Aussois (France), 17-21 juin 2009

Régulation et fonction des petites protéines G

Regulation and function of small GTPases

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RESUME DU RAPPORT

Conférence Jacques Monod intitulée : Régulation et fonction des petites protéines G

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Les petites protéines G de la super-famille Ras (SMGs) sont des régulateurs essentiels de multiples processus cellulaires comme la transduction du signal, la dynamique du cytosquelette et le trafic membranaire. Ils sont de ce fait impliqués dans de nombreux processus physiologiques comme la prolifération, l'adhésion, la migration et la différenciation de nombreux types cellulaires. Connaissant leur rôle central dans ces processus développementaux, il n'est pas surprenant que ces mêmes protéines soient également impliquées dans un nombre extrêmement varié de pathologies telles que la prolifération cellulaire incontrôlée, les métastases et l'angiogénèse au cours de la tumorigénèse, l'inflammation et les problèmes vasculaires, le retard mental ou les infections. Pour toutes ces raisons, la thématique des SMGs a toujours été un domaine de recherche très compétitif.

Les défis à l'heure actuelle dans le domaine des SMGs sont de comprendre comment les SMGs, leurs régulateurs et leurs effecteurs interagissent, comment ils s'organisent dans les complexes moléculaires, fonctionnent in vivo, et comment la spécificité des interactions est contrôlée. Dans les 20 dernières années, les efforts ont porté essentiellement sur l'identification des protéines de la famille et de leurs régulateurs, mais la fonction physiologique de ces protéines reste obscure pour la plupart. De nombreuses questions restent sans réponse, telles que la spécificité des SMGs vis-à-vis de leurs cibles in vivo, leur régulation spatio-temporelle, leur participation à des réseaux d'interaction, leur contribution exacte dans le développement de processus pathologiques, et finalement le design d'inhibiteurs spécifiques à des fins thérapeutiques.

La conférence "Fonction et Régulation des petites GTPases" a été une excellente occasion de réunir de nombreux spécialistes du domaine des SMGs et de faire le point sur les nouvelles approches pour répondre à ces questions essentielles de physiologie cellulaire. La conférence a réuni une centaine de participants de 14 pays différents, montrant l'attrait des thématiques développées dans la communauté scientifique internationale. Les scientifiques provenaient d'horizons culturels et scientifiques très différents, ce qui a permis de couvrir une grande diversité de systèmes biologiques et d'approches technologiques, ce qui a été très apprécié par les participants. Ces différentes contributions ont été réparties sur neuf sessions thématiques :

- Etudes des GTPases in vivo
- Rho GTPases dans les pathologies humaines
- Vers la biologie systémique
- GTPases et membranes
- GTPases et trafic
- GTPases et infections
- Boîte à outils et ses applications
- Nouveaux aspects dans la régulation des Rho GTPases
- Signalisation des GTPases Ras

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled: Regulation and function of small GTPases

Aussois, June 17-21, 2009

Summary

The CNRS-Jacques Monod conference "Regulation and Function of Small GTPases" was held in Aussois (French Alps), June 17-21, 2009. The function of small GTPases (SMGs) is mediated by their ability to cycle between an inactive GDP-bound state and an active GTP-bound state, which allows them to act as cellular switches. Each SMG subfamily is regulated by three types of proteins: guanine nucleotide exchange factors (GEFs), which carry a conserved catalytic domain that stimulates their intrinsically slow GDP/GTP exchange, GTPase activating proteins (GAPs) that activate the intrinsic GTPase activity, and guanidine dissociation factors (GDIs) that solubilize the GTPase in the cytosol. Each of these three types of SMG regulators usually belongs to extended families, and often they outnumber their SMG targets. The regulators of SMG proteins are in general complex proteins with various other domains in addition to the catalytic motifs, suggesting that they participate to other signaling pathways. Intriguingly, these specific features suggest that the regulation of SMG function is highly complex and regulated by many different pathways.

Currently, the challenge is to understand how SMGs and their regulators and effectors interact, assemble signalling pathways and eventually function in vivo. Specificity of interactions and signalling remains one of the prominent issues. In the last 20 years most of the research effort has been on identifying proteins, yet the function of each member of these families it is still not clear. Questions such as specificity towards their target *in vivo*, spatio-temporal regulation within the cell, contribution of these proteins in interaction networks remain to be solved. The meeting on SMGs was an occasion to discuss these issues and find new technical approaches to unravel these questions. The main objective of this meeting was aimed at covering the most recent developments and exciting results in the small GTPase field.

A particularly valuable aspect of the meeting was that it brought together scientists from widely different cultures and scientific backgrounds, and that specialists in both eukaryotic and prokaryotic biological systems were communicating vividly. In planning this conference, we favoured diversity and interdisciplinary approaches, and such a broad coverage was certainly appreciated and considered to be an 'added value' to this conference. Along these lines, we took care to balance contributions and dispatched them into 9 topic headings divided into a total of 11 sessions:

- In vivo studies
- Rho GTPases in human disorders
- Systems Biology
- GTPases and membranes
- GTPases and cellular traffic

- GTPases and infection
- Toolbox and its applications
- Novel aspects of Rho GTPase regulation
- Signalling by the Ras family

A total of 29 speakers were invited to give 30 min talks. Of these, six came from North American laboratories (USA, Canada) ten came from French laboratories and 13 from other European laboratories. **Alfred Wittinghofer** (Max-Planck, Dortmund, Germay) was the keynote speaker and as such was invited to give a one hour presentation. In addition, 19 applicants were selected from submitted abstracts to give 15 minute talks. Finally, there were two (two hour) poster sessions where a total of 42 posters rounded-out the scientific program.

Scientific report

Small GTP-binding proteins (also known as small GTPases) are one of the largest and most ancient families of signalling proteins with well over 100 mammalian family members. These signalling proteins are essential for multiple cellular processes such as cell proliferation (Ras), dynamics of the cytoskeleton (Rho and Rop), membrane trafficking (Arf and Rab), and nucleo-cytoplasmic transport (Ran). Because of this, they are involved in numerous physiological processes including embryogenesis, establishment and/or maintenance of polarity, adhesion, migration, and differentiation of numerous cell types. Given their pivotal role in these normal and developmentally regulated processes, it is not surprising that small GTP-binding proteins are also involved in an amazing variety of pathological human conditions such as uncontrolled cell proliferation, metastasis or angiogenesis during tumor development, inflammation and vascular diseases, mental retardation, and infections.

The Conference "Regulation and Function of Small GTPases" was aimed at covering the most recent exciting developments in this field. The work presented and discussed by the selected speakers addressed biological roles and mechanisms of Small GTPase signalling. The talks were organized in 11 sessions covering nine themes.

Session I – In vivo studies of GTPases

During the last 20 years most small GTPase research focused on identifying novel small GTPases, yet the function of each member of the various small GTPase families remained unclear. Questions such as specificity towards their targets *in vivo* and spatiotemporal regulation remain to be solved. The challenge of today's researcher is to understand the function and regulation of this multifunctional family under physiological conditions. *In vivo* models are absolutely required to finally understand the physiological function of the Rho GTPases and their regulators. For this session we invited speakers who made pioneering contributions in the last few years in the area of *in vivo* small GTPase signalling using either mouse or Drosophila model systems. Rho-family GTPases regulate a large number of processes including cytoskeletal changes, cell motility and cell proliferation. Most Rho-family GTPases cycle between GDP-bound inactive and GTP-bound active states, and altering this cycling process is known to contribute to various pathological states. **Xose Bustello** (CSIC-University of Salamanca, Spain) showed that the family of Vav proteins regulate crucial new pathways necessary for homeostasis of the cardiovascular system. He went on to discuss the role of the Vav oncoprotein in cancer-related biological processes. **Michael J Williams** (University of Aberdeen, UK) discussed his labs pioneering work using the Drosophila cellular immune response as a model system to further our understanding of the role Rho-family GTPases play in regulating migration and adhesion during a cellular immune reaction.

He also presented preliminary studies studying the role of the Drosophila Rho GTPases in ageing. **Anne Debant** (CRBM-CNRS, University of Montpellier, France) presented data on the role phosphorylation has in regulating the function of the complex RhoGEF Trio during netrin-1/DCC-mediated axon outgrowth and guidance. She went on to discuss the role of alternative splicing in Trio function and activation of Rho GTPases. Two short talks were presented during this session, **Deike Hesse** (German Institute of Human Nutrition, Potsdam, Germany) presented work on ADP-ribosylation factor related protein 1 (ARFRP1) which is involved in the regulation of intracellular protein trafficking, specifically in Golgi function. They have demonstrated that in adipocytes the lipodystric phenotype of Arfrp1 deficient mice is due to defective lipid storage and that ARFRP1 is required for proper lipid drop formation. **Arno Muller** (University of Dundee, UK) gave the final talk of the session. They have found that the function of the RhoGEF proto-oncogene ECT2 (known as Pebble in Drosophila) in mesoderm movements is independent of its function in cytokinesis and does not require Rho1. Genetic and biochemical experiments indicated that Pbl acts through the Rac pathway in this process.

Session II– Rho GTPases in Human disorders

Given their pivotal role in these normal and developmentally regulated processes, it is not surprising that small GTP-binding proteins are also involved in an amazing variety of pathological human conditions such as uncontrolled cell proliferation, metastasis or angiogenesis during tumor development, inflammation and vascular diseases, mental retardation, and infections. For this session we invited speakers who are considered to be world leaders in the field of Rho-family GTPase signalling. **John G. Collard** (Netherlands Cancer Institute, Amsterdam, Netherlands) presented data on the Rac-activator Tiam-1 during T-cell trafficking and trans-endothelial cell migration. Intriguingly, they discovered that Tiam1 is required for both chemokine and sphingosine-1-phosphate induced Rac1 activation during cell migration. T-cells migrate through the endothelium either through the endothelial cells (transcellular migration) or more often via the cellular junctions between cells (paracellular migration). In Tiam1-deficient cells T-cells undergo a much higher rate of transcellular migration. Since Tiam1 regulates T-cell polarisation, this leads to the possibility that T-cells change their route of trans-endothelial migration depending on their polarisation status. **Gervaise Loirand** (Inserm, Nantes, France) presented compelling evidence that Angiotensin II play a central role in the overactivation the RhoA/Rock pathway during hypertension. Loirand's lab has shown that this RhoA activation is mediated by a specific RhoGEF, whose activation is regulated by phosphorylation. **Anne Ridley** (King' College London, UK) presented her work showing that Rac1/Rac2 are not necessary for macrophage migration, and also presented evidence that Pak1 is involved in phospho-Erk1/2 localisation leading to lamellipodia stabilisation in T-cells. **Linda van Aelst** (Cold Spring Harbor Laboratory, New York, USA) talked about her labs continuing work to understand the role of the RhoGEF Dock7 and the RhoGAP OPHN1 in determining neuronal axon formation. **Cécile Gauthier-Rouvière** (CRBM/CNRS, Montpellier, France) talked about Rhabdomyosarcomas (RMS), which are the most common soft tissue tumours in children. R-cadherin has recently emerged as a marker for RMS. Dr. Gauthier-Rouvière presented compelling evidence that R-cadherin adhesion induces Rac1-activity, and this possibly inhibits myogenesis and induces myoblast transformation. This R-cadherin induced Rac1 activity may lead to in vivo tumour formation and increased cell motility. **Violaine Moreau**'s talk (Inserm, Bordeaux, France) was about Hepatocellular carcinoma (HCC), the main primary malignancy of the liver and one of the most common and aggressive cancers worldwide. Dr. Moreau gave a short presentation on the role of RhoE/Rnd3 in HCC development, presenting data demonstrating that knockdown of RhoE/Rnd3 affects

proliferation, 2D migration and invasion of HCC cells. **Jaap D. van Bull** (Sanguin Research & Landsteiner Laboratory, Amsterdam, the Netherlands) presented work showing that ICAM-clustering on leukocytes induces stress fiber formation via RhoA, and that downstream of ICAM-clustering signals to RhoG via RhoA.

Session III - Towards Systems Biology

In this session we explored for the first time how the new era of systems biology is starting to impact the GTPase field. It is essential to discuss what systems biology can tell us about the function of the small GTPases. The exciting challenge is to use new techniques to be able to find the cellular protein interaction networks where the small GTPase family is going to be involved. **Chris J. Marshall** (CRUK, London) In order to understand which Rho-family GTPases are involved in cell migration and how they are controlled the Marshall lab is carrying out a systematic analysis of Rho-family GTPases and their regulators. They have used RNAis targeting all the mammalian Rho-family GTPases, guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) in order to study their requirement for cell motility and morphology in a number of different systems. Using this technique, they have shown that mesenchymal-type movement is driven by Rac activation mediated by a pathway containing the adaptor protein NEDD9 and the exchange factor DOCK3, while amoeboid movement is suppressed by Rac activation and driven by Rho and Cdc42 activation. This is a powerful technique that should help us in our understanding of the tight interplay between Rho and Rac signalling that determines cell movement. **Bernard Hoflack** (Technical University of Dresden, Germany) has combined liposome-based *in vitro* reconstitution systems that recapitulate the fidelity of protein sorting as seen *in vivo* and proteomic screens to identify protein networks supporting AP-1 and AP-3 coat function in targeting transmembrane proteins to the endosomal/lysosomal system. Their proteomic screen revealed that the same membrane domains account not only for a selective AP coat assembly but also for the concomitant recruitment of selected machineries required for actin-based or septin-regulated movement and subsequent membrane fusion (Rab GTPases). **Christina Kiel** (CRG, Barcelona, Spain) presented an interesting talk about their *in silico* work. They performed a 3D reconstruction of the human EGF signal transduction pathway. From a core set of 180 proteins, they have predicted domains and experimentally recorded phosphorylation sites. Next, they have identified domain-domain and phospho(P)peptide-domain interactions, based on experimental databases. Their structural reconstruction enables to distinguish between direct and indirect interactions and more importantly to decide if a target protein or complex can interact simultaneously with two or more proteins or complexes, adding a spatial dimension. Together with determining absolute protein concentrations, and localisation experiments they can derive conclusions regarding competition between proteins and within pathways. **Oliver Rocks** (Samuel Lunenfeld Research Institute, Toronto, Canada) on the Pawson lab's aim to integrate mass spectrometry with live cell imaging and functional assays to study the RhoGAP/GEF interaction networks that orchestrate localized Rho protein signaling and to understand the cellular functions associated with individual regulatory proteins. **Andrew B, Goryachev** (University of Edinburgh, UK) gave the other short-talk in this session discussing his work showing that the nucleotide cycling of Cdc42 converts cellular energy into a stable cluster of activated Cdc42 by driving a continuous membrane-cytoplasmic flux of cluster components that counteracts the diffusive spread of the cluster on the membrane. Furthermore, they have identified a molecular motif, a complex between the Cdc42 effector and its GEF, as a fundamental prerequisite for the cluster formation.

Session IV - GTPases and membranes

Most GTPase functions are intimately communicating with their membrane environment. In many cases membranes act as regulatory components by up- or down-regulating GEFs, GAPs or GDIs. GTPases also contribute to modifying the composition of membranes, and are thus critical for defining the identity of a subcellular organelle. The recognition that GTPases themselves, by means of their effectors, are actively detecting and/or modifying biophysical properties of membranes is much more recent. This is a fascinating field that emerges at the interface of biology and physics. **Bruno Antonny** (CNRS, Sophia Antipolis, France) gave an exquisite and very fun talk presenting data showing that the lipid membrane is not a passive surface where Arf-dependent reactions take place. Many GAPs, GEFs and Arf effectors contain regions that sense the changing physicochemical properties of the underneath membrane. Interestingly, the PH domain of the ArfGEF Arno senses not only the density of negatively charged lipids but also the density of Arf-GTP. **Cecile Sykes** (Institut Curie/CNRS/Université Paris 6, France) gave a talk on her lab's work using red blood cells and an optical tweezers inspired system to show that membranes are softened or stiffened depending on the structure of the underlying cytoskeleton. **Antonella Demattis** (Santa Maria Imbaro, Italy) presented her lab's work on the molecular mechanisms that underlie the incorporation of pre-Golgi membranes into the Golgi complex, and the role of Arf GTPases and lipids in the maintenance of this organelle.

Katarina Trajkovic (University of Geneva, Switzerland) presented a short-report on their lab's aim which is to test the role of Rab proteins in intra-endosomal dynamics and in the coordination with overall endosomal trafficking by using in vivo and in vitro approaches. She presented preliminary observations indicating that Rab5 and Rab7 play a role in the mechanisms of intraluminal vesicle formation. **Frederique Gaits-Iacovoni** (INSERM, Toulouse, France) presented work on how the phosphoinosotide phosphatidylinositol 5-phosphate (PtdIns5P) regulates Rac1 and Cdc42 activation, possibly via regulating the RhoGEFs Vav1, Vav3, Trio and Tiam1.

Session V – GTPases and traffic

Bruno Goud (Institut Curie, CNRS, Paris, France) lab has shown that Rab6A, a Golgi-associated Rab, controls the fission of its own transport carriers from Golgi membranes, and that myosin IIA is an effector of Rab6A in this process. They have also observed a transient F-actin accumulation at the Rab6 vesicle fission sites in living cells. Their results provide evidence that the acto-myosin system is required in vesicle biogenesis at the Golgi and uncover a new function for Rab GTPases in vesicle fission. **Cathy Jackson** (CNRS, Gif-sur-Yvette, France) presented evidence that the GBF1-Arf1-COPI cascade is involved in a novel trafficking pathway that delivers the triglyceride lipase ATGL to lipid droplets from the ER, and that branches from the secretory pathway at the level of ERGIC. She proposed a model whereby competition between different interacting partners of GBF1 directs membrane maturation towards different pathways that diverge from the ERGIC compartment. **Crislyn D'Souza-Schorey** (University of Notre Dame, USA), **Julie G. Donaldson** (NIH, Bethesda, USA) and **Julie Ménétrey** (Institut Curie, Paris, France) all presented work on Arf6. Dr. D'Souza-Schorey used cell lines and animal model systems to examine the mechanisms of ARF6-regulated membrane remodeling and protease release during metastasis. Dr. Donaldson presented data from a recent proteomic analysis of the CIE endosomal system which reveals that multiple Rab GTPases function with Arf6 in facilitating membrane traffic. Finally, Dr. Ménétrey presented the crystal structure of ARF6 in complex with its effector JIP4, which regulates endosome traffic in cytokinesis. She compared the ARF6–JIP4 interface with the equivalent region of ARF1, which binds JIP4 with 21-fold lower affinity, which revealed the

structural basis of JIP4's specificity for ARF6. **Roger Goody** (Max-Planck Institute, Dortmund, Germany) presented work on the process of Rab prenylation, delivery of Rab proteins to membranes and the reverse process of extraction, as well as exchange of GDP for GTP catalyzed by guanine nucleotide exchange factors (GEFs). **Dan Cassel** (Technion, Haifa, Israel) is trying to understand why Coat dissociation is regulated by three ArfGAPs, the previously characterized ArfGAP1 and the closely related ArfGAP2/3, while **Amir Kahn** (Trinity College, Dublin, Ireland) discussed structural studies of Rab-effector complexes to understand contradictory properties of Rab GTPase specificity and promiscuity.

Session VI – GTPases and infection

Pathogens use tricky methods to survive by acquiring the ability to intercept, disrupt or mimic the cell machinery of their host. Numerous studies have demonstrated that pathogens manipulate SMG pathways to accomplish different processes during their infectious cycles. Studies of host/pathogen interactions are not only essential to understanding the pathogenicity of infectious diseases, but also provide interesting insights into basic cellular function. **Michael Way** (CRUK, London, UK) discussed his lab's recent findings concerning the role of vaccinia viral protein F11 ability to inhibit RhoA signalling in the cell-to-cell spread of vaccinia *in vitro* as well as *in vivo* in mouse models of infection. **Craig R. Roy** (Yale University, USA) discussed the mechanisms by which *Legionella pneumophila* delivers bacterial proteins into the host cell that directly modulate Arf and Rab GTPase signaling. These proteins include an ARF guanine nucleotide exchange factor (GEF) called RalF, a Rab1 GEF called DrrA, and a Rab1 GTPase activating protein called LepB that control cellular GTPases to subvert the transport of membrane vesicles between the endoplasmic reticulum (ER) and the Golgi apparatus thereby creating a specialized vacuole that supports bacterial replication. **Madhavi Maddugoda** (INSERM, Nice, France) gave a short talk on the exotoxin EDIN, isolated from a strain of S. aureus and a screen they performed for important regulators implicated in the dynamics of macroaperture opening and closure. They have found a clear involvement of lipid metabolism and of the GTPase ARF6, a master regulator of actin dynamics. **Laurent Boyer** (Harvard Medical School, USA) discussed his work on how Drosophila Rac2 GTPase is a key regulator of the innate immune signaling pathways induced during *bacterial* infections in vivo.

Session VII – Toolbox and its applications

Many different GTPases and their regulators have been identified since the human genome was sequenced, but the function, the specificity, and the putative involvement in pathology of this huge family remains unclear. Tools allowing for the manipulation of small GTPases and their regulators are desperately needed to follow activation *in vivo* of the small GTPase family and to better understand their exact role in cellular physiology. **Anne Blangy** (CNRS-CRBM, Montpellier, France) has developed a yeast-based assay that will allow for medium to high throughput screens to identify RhoGEF inhibitors. Using this method, they identified a series of compounds that proved to be efficient inhibitors of the Trio N-terminal GEF domain. They propose that this method can be used for the identification of inhibitors of a wide range of exchange factors in particular those with potential therapeutic interest. **Olivier Pertz** (University of Basel, Switzerland) reported on fluorescent biosensors that detect Rho GTPase activation with high spatial and temporal resolution in single living cells, which have provided new insights in Rho GTPase biology. Furthermore, work was presented on a universal strategy to construct genetically-encodable FRET sensors for a large variety of signaling molecules. **Katharina Uhlenbrock** (Max-Plank, Dortmund, Germany) and **Fried**

ZwartkRuis (UMC Utrecht, the Netherlands) both presented short talks on their work with the Ras-like GTPase Rheb.

Session VIII - Novel aspects of RhoGTPase regulation

Nathalie Lamarche-Vane (McGill University, Quebec, Canada) talked about her lab's work with CdGAP (Cdc42 GTPase-activating protein) a RhoGAP protein showing GAP activity against both Cdc42 and Rac1 but not RhoA. Dr. Lamarche-Vane suggested that CdGAP is required for TGF-β-induced cell motility and invasion in mammary breast cancer cells and may function as an oncogene in breast cancer. **Rafael García-Mata** (University of North Carolina, Chapel Hill, USA) gave an interesting presentation on the nuclear-localized RhoGEF Net1. Nuclear localised Net1 is in an active form and can induce nuclear RhoA activation upon overexpression, demonstrating for the first time a role of RhoA and its exchange factor Net1 within the nucleus of the cell and suggesting they may function in the DNA damage response. Two short-talks were presented in the session by **Harry Mellor** (University of Bristol, UK) and **Etienne Boulter** (University of North Carolina, Chapel Hill, USA). Dr. Mellor talked about his work on RhoBTBs, which are large multi-domain proteins containing two copies of the BTB/POZ protein-protein interaction domain downstream from a small GTPase domain of the Rho family. RhoBTB1 and RhoBTB2 are transcriptional regulators and recruit factors through their BTB domains to control the expression of an overlapping, but distinct set of genes. Dr. Boulter' talk highlighted a new function of RhoGDI (Rho GDP-dissociation inhibitor) acting as a "GTPase Degradation Inhibitor", raising the concern about the conclusions that have been drawn from Rho protein overexpression, microinjection or silencing studies during the past two decades, since alteration of the level of one Rho GTPase will affect others via the activity of RhoGDI.

Session IX -Signalling by the Ras family

In 1982 it was discovered that mutated forms of the small GTPase Ras were present in human cancers. This discovery led to intense research in an attempt to dissect Ras function and towards the development of Ras inhibitors for cancer treatment. Given their pivotal role in normal and developmentally regulated processes, it is not surprising that Ras-like small GTP-binding proteins are involved in an amazing variety of pathological human conditions including oncogenesis, inflammation, and infection. The final seminar session of the conference focussed on important advances made in the Ras GTPase field. **Dafna Bar-Sagi** (New York University, USA) talked about Ras proteins in health and diseases. Ras proteins are highly conserved membrane-bound nucleotide binding proteins essential for the transduction of diverse extracellular signals that control cell growth. It is now well established that Ras proteins control cell growth through the activation of multiple effector pathways which form a signaling network enabling cells to interpret biological inputs in a context-dependent manner. Dr. Bar-Sagi described her lab's efforts to define a molecular framework for the regulation of Ras-dependent signal output and to understand how this output is modified in cancer cells. **Joannes L. Bos** (University of Utrecht, the Netherlands) gave an interesting presentation on his lab's work to understand the Ras GTPase family member Rap1. Rap1 is known to be regulated by multiple RapGEFS, including EPAC, C3G, and Dock4, and its function is necessary to control of cell-cell and cell-matrix adhesion. **Neus Agell** (University of Barcelona, Spain) reported that calmodulin inhibits the phosphorylation of another Ras-family member K-Ras by PKC, and that this phosphorylation is essential for the activation of K-Ras. They also analyzed the effects of this phosphorylation on K-Ras signaling, showing that the non-phosphorylable K-Ras mutant has a less sustained activation profile after growth factor addition. **Michelle de la Vega** (Queen's University, Belfast,

Ireland) gave a short-talk about her work on the DUB-3/USP17 family of deubiquitinating enzymes demonstrating that USP17 overexpression results in a block in cell growth in cells transfected with the H-Ras, N-Ras, and K-Ras4b isoforms. **Kris A Reedquist** (University of Amsterdam, the Netherlands) presented his lab's work on the regulation of T-cell activity by RapGAPs and suggested that T cell activation and differentiation is "hard-wired" to expression of specific Rap1GAPs in T cell.

Keynote Address

Alfred Wittinghoffer (Max-Planck, Dortmund, Germany) was invited to present the conference keynote address. Pr Wittinghofer's carrier and achievements were pleasantly introduced by his friend and colleague Hans Bos. Professor Alfred Wittinghofer studied chemistry receiving his PhD degree from the German Wool Research Institute in Aachen with a dissertation on the chemical synthesis of insulin. In 1971 he became a postdoctoral fellow at the University of North Carolina, where his main research interest was the modification of proteins. In 1974 he returned to Germany to work as a research staff member at the Max Planck Institute for Medical Research in Heidelberg. In 1980 he became group leader of an independent working group there, where he focused on the structural functional relationships on oncoproteins and on GTP-binding proteins. In 1992 he qualified as professor at the University of Heidelberg. Since 1993 he has been head of the Department of Structural Biology at the Max Planck Institute of Molecular Physiology in Dortmund. In 1986 he published the first work studying the structure of the HA-Ras oncogene (RöschP et al, 1986). Since then, he has carried out a systematic biochemical and structural analysis of many small GTPases and their regulators and effectors, notably dissecting the mechanisms by which small GTPases are turned off by their GAPs. In his presentation Professor Wittinghofer talked about the large family of G-proteins. A very large and diverse class of G proteins is regulated by homodimerization, which he categorizes as G proteins activated by nucleotide-dependent dimerization (GADs). This class includes proteins like SRP, dynamin, septins and the newly discovered Roco proteins. He went on to talk about how the G domain juxtaposition across the GTP binding site activates the biological function and the GTPase reaction.

Conclusion of the meeting and general discussion

The meeting concluded with a session to discuss between the attendees if the meeting was worthwhile and the possibility to have another meeting in two to three years. The final session was well attended.

The responses received from the attendees indicated the enthusiasm of the participants for having attended this meeting. A particularly valuable aspect of the meeting was that it brought together scientists from widely different cultures and scientific backgrounds, and that specialists in both eukaryotic and prokaryotic biological systems were communicating vividly. The selected topics were of high interest to the scientific community, indicated by turnout of applicants, a total of 100 participants from 14 different countries (Canada, Denmark, Finland, France, Germany, Israel, Italy, Hungary, Spain, Switzerland, The Netherlands, Taiwan, USA, United Kingdom). This conference featured many of the most highly recognized specialists in the fields covered.

There was time and opportunity for questions and discussions in both platform talks and poster sessions, as well as during breaks and over lunch and dinner, eventhough the schedule was rather tight. The oral and poster presentations were of outstanding quality. In addition,

the relatively small scale of the meeting, the intimate and peaceful atmosphere, and the excellent facilities provided by the CNRS in Aussois, stimulated many in-depth discussions and initiated future collaborations. Much of this must be credited to Mrs Dominique Lidoreau and the personnel of the Aussois centre who took care of the practical organization (administration, lodging, poster room, computer facilities). We also want to emphasize the very good quality of housing and the excellent food.

It was decided that the meeting was definitely worthwhile and that it would be important to cover this topic, with a timely focus, again in two years. To this end Michael Williams (Aberdeen, UK) and Bruno Goud (Paris, France) were elected to organize the next European Small GTPase meeting as a conference Jacques Monod in Roscoff