation and fluorescence resonance energy transfer. With these sophisticated techniques they can analyse the binding interactions and

PROOF

MEMBRANE TRAFFICKING

Regulatory mechanisms in clathrin-mediated endocytosis at synapses

V. HAUCKE*, BERLIN, GERMANY

Synaptic transmission requires calcium-stimulated exocytosis of presynaptic neurotransmitter-filled vesicles. Synaptic vesicle components are then recycled locally and refilled with neurotransmitter to sustain further rounds of release. Clathrin-mediated endocytosis represents a major mechanim by which synaptic vesicle recycling occurs. Recent studies have identified a plethora of endocytic proteins as well as membrane lipids that regulate clathrin-dependent vesicle cycling at synapses and have provided new insights into the regulatory mechanisms governing plastic changes of synaptic transmission.

Clathrin-mediated endocytosis is a process by which most eukaryotic cells internalize nutrients, growth factor and signaling receptors, as well as pre- and postsynaptic proteins within the nervous system. In many cell types endocytosis of plasmalemmal proteins such as the transferrin receptor occurs constitutively by incorporation of receptor proteins into nascent clathrin-coated pits. Coated pits then successively invaginate and finally bud off to yield clathrin-coated vesicles. Following uncoating these plasmalemmal-derived vesicles are directed to endosomal populations destined for recycling or lysosomal degradation. At chemical synapses both pre- and postsynaptic membrane proteins undergo clathrin-mediated internalization in a largely stimulus-dependent manner. Synaptic vesicles (SVs) undergo local recycling which at least on the long run requires the clathrin pathway. Perturbation of clathrin-mediated endocytosis by mutation, expression of dominant-negative mutants, local injection of endocytic protein domains into lamprey and squid nerve terminals, or modulation of phosphatidylinositol 4,5-bisphosphate (PIP_2; a membrane lipid required for clathrin-mediated endocytosis) levels results in SV depletion, morphological changes of the presynaptic compartment, and defects in neurotransmission. Likewise, a number of postsynaptic receptors including ionotropic glutamate receptors have been shown to undergo clathrin- and dynamin-dependent internalization followed by recycling or targeting for lysosomal degradation. The fate of these endocytosed receptors appears to be critically determined by so far ill-defined regulatory mechanisms elicited by complex signaling networks within the postsynaptic neuron.

We have set out to identify regulatory networks that affect the synthesis of phosphoinositides, in particular phosphatidylinositol 4,5-bisphosphate (PIP_2), at the synapse and to analyze their effects on the endocytic machinery. Using a combination of affinity chromatography, chemical crosslinking, and lipid kinase activity assays we have identified the small GTPase Arf6 as an activator of the synaptic phosphatidylinositol 4-phosphate 5-kinase Iγ resulting in the local production of PIP_2 at presynaptic sites and the recruitment of endocytic coat proteins such as clathrin and the adaptor proteins AP-2, AP180, and epsin to the plasmalemma. We currently favor a model according to which Arf6-mediated PIPKIγ activation is controlled by synaptic activity preceding calcium-triggered vesicle fusion. Recent evidence suggests that phosphoinositide metabolism in addition may be controlled by a transsynaptic signaling cascade downstream of activated NMDA-type glutamate receptors elicited by postsynaptically released nitric oxide (NO). NO then diffuses to presynaptic neurons where it leads to increased PIP_2 levels at the nerve terminal and enhanced rates of vesicle cycling.

Dr. Volker Haucke

PIP_2 not only binds to several components of the endocytic machinery but may also play a pro-active role in mediating their conformational activation. The heterotetrameric AP-2 adaptor complex composed of four chains ($\alpha, \beta 2, \mu 2, \sigma 2$) harbors two independent binding sites for PIP_2 within the α and $\mu 2$ subunits of its brick-like core domain. A phosphorylation-induced conformational change within the linker region of $\mu 2$ may allow the simultaneous engagement of both PIP_2-binding sites and arrest AP-2 in an active conformation that displays a high affinity for tyrosine-based endocytic sorting motifs of transmembrane cargo proteins **(fig.1)**.

A largely unresolved question pertains to the identity of the sorting signals that direct presynaptic membrane proteins to neurosecretory vesicles and allow them to be internalized via the clathrin pathway. Tyrosine- (Yxxø or NPxY) and dileucine based signals within the cytoplasmic domains of plasmalemmal receptors so far represent the best-characterized examples of internalization motifs. However, these motifs are rarely found in SV proteins and the most abundant vesicle membrane proteins lack these altogether. More recently we and others have identified basic signals within the cytoplasmic domains of the presynaptic vesicle protein synaptotagmin and postsynaptic ionotropic receptors which appear to be recognized by AP-2m and/ or the endocytic accessory protein stonin 2. Stonin 2 is a mammalian ortholog of *Drosophila* stoned B, a presynaptic protein involved in SV recycling and sorting of synaptotagmin. In transfected neurons stonin 2 localizes to presynaptic vesicle clusters **(fig 2)** and gets recruited to the membrane at least in part via interactions with synaptotagmin. It also binds to endocytic proteins such as Eps15 and AP-2 via repeated peptide motifs that define a novel class of accessory factors. Preliminary data from our laboratory suggest that stonin 2 targets synaptotagmin for clathrin / AP-2-dependent internalization and that this activity requires both its AP-2-binding WxxF motifs as well as a functional synaptotagmin-binding μ-homology domain. Therefore stonin 2 may represent one of the first examples of an endocytic adaptor protein specifically implicated in the regulated internalization of a presynaptic vesicle protein.

Outlook

The past decade has witnessed enormous progress in the identification of endocytic trafficking pathways, the molecular components involved in these pathways, and the protein-protein and protein-lipid interactions that govern vesicle assembly and dynamics. With this knowledge and new molecular tools including RNAi, mouse genetics, viral vectors etc. in hand we should soon be able to unravel the regulatory networks and plastic changes underlying signal transmission in the nervous system. Using advanced 4D-imaging technologies we are starting to gain insights into endocytic trafficking events in living cells, tissues, or even intact animals that will revolutionize our understanding of signaling processes in the brain and may help to design novel therapeutic approaches for the cure of neurological disorders.

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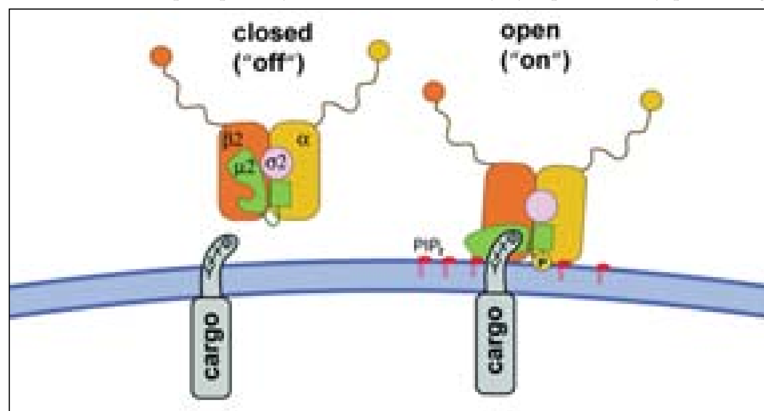

Fig 1: Hypothetical model for conformational activation of endocytic AP-2 adaptor complexes at PIP2-rich membrane sites (see text for details).

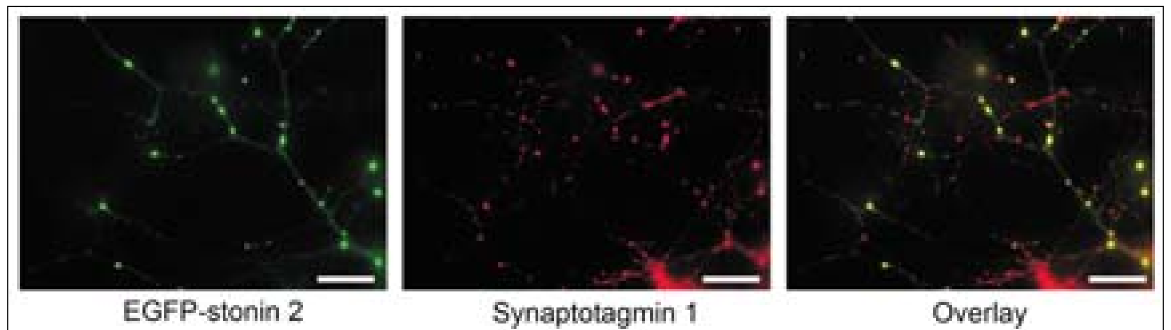

Fig 2: Co-localization of EGFP-stonin 2 and synaptotagmin I at presynaptic vesicle clusters. Immunofluorescent images of primary cortical neurons transfected with a plasmid encoding EGFP-stonin 2 and counter-stained for endogenous synaptotagmin I (red). Scale bar, 20 mm. Synaptotagmin I-immunopositive puncta (red) lacking EGFP-stonin 2 originate from non-transfected neurons.

Bio-TecReport INFODIENST

biosyn plant stärkeres Engagement im Ausland

Eine deutliche Ausweitung seines internationalen Engagements hat das Arzneimittel-Unternehmen biosyn angekündigt. Aus Anlass des zwanzigjährigen Firmenjubiläums teilte da Fellbacher Unternehmen mit, in diesem Jahr weitere Lieferverträge mit amerikanischen und europäischen Biotech-Unternehmen abzuschließen. Außerdem steht die Gründung eines Gemeinschaftsunternehmens mit einer australischen Firmengruppe in Kalifornien bevor.

Mit dem verstärkten internationalen Engagement reagiere biosyn auf die jüngste Gesundheitsreform, betonte Geschäftsführer *Dr. Thomas Stiefel*, die auch biosyn getroffen hat. Bei den Arzneimitteln, die biosyn über seinen eigenen Ärzte-Außendienst vertreibt, verzeichne das Unternehmen Umsatzrückgänge von über 10 %. Kompensiert werde dies jedoch durch den Erfolg im Ausland und durch die neu eingegangenen Vertriebskooperationen, die dem Unternehmen neue Zielgruppen erschlossen hätten. Dr. Stiefel: „Der pharmazeutische Mittelstand kann nur überleben, wenn er seine Stärken bündelt, aber auch seine Chancen im Ausland sucht.“

Ohne das Auslandsgeschäft, das bislang rund 20 % zum Gesamtumsatz von 13,3 Millionen Euro beitrug und in den nächsten Jahren kräftig wachsen soll, sei die Zukunft des 80-Mitarbeiter-Unternehmens kaum zu sichern. In den vergangenen zehn Jahren hatte das Unternehmen seine Umsätze von 7,8 Millionen Euro im Jahr 1993 durchschnittlich um 5 % pro Jahr steigern können. Für das laufende Geschäftsjahr ist ein Umsatz von 14 Millionen Euro geplant. Für das Jahr 2010 erwartet Stiefel eine Verdreifachung des Umsatzes.

Der Optimismus gründet auf neuen Erkenntnissen über den Zusammenhang von Krebserkrankungen, aber auch von Sepsis, mit der Konzentration des Spurenelements Selen im Körper. Das mittelständische Pharma-Unternehmen erwartet daher eine sprunghaft steigende Nachfrage nach Selenpräparaten. Bereits heute ist ein Selenmedikament – neben einem humanen Beta-Interferon – Hauptumsatzträger des Fellbacher Unternehmens. biosyn stellt in erster Linie Medikamente für die Krebstherapie sowie für die Intensivmedizin her. Rund 30 Präparate umfasst das Angebot.

Das geplante Gemeinschaftsunternehmen in Kalifornien, das mit der australischen Firmengruppe Tasmanian Seafoods Pty Ltd, Smithton, Tasmania, geplant ist, soll pharmazeutische Wirk- und Trägerstoffe aus einer Meeresschnecke gewinnen, der als Delikatesse bekannten Abalone. Der Beitrag von biosyn besteht im Wesentlichen im Know-how-Transfer, betonte biosyn-Geschäftsführer *Ortwin Kottwitz*.

Spezielles Wissen mit Wirkstoffen aus dem Meer hat biosyn bereits seit nahezu 20 Jahren durch Forschungen an einer anderen kalifornischen Meeresschnecke, *Megathura crenulata* **(Abb.)**, gesammelt, aus deren Lymphe biosyn ein Medikament gegen Blasenkrebs sowie ein Trägermolekül für therapeutische Impfstoffe gegen Krebs herstellt. biosyn hat Mitte der neunziger Jahre im kalifornischen Carlsbad die Tochtergesellschaft biosyn Corporation aufgebaut. Dort wird den Schnecken die Lymphe entnommen. Kottwitz hatte in den neunziger Jahren die technischen Voraussetzungen entwickelt, um das aus der Lymphe gewonnene Glykoprotein in der für den klinischen Einsatz erforderlichen Qualität herzustellen.

Die kalifornische Meeresschnecke *Megathura crenulata* liefert durch nichtletale Punktion den blauen Blutfarbstoff Keyhole limpet hemocyanin (KLH), aus dem der Wirkstoff Immunocyanin entwickelt wird. Das Protein hat sich als wirksam gegen Harnblasenkrebs erwiesen.

Dieses Trägermolekül für Impfstoffe entwickele sich zum Verkaufsschlager, sagte *Thomas Stiefel*, Geschäftsführer. Bereits seit einigen Jahren beliefert biosyn auch andere Biotech-Unternehmen mit dem Trägermolekül, unter anderem Genitop aus San Francisco. Die derzeit gelieferten Mengen werden für die Herstellung von Impfstoffen für klinische Prüfungen verwendet. Der Abschluss eines Liefervertrags mit einem weiteren Biotech-Unternehmen in San Diego steht unmittelbar bevor. Nach Zulassung eines Impfstoffes rechnet biosyn mit einem jährlichen Lieferumfang von 2,5 bis 4 Millionen Dollar pro Impfstoff.

In and out of Cajal bodies

KARLA NEUGEBAUER, DRESDEN, GERMANY

Nuclear bodies are non-membrane bound inclusions that contain specific sets of factors involved in diverse regulatory events in gene expression, yet the molecular activities occurring within nuclear bodies are largely unknown. Because the factors concentrated within nuclear bodies are also distributed throughout the surrounding nucleoplasm and because many factors exist within the nucleus in various states of assembly and/or activity, the mere fact of their localization does not establish their function at any given site.

Indeed, recent research on the mobility of nuclear proteins in living cells indicates that factors diffuse rapidly among nuclear compartments. This situation suggests that nuclear structures are highly dynamic, arising and maintaining their form through a constant flux of molecules that differ in activity in and out of nuclear bodies. In order to understand the location of molecular function, assays that can pinpoint different functional states and/or activities of each factor are needed. Only these approaches can begin to explain the function(s) of the bodies themselves.

Most cell nuclei contain 1–2 Cajal bodies (CBs), electron dense spheres 0.5–1.0 microns in diameter that are enriched in factors required for RNA processing and transcription.

How and why are molecules targeted to CBs?

We have addressed these questions, by examining the possibility that CBs play a role in spliceosomal small nuclear ribonucleoprotein particle (snRNP) assembly. Each snRNP is required for pre-mRNA splicing and contains a small nuclear RNA tightly bound by about 10 specific snRNP proteins. Several snRNPs undergo assembly steps within the nucleus; for example, the U4 and U6 snRNPs assemble together to form the U4/U6 snRNP, with which the U5 snRNP subsequently assembles to form the U5• U4/U6 snRNP that is active in splicing. Moreover, the splicing reaction itself disassembles the U5• U4/U6 snRNP into its constituent snRNPs, such that the U5• U4/U6 snRNP must be regenerated in order to participate in subsequent rounds of splicing. At steady state, spliceosomal snRNPs are distributed throughout the nucleoplasm, where splicing occurs, and are also concentrated in CBs. We have used fluorescence resonance energy transfer (FRET) microscopy to investigate the subnuclear distribution of specific snRNP intermediates in the assembly pathway. Two distinct complexes containing the protein SART3 (p110), which is required for U4/U6 snRNP assembly, were localized by FRET microscopy: SART3•U6 snRNP and SART3•U4/U6 snRNP. These complexes segregated to different nuclear compartments, with SART3•U6 snRNPs exclusively in the nucleoplasm and SART3•U4/U6 snRNPs preferentially in CBs. Experiments with dominant negative mutants of SART3 indicate that SART3 is required for U6 (but not U4) snRNP accumulation in CBs, suggesting that nucleoplasmic SART3•U6 snRNP translocates to CBs where U4 is concentrated by a distinct mechanism. Taken together, the results suggest that U4/U6 snRNP assembly occurs in CBs.

Dr. Karla Neugebauer

Based on these results, we speculate that the concentration in CBs of snRNP intermediates, which are inactive for splicing, benefits the cell by removing inactive snRNPs from the splicing environment and increasing the efficiency and/or fidelity of assembly within the CB.

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BEILAGENHINWEIS

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2-D-Chip for High-Throughput Proteom Analysis

Marion Ritzi, Klaus Stefan Drese

Conventional 2-D-electrophoresis usually uses isoelectric focusing [1] in the first dimension combined with SDS-gel electrophoresis [2] in the second dimension. This method is often followed by mass spectrometry analysis in a third dimension [3]. Due to its ability to separate complex protein mixtures 2-D-electrophoresis is still the commonly used tool in proteome analysis. Main reasons for this method not being a routine method for fast characterization of protein mixtures are high costs, long analysis time, extensive handling steps and low interlaboratory reproducibility [4]. Miniaturization of bioanalytical devices often leads to cheap, convenient and time-sparing tools usable for high-throughput sample screening and thereby opens up a route for improvement of 2-D performance [5]. Supported by the BMBF grant „Analysis of the human proteome" (FKZ 01GG9836) we developed an easy-to-handle microstructured 2-D-electrophoresis chip which offers these features [6]. Manufacturing via injection compression molding allows mass production of the credit card-sized PMMA-chip as a disposable tool. In addition to low costs mass production ensures constant quality, a condition precedent for reproducibility.

The 2-D-chip combines the advantages of immobilized pH gradients (IPG), such as high resolution and long supportability for the separation in the first dimension (IEF), with the speed of highly parallel SDS capillary gel electrophoresis (SDS-CGE) in the second dimension. A cavity for an especially developed IPG strip and two buffer reservoirs for SDS-CGE are physically connected via 300 parallel channels **(fig. 1)**. The channels are of 50 µm x 50 µm cross section. Buffer reservoirs and the IPG-cavity are arranged perpendicular to the 300 parallel channels. IEF cavity and channels are connected via a small gap for the protein-transfer from first to second dimension. A special coating of the channel wall surfaces eliminates both, electro-osmotic flow in the presence of SDS and protein-polymer interactions (Serva Electrophoresis GmbH, Heidelberg, Germany [7, 8]). The handling system contains three programmable power supplies controlling two electrodes for IEF and three electrodes for transfer and CGE. The electrode arrangement was realized according to optimal results of simulations. For real-time detection proteins are labeled with Alexa 488. An Argon laser serves as fluorescence excitation light source and fluorescence signals are detected by a CCD camera with special filter.

Due to the possibility of preparing different pH ranges between pH 3 and 10, according to the sample investigated, IPG strips are a flexible tool in 2-D electrophoresis for high resolution separation. We developed special IPG strips with the gradient immobilized on a PES membrane permeable for protein-transfer. IPG strips have to be rehydrated with the protein solution (8M urea, 2 % CHAPS, 10 mM DTT) at least 2 hours before use in a disposable cassette where no mineral oil overlay is needed. The actual IEF experiment takes only about 1 hour (50 µA, 200–2000 V increasing in stages; 8 °C) compared to 2–8 hours needed to focus proteins in a conventional oil-immersion system. Besides the simplified handling, the reproducibility of the IEF results is increased as well.

During IEF first and second dimension are separated by a membrane. Transfer and SDS-CGE are performed at 8 °C at a maximum voltage of 1000 V and open maximum current. Via a gap of 50 µm proteins are transferred from the IEF cavity into the 300 microchannels where the CGE is carried out in 15 % (w/v) pullulan, 100 mM Tris, 100 mM CHES and 0,1 % SDS (pH 8,8) [9]. In comparison to slab gels CGE can be performed at high electric field, due to the efficient removal of Joule heat [10]. This allows rapid sample separation and therefore increases transfer rate.

Transfer is highly efficient for that about 50–75 % of the protein concentration in the first dimension can be transferred to the second dimension. Protein peaks separated in the second dimension contain about 0.5–1 fmol of protein or less. Miniaturized first and second dimensions were first established separately and now brought together for complete 2-D-analysis on chip.

Reduction of analysis time to only about two hours leads to a tool for high throughput 2-D-analysis whereby automation of most handling steps provide increased reproducibility. Furthermore, the chip system bears the potential of subtractive analysis of patient samples for diagnostic purposes. To obtain data of the separated proteins we look ahead to combine our 2-D-chip with mass spectrometry analysis in a third dimension. This extension would lead to a tool suitable for all kinds of protein analysis regarding cancer, hereditary diseases and other diseases caused by changes in protein expression.

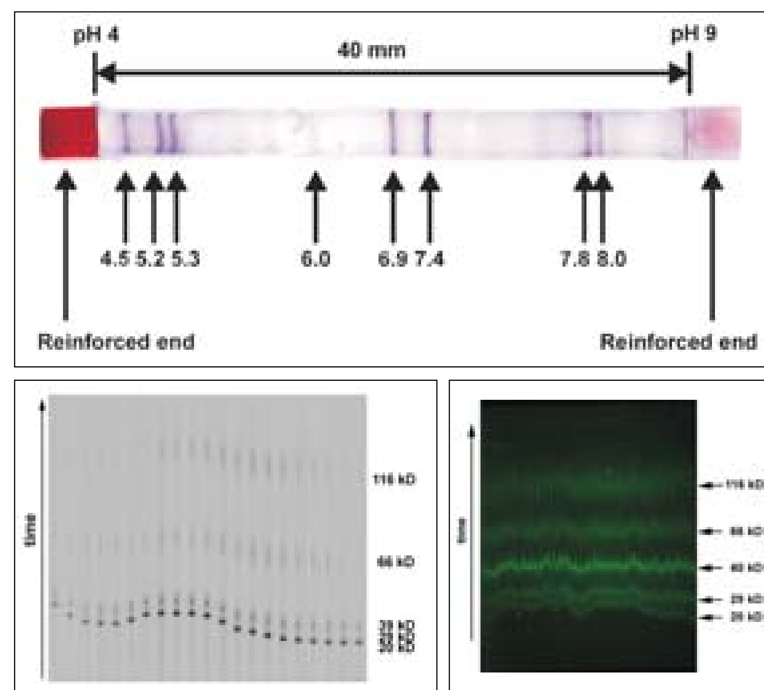

Fig 2: Separation of a high molecular weight marker protein mix. A: Result of IEF. B: Result of CGE; picture taken by the handling system (left) and a fluorescence-Stemi SV 11 (Zeiss, enlargement 6.25)(right).

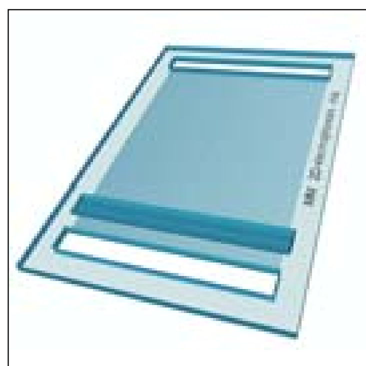

Fig 1: Chip-Design. A: Schematic representation, B: Photographical representation. L/B/H: 88 mm x 56.5 mm x 2mm; 300 parallel microchannels: 64 mm x 50 µm x 50 µm; separation length 60 mm. Buffer reservoirs: 46.5 mm x 6 mm; IEF-cavity: 47 mm x 6 mm x 1.5 mm; Transfer gap: 47 mm x 100 µm x 200 µm; (reduction of width in 200–250 µm depth from 100 µm to 50 µm).

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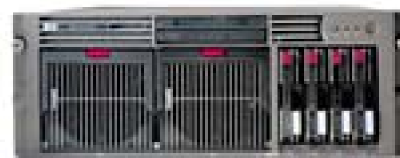

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