To the centre of the volcano

Workshop on the Mechanisms of Nucleocytoplasmic Transport

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The EMBO Workshop on the Mechanisms of Nucleocytoplasmic Transport took place between 27 and 31 October 2007, in Taormina, Sicily, and was organized by E. Hurt and V. Doye.

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the import of transcription factors. Besides this, many regulatory and biosynthetic pathways take place partly in the nucleus and partly in the cytoplasm, leading to a substantial flux of macromolecules between these two compartments. Most macromolecules use a nuclear import or export receptor to facilitate nuclear transport (Macara, 2001). All interphase macromolecular transport to and from the nucleus takes place through nuclear pore complexes (NPCs), which are large protein complexes composed of nucleoporins (Nups; Suntharalingam & Wenthe, 2003). If the nucleus is at the heart of the cell, and all transport goes through NPCs, then nuclear import and export pathways must have great importance, and the centre of the NPC might contain a crucial secret.

This sense of urgency pulls together scientists from all across the world every two years to a meeting that alternates between the United States and Europe, and is unique for several reasons. First, almost the entire field is represented and the participants are mainly principal investigators (PIs). Second, everyone has the opportunity to present their work. Third, most of the data presented are unpublished (Fig 1A); these ingredients have so far resulted in a series of highly successful meetings.

Superficially, the 2007 meeting was a carbon copy of the 2003 meeting, as both the organizers and venue were the same, under the watch of the magnificent Mount Etna. But was that really true? Indeed, roughly 70% of PIs present four years ago were present again in 2007, but their focus had clearly shifted. Four years ago, one of the main issues discussed was the mechanism of mRNA export and its relation to splicing and translation, whereas in 2007 the most hotly debated issues were the structure and function of the NPC, perhaps the most crucial questions of the entire field. An overall view of the 2007 meeting (Fig 1B) shows that, in numeric terms, the main topics were the role of specific nucleoporins—in particular in NPC assembly and disassembly—and regulated RanGTP-dependent nuclear transport. Emerging topics were the links between mRNA export and the proteasome, transport of large cargoes and new quantitative model systems for nuclear transport.

NPC structure: getting there quickly or slowly?

Two parallel approaches are being used to elucidate the structure of the NPC. M. Rout (New York, NY, USA) presented a structure of the entire NPC that was computed by using a large proteomic data set.

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Introduction

Transport between the cell nucleus and cytoplasm has captured the imagination of researchers ever since the discovery of the cell nucleus by Robert Brown in the early 1830s (Brown, 1866). In eukaryotes, transcription and translation are spatially separated by the double membranes of the nuclear envelope. This alone necessitates a large amount of nuclear transport—that is, the export of protein-encoding RNAs and

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Fig 1 | The meeting at a glance. (A) An important indicator to the continued success of the bi-annual nuclear transport meetings is that what is presented is largely unpublished. (B) Network representation of the meeting. Talks are categorized in two or more topics (circles) that are connected (lines). The surface area of the circles is proportional to the frequency of the topic in the meeting, whereas the width of the line is proportional to the number of times a connection between topics was made within a talk. The network was generated using Cytoscape (www.cytoscape.org). NPC, nuclear pore complex.

This ‘fast-track’ method requires only limited information on the individual structure of nucleoporins and their interaction surfaces, and makes innovative use of computational modelling. Rout’s results suggest, among other things, that the NPC is constructed from ancient duplications of simpler modules. A different approach, which is relatively ‘slow-track’ but methodical, involves solving the structure of subcomplexes of the NPC by crystallography to build up a complete high-resolution structure. This approach has been used by E. Hurt (Heidelberg, Germany) and T. Schwartz (Cambridge, MA, USA) who presented new crystal structures of architectural nucleoporins, namely Nic96/Nup93 (Nic, for nucleoporin-interacting protein; Schwartz and Hurt) and the interaction complex of Nup107 and Nup133 (Schwartz). These results provide

encouragement that the architecture of the NPC scaffold could be determined by using this approach. It will be interesting to see where and how the fast- and slow-track methods of determining NPC structure converge in the future.

The heart of the matter: a brush or a gel?

Perhaps the main question in nuclear transport is how the NPC actually works—that is, how the selective passage of cargoes occurs. During the past decade, many models have been laid to rest, but two remain standing: virtual gating and phase exclusion (Becksei & Mattaj, 2005). Virtual gating assumes that the nuclear pore is blocked by a brush of nanofilaments consisting of nucleoporin phenylalanine-glycine (FG) domains, which only allows cargoes that interact with FG repeats to enter. Phase exclusion assumes that the centre of the NPC is filled with a gel-like nanonetwork of nucleoporin FG repeats, which can only be partitioned by cargoes that interact with FG repeats. Clearly, the biophysical characteristics of nucleoporin FG repeats must be evaluated to differentiate between these two models. R. Lim (Basel, Switzerland) presented nanoscopic evidence obtained by using atomic force microscopy, showing that surface-anchored FG-repeat domains of Nup153 and Nsp1 resemble polymer brushes that collapse after the addition of nuclear transport receptors. This would be consistent with virtual gating. However, D. Görlich (Göttingen, Germany) showed that nucleoporin FG-repeat domains can form a hydrogel that allows a transport receptor–cargo complex to enter up to 20,000-fold faster than the cargo alone. This is consistent with a selective-phase model. In conclusion, both models obtained new supportive data at the meeting, but the phase-exclusion model can now boast reductionist experiments that mimic cargo selectivity.

Nuclear transport: single molecules and big cargoes

Nuclear transport is a dynamic system with two easily identifiable cellular compartments, relatively few components and a driving system—the RanGTPase system—which is enzymatically well characterized. Nuclear transport has therefore proven to be an attractive system to pioneer quantitative modelling of cellular processes (Becksei & Mattaj, 2005). In this tradition, S. Musser (College Station, TX, USA) used a single-molecule fluorescence resonance energy transfer (FRET)-based assay to study the dissociation of importin- α from a cargo bound to a nuclear localization signal (NLS). The nuclear export receptor of importin- α , CAS, which recycles importin- α back to the cytoplasm, was found to stimulate the dissociation of cargo–importin- α complexes in permeabilized cells. In the presence of CAS, FRET disappeared on, or near, the nuclear side of the NPC. This indicates that CAS-stimulated dissociation of cargo from importin- α occurs near the NPC exit point, providing a beautiful example of how single-molecule methods can give insights into the spatio-temporal execution of nuclear transport. K. Berland (Atlanta, GA, USA) used fluorescence correlation spectroscopy to determine the diffusion of nuclear transport receptors and cargoes. In particular, importin- α and - β diffused in the cytoplasm much more slowly than anticipated from their sizes. This behaviour can be attributed to their binding to cytoplasmic factors. Importantly, binding of import cargo increased diffusion of the importins. This suggests that interactions of import receptors in the cytoplasm could be a mechanism to prevent the import of cargo-less importins. R. Kehlenbach (Göttingen, Germany) studied the specific roles of cytoplasmically oriented nucleoporins in nuclear import and export in cultured

human cells. By using RNA interference (RNAi) to deplete NUP214 and NUP358/RanGTP-binding protein 2 (RanBP2), he reported that NUP214 is required for nuclear export signal (NES)/chromosome region maintenance protein 1 (CRM1)-mediated nuclear export and that NUP358/RanBP2 is required for NLS-mediated nuclear import. These data indicate that peripheral nucleoporins function as specific assembly and disassembly sites for import and export complexes. However, C. Samakovlis (Stockholm, Sweden) came to the almost opposite conclusion using a *Drosophila* system. His data indicate that depletion of Nup214 results in a stimulation of Nes/Crm1-mediated nuclear export rather than a reduction. Interestingly, this stimulatory effect could be counteracted by the depletion of RanBP3, an intranuclear Crm1-interactor, indicating that Crm1 activity is regulated by absorption to intranuclear and NPC-linked factors.

Although we have a basic understanding of how nuclear transport of regular proteins occurs, our understanding of the import and export of large cargoes is still limited. Important cargoes such as pre-ribosomal subunits and viruses fall into this category. A. Fassati (London, UK) addressed the question of how human immunodeficiency virus (HIV) pre-integration complexes of approximately 100 nm are imported into the nucleus. Previous work has indicated an involvement of the import receptor importin γ . Surprisingly, biochemical fractionation and subsequent functional assays identified tRNAs as nuclear import factors. Inclusion of tRNAs in HIV was essential for HIV import into non-dividing cell nuclei and exogenous expression of tRNAs promoted nuclear import. On their own, tRNAs were also actively imported into human cell nuclei, indicating that tRNAs act as adaptors to feed HIV into the tRNA retrograde transport pathway (see also A. Hopper below). A. Johnson (Austin, TX, USA) presented new insights into the nuclear export of 60S pre-ribosomes, which are about 25 nm in diameter. Export of 60S pre-ribosomes is known to rely on the Crm1 pathway through nonsense-mediated decay 3 (Nmd3) and also requires mRNA transport regulator 2 (Mtr2). Johnson identified a third independent pathway for export, involving the protein ribosomal export complex 1 (Arx1), which was shown to interact with both 60S particles and nucleoporins. The involvement of many transport factors in the movement of large cargoes suggests further challenges to understanding events at the NPC. In this respect, the best-understood large cargoes are spliceosomal small nuclear ribonucleoproteins (snRNPs or Snurps) that are imported after their assembly in the cytoplasm. Rather than two different import receptors, nuclear import requires two copies of importin- β , one connected to the trimethyl cap through the adaptor protein snurportin 1, and one connected to the Sm core (Palacios *et al.*, 1997). In contrast to other importin- β cargoes, snRNPs do not require RanGTP for nuclear entry *in vitro*. A. Dickmanns (Göttingen, Germany) analysed the crystal structure of snurportin 1 with importin- β and found that snurportin 1 facilitates a conformation of importin- β that mimics the RanGTP-bound state, preventing its stable interaction with the nuclear face of the NPC. Possibly, the NPC interaction is prevented to avoid obstruction of the NPC by this sizeable cargo.

Regulation: the closer we look the more we see

Perhaps a sign that the nuclear transport field is still in its infancy is that everywhere one looks, one finds new forms of regulation. A. Hopper (Columbus, OH, USA) reported that retrograde nuclear accumulation of cytoplasmic tRNAs in yeast and vertebrate cells is regulated by their metabolic state, and might act as a way of nutritional regulation. Y. Yoneda (Osaka, Japan) reported that nuclear import of Snail,

a transcriptional regulator of E-cadherin, is dependent on importin- β , but is specifically inhibited by two isoforms of importin- α , α -3 and α -5. Yoneda also reported that ultraviolet irradiation of cells leads to a drop in the levels of RanGTP and an accumulation of importin- α and its export factor CAS in the nucleus. *In vitro*, importin- α binds to DNA and forms a heterotrimer with an NLS cargo independently of importin- β . This suggests a new, perhaps primordial, role for importin- α in targeting proteins to DNA. D. Goldfarb (Rochester, NY, USA) studied the effects of heat stress on nuclear import in yeast. Importin- α / β -mediated import was stimulated in heat-shocked cells, whereas karyopherin (Kap) 104-mediated import was decreased. Overexpressing heat-shock protein Hsp70, which is known to selectively stimulate α / β -mediated import, was sufficient to recapitulate the effect of heat shock. Elevating cellular levels of Kap104 or importin- α / β indicated that these karyopherin pathways are in direct competition for limited binding sites at the NPC. Interestingly, the FG domain of the cytoplasmic Nup42 was required for the differential effect, indicating that heat-shock competition during import takes place at these specific FG repeats at the cytoplasmic face of the NPC. Together, this indicates that nuclear transport is responsive to nutritional status and cellular stress. It seems highly probable that there are general, as well as cargo-specific, mechanisms linking nuclear transport to cell growth and proliferation.

Export of mRNA: links to chromatin and the proteasome

A mechanistic understanding of mRNA export has turned out to be much more difficult than that of simple protein cargoes (Vinciguerra & Stutz, 2004). Viral RNA transport elements, together with yeast genetics, have been instrumental in forming a basic framework of cellular mRNA export, in which TAP/NXF (higher eukaryotes) or Mex67 (yeast) are now well-established essential export factors that interact with both the NPC and nuclear mRNAs. A domain of TAP/NXF and Mex67 that has long attracted attention is its UBA domain, which is predicted to bind to ubiquitinated proteins. But which proteins? C. Dargemont (Paris, France) reported that the Mex67 UBA domain interacts with specific ubiquitinated targets, one of which is Hpr1, a component of the THO-TREX complex. The interaction occurs co-transcriptionally and helps to coordinate transcription elongation and mRNA nuclear export. Interestingly, Mex67 interacts with a protein called Swd2, the ubiquitination of which is required for trimethylation of histone H3 lysine 4 (H3K4), indicating a link between mRNA export, ubiquitination and chromatin metabolism. C. Guthrie (San Francisco, CA, USA) performed a high-throughput genetic interaction screen of genes involved in mRNA processing. Surprisingly, some of the highest genetic correlations of genes involved in mRNA export occurred with genes encoding components of the proteasome pathway. In particular, the proteasome lid component Sem1/DSS1 was highly genetically correlated with the Sac3-Thp1 mRNA export complex, and Sem1/DSS1 was found to have a role in mRNA export in *Saccharomyces cerevisiae*. F. Stutz (Geneva, Switzerland) also reported a link between mRNA export and proteasomal function. In yeast, Nab2 and Yra1 are other essential mRNA export factors that bridge Mex67 and mRNA. Stutz presented evidence that Yra1 not only recruits Mex67 to the mRNA but also has to dissociate from the mRNA before export, indicating a licensing step. Interestingly, this dissociation is dependent on the ubiquitination of Yra1 by the ubiquitin ligase Tom1. The proteasomal pathway is therefore emerging as a crucial regulator of mRNA export. This function might be separate from its function in protein degradation, raising the question of which came first.

Nab2 is also known to interact with Mlp1, a nucleoporin that forms the nuclear basket of the NPC. It is therefore thought that Nab2 assists in docking the mRNP to the NPC. M. Stewart (Cambridge, UK) reported the crystal and nuclear magnetic resonance structures of this protein, and mapped its interaction sites with Gfd1 and Mlp1. Gfd1 interacts with a nucleoporin subcomplex at the cytoplasmic site of the NPC, and the structural studies by Stewart indicate that Nab2 could join this complex, potentially bringing the mRNP particle close to its disassembly site at the cytoplasmic face of the NPC. D. Libri (Gif-sur-Yvette, France) investigated the role of the THO complex in the assembly and export of the mRNP. In THO and Sub2 mutants, several genomic loci are non-productively docked to the nuclear pore, which leads to alterations in chromatin structure. This probably results from a failure to release the mRNP from the site of transcription because a crucial remodelling step that follows commitment to 3'-end processing cannot be performed. M. Fornerod (Amsterdam, The Netherlands) provided genome-wide evidence that nucleoporins interact with active chromatin and that these interactions predominantly occur in the nucleoplasm rather than at the nuclear periphery.

NPC assembly and disassembly

In most animal and plant cells, the compartmentalization of the nucleus breaks down during mitosis, with the disassembly of NPCs and the nuclear envelope becoming fragmented or adsorbed into the endoplasmic reticulum. Around telophase, the nuclear envelope and NPCs are reassembled and nuclear transport restarts. J. Ellenberg (Heidelberg, Germany) presented a tour-de-force of imaging in which his group has characterized the timing of NPC reassembly after mitosis in live cells. By following the incorporation of certain green fluorescent protein (GFP)-tagged nucleoporins relative to the import of a specific cargo (IBB, the importin- β -binding domain of importin- α), he found that Nup133 was concentrated early—long before transport started—on chromatin, whereas Pom121 was later and Nup58 accumulated along with IBB. Interestingly, Nup153 and Nup50 partly accumulated at an early stage, indicating that they have a role early in NPC assembly but then they accumulate in the nucleus mainly by import. Disassembly in prometaphase was not simply the reverse process, as most nucleoporins were lost simultaneously.

Genetic analysis of amenable organisms can be used to dissect the requirements for the insertion of NPCs into the nuclear envelope and for NPC maintenance. S. Osmani (Columbus, OH, USA) reported studies of *Aspergillus nidulans*, in which there was a partial disassembly of the NPC during mitosis. By using live-cell imaging of GFP–nucleoporin fusions combined with deletion of specific nucleoporin genes, Osmani found that the deletion of ELYS and Nup37 released virtually all nucleoporins specifically during mitosis. However, Nup170 and Gle1 remained at the nuclear envelope, identifying them as potential 'core components' for NPC reassembly on exit from mitosis. Indeed deletion of Nup170 resulted in strong NPC reassembly defects. The two known transmembrane nucleoporins in *Aspergillus*—Pom152 and Ndc1—were also studied. Surprisingly, double deletion of these did not visibly affect viability or NPC function, raising the question of what is the true membrane anchor of the NPC.

In *S. cerevisiae*, there are three transmembrane nucleoporins: Pom152, Pom34 and Ndc1. K. Weis (Berkeley, CA, USA) described how deletion of Ndc1 and Pom152 causes severely aberrant and dilated pores. Ndc1 seems to have a crucial role in NPC assembly

through interactions with Pom34, Pom152 and several soluble nucleoporins—Nup170, Nup157, Nup53 and Nup59. R. Wozniak (Edmonton, Canada) reported that Nup155 interacts directly with Pom121 and Ndc1, and deletion of its yeast counterparts, Nup170 and Nup157, blocks NPC assembly. Loss of these nucleoporins leads to the formation of 'prepore' structures that fail to form mature NPCs. A model was proposed describing a role for these nucleoporins in forming pores together with Kap95 and the membrane protein Apq12. Apq12 was first implicated in NPC assembly by C. Cole (Hanover, NH, USA), who suggested that it might affect lipid ordering in the nuclear envelope. In *Caenorhabditis elegans*, a multicellular animal commonly used for genetic analysis, M. Hetzer (La Jolla, CA, USA) reported that scaffold nucleoporins are downregulated in post-mitotic cells, indicating that there is no new NPC assembly. Remarkably, NPC stability in these cells was calculated to exceed lifespan.

The mechanisms controlling NPC disassembly in mitosis are still poorly understood. U. Kutay (Zürich, Switzerland) presented a human-cell extract system to analyse nuclear envelope breakdown and NPC disassembly. Through the use of protein kinase inhibitors and dominant-negative kinase mutants, Kutay and colleagues found evidence for an involvement of at least four different kinases in NPC disassembly—a starting point to characterize the contribution of nucleoporin phosphorylation to nuclear envelope breakdown. E. Hallberg (Huddinge, Sweden) reported that the single mitotic phosphorylation site on the nucleoporin gp210 is important in controlling its interaction with the NPC.

Nuclear fusion: first a dry kiss, then a wet one

In one of this meeting's highlights, A. Tartakoff (Cleveland, OH, USA) presented pioneering work on the fusion of haploid yeast nuclei, leading to diploid yeast formation. By using real-time microscopy and differently coloured outer nuclear envelope (ONE), inner nuclear envelope (INE), NPC, spindle pole body (SPB) or nucleoplasmic proteins, Tartakoff determined a sequence of events. Focal–nuclear contact is first established at a nexus that is at or immediately adjacent to the composite SPB. Proteins of the ONE intermingle at once, followed several minutes later by INE proteins, concurrent with the first exchange of nucleoplasmic content. Last, the nexus dilates, the SPB disengages from one face of the nuclear envelope, and NPC components intermingle. The SPB seems to orchestrate this orderly sequence of events.

Mitotic roles for the nuclear transport system

In recent years, RanGTPase, transport factors and certain nucleoporins have been shown to have special roles during mitosis that are distinct from their functions in nuclear transport during interphase. For example, the Nup107–Nup160 complex has been characterized as important for microtubule attachment to kinetochores during mitotic spindle assembly. Although this area was not a strong focus of the meeting, some new progress was reported. M. Powers (Atlanta, GA, USA) identified a new and unexpected role for Nup98 in mitotic spindle assembly by using *Xenopus* egg extracts. N. Imamoto (Saitama, Japan) reported the role of importin- β and RanGTP in the loading of the human chromokinesin KID to chromosomes, a strong indication that importins act as targeting proteins and not just inhibitors of crucial mitotic factors during mitosis. P. Clarke (Dundee, UK) described RCC1- γ , a new isoform of the guanine nucleotide exchange factor for Ran that might have a specialized mitotic function.

Fig 2 | Inner surface of a *Xenopus* oocyte nuclear envelope showing lamin filaments tightly associated with the membrane and interconnecting nuclear pore complexes, visualized by field emission scanning electron microscopy. (Photograph courtesy of Martin Goldberg, University of Durham, UK.)

Notable work reported by J. van Deursen (Rochester, MN, USA) showed that, although deletion of RanBP2/Nup358 in mice is embryonically lethal, a hypomorph expressing approximately 30% of normal levels of RanBP2 apparently develops normally, and nuclear transport seems active. However, there is a significant increase in the formation of lung and skin tumours in response to the tumour promoter DMBA, as well as an increase in spontaneous tumours. This coincides with a marked increase in aneuploidy in RanBP2-hypomorphic cells and with the formation of anaphase chromosome bridges. Interestingly, the E3 SUMO ligase domain of RanBP2 is required for SUMO modification of topoisomerase II α (Topo II α), a protein involved in decatenating DNA. Moreover, SUMO modification of Topo II α is required for its localization to inner centromeres during mitosis. This suggests that one function of RanBP2 is to localize correctly to Topo II α during mitosis, allowing it to function in decatenation of metaphase chromosomes. Owing to its role in preventing aberrant mitosis, RanBP2 is predicted to be a tumour suppressor protein. Indeed, van Deursen and colleagues found that approximately 25% of human lung tumours analysed have more than a twofold reduction in RanBP2 and there is frequent loss of RanBP2 in cultured cell lines. M. Matunis (Baltimore, MD, USA) reported that RanGAP1, the GTPase-activating protein of Ran and a partner of RanBP2, is preferentially modified by SUMO1 *in vivo*. However, no preference for SUMO1 compared with SUMO2 was detected *in vitro*. This paradox could be explained by the observation that RanBP2 has a higher affinity for SUMO1-modified RanGAP1 and that RanBP2 binding protects against SUMO deconjugation. Therefore, the preference of SUMO1 over SUMO2 might be due to differences in the rate of SUMO deconjugation rather than conjugation.

In and around the nuclear lamina

Owing to its proximity to the NPC, the nuclear lamina has been a popular excursion for investigators in the nuclear transport field. However, so far the NPC and the nuclear lamina have been similar to good neighbours, often saying hello, but living their own lives. Is this still the case? S. Adam (Chicago, IL, USA) showed data that connected nuclear lamina assembly with the nuclear transport machinery. The depletion of an importin- α isoform in *C. elegans* severely reduced nuclear lamina formation. *In vitro*, *Xenopus* Lamin L(III) forms large complexes that are dependent on the importin- α -LIII NLS interaction. This opens the possibility that importin- α is required to deposit lamins at the nuclear lamina. M. Goldberg (Durham, UK) presented the first new electron microscope data in 25 years on the *in vivo* structure of the nuclear lamina (Fig 2). His images of *Xenopus laevis* oocyte nuclear laminae suggest that the two-dimensional lattice of the nuclear lamina does not consist of 'woven' filaments but rather of parallel cross-linked ones. Y. Gruenbaum (Jerusalem, Israel) presented *in vitro*-assembled structures of *C. elegans* lamin that formed large regular sheets of lamin filaments in antiparallel orientation. A mimic of a human laminopathy disease protein could only form irregular structures. Several laminopathy mutations could be mimicked in the worm, showing many different nuclear phenotypes. Laminopathy mimics in *C. elegans* could therefore be used to correlate *C. elegans* lamin assembly defects with nuclear phenotypes in the worm and disease outcome in humans. B. Paschal (Charlottesville, VA, USA) reported that the loss of lamin A or expression of certain lamin A mutants causes a marked redistribution of Ran from the nucleus to the cytoplasm. Changes in Ran were correlated with histone H3K9 trimethylation, indicating a link between the function of lamin A, the Ran protein gradient and chromatin modification.

The INE contains proteins that are connected to the nuclear lamina, forming a physical link between these structures. L. Gerace (La Jolla, CA, USA) reported that several INE proteins are upregulated during muscle differentiation and that knockdown of one of these resulted in a block in muscle differentiation, indicating that INE proteins might be important components in the regulation of differentiation. E. Schirmer (Edinburgh, UK) also reported tissue-specific variation in the nuclear envelope proteome. In addition, he identified several new putative nuclear envelope proteins, some of which were expressed at different levels in resting and activated human blood cells.

P. Lusk from G. Blobel's laboratory (New York, NY, USA) addressed the question of how INE proteins are targeted in yeast. He showed that the localization of a GFP-tagged INE protein to the INE was dependent on its NLS. An NLS mutant could be rescued by a heterologous NLS but only by those recognized by kap- α / β or kap- β , indicating specificity in NPC interaction or INE retention. Indeed, certain nucleoporin mutants affected INE localization of the reporter but not NLS-dependent protein import in general. B. Burke (Gainesville, FL, USA) discussed the two isoforms in human cells of SUN, another INE protein that interacts with the nuclear lamina. Burke showed that the depletion of Sun1, but not of Sun2, resulted in a clustering of NPCs. In conclusion, several new connections have been made between nuclear transport and the nuclear lamina. However, so far it seems that the NPC and the nuclear lamina are two entities that are linked more by physical rather than by mechanistic proximity.

Conclusions

This meeting confirmed that nuclear transport is still a vibrant and rapidly progressing field of cell biology. At the heart of the subject are the structure of the NPC and the mechanism of translocation across the nuclear pore. The new developments reported in Sicily are clearly significant advances in this area. In the future, perhaps

the final model for translocation will be a synthesis of the two polarized views of 'brush' and 'gel' that are now in vogue. A combination of structural biology and computational modelling will hopefully converge with visual techniques to provide a comprehensive structure of the NPC. In other areas, it is increasingly clear that the molecular components of the NPC and transport system have wider roles in coordinating gene expression as well as cytoplasmic processes, particularly during mitosis, and are subject to regulation.

REFERENCES

- Becskei A, Mattaj JW (2005) Quantitative models of nuclear transport. *Curr Opin Cell Biol* **17**: 27–34
- Brown R (1866) On the organs and mode of fecundation of *Orchidex* and *Asclepiadea*. *Miscellaneous Botanical Works* **1**: 511–514
- Macara IG (2001) Transport into and out of the nucleus. *Microbiol Mol Biol Rev* **65**: 570–594
- Palacios I, Hetzer M, Adam SA, Mattaj JW (1997) Nuclear import of UsnRNPs requires importin β . *EMBO J* **16**: 6783–6792
- Suntharalingam M, Wente SR (2003) Peering through the pore: NPC structure, assembly, and function. *Dev Cell* **4**: 775–789
- Vinciguerra P, Stutz F (2004) mRNA export: an assembly line from genes to nuclear pores. *Curr Opin Cell Biol* **16**: 285–292

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