EMBO WORKSHOP REPORT

Membrane trafficking and the cytoskeleton: an integrated view

ASCB/EMBO/Dudley Wright Summer Research Conference Santa Maria Imbaro, Italy, June 26–30, 1999

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Introduction

The beautiful grounds, facilities and location of the Consorzio Mario Negri Sud in Santa Maria Imbaro, on the Adriatic Coast of Italy, were the site at the end of June for a stimulating, interdisciplinary meeting entitled 'Membrane trafficking and the cytoskeleton: an integrated view'. This meeting represented the fifth and final offering of biannual summer research conferences held this decade, arranged and supported jointly by the European Molecular Biology Organization, the American Society for Cell Biology (ASCB) and the H.Dudley Wright Foundation of Switzerland. Daniela Corda and Alberto Luini of the Department of Cell Biology and Oncology of the Consorzio hosted the meeting, supported by the excellent administrative assistance of Raffaella Bertazzi and Dorothy Doyle.

The meeting topic was conceived and the sessions were organized by George Bloom (University of Texas Southwest Medical Center, Dallas, TX), Viki Allen (University of Manchester, Manchester, UK), Jennifer Lippincott-Schwartz [National Institutes of Health (NIH) Division of Child Health and Human Development] and the late Thomas Kreis (University of Geneva, Geneva, Switzerland) who lost his life in the SwissAir crash last year. The meeting was attended by ~175 principal investigators, postdoctoral and graduate students representing 14 European countries plus the USA, Canada, Japan, Israel and New Zealand. Additional funding for the meeting was provided by the National Cancer Institute of the NIH, the National Science Foundation and the Keith Porter Foundation.

The purpose of these topic-oriented summer research conferences, as envisioned by the late Mr Dudley Wright, is to foster scientific exchange between various groups of scientists isolated from each other by geographical constraints, or differences in fields or approaches to a problem. The meeting in Italy certainly succeeded in fulfilling Mr Wright's intent and was notable for its inclusion of many scientists at the postdoctoral and graduate student levels. It focused attention on two previously disparate but now frequently intersecting fields of

investigation: cell motility and protein targeting. Productive interactions occurred between scientists studying the cytoskeleton, cell motility, motor enzymes, protein targeting, secretion and endocytosis. The following highlights of selected presentations provide a sense of the dynamic, interactive and timely nature of the meeting.

Visualizing membrane and cytoskeletal dynamics in real time

In a session on the visualization of cellular dynamics, speakers focused on the use of contemporary techniques and technologies to observe and study movements of membrane-bound organelles relative to cytoskeletal components in living cells. Interestingly, two reports presented apparently conflicting conclusions. Jennifer Lippincott-Schwartz (NIH), employing a galactosyl transferase–GFP fusion protein, followed dispersal of the Golgi membranes during mitosis. She showed that the Golgi breaks down at prophase into minute fragments that disperse equally throughout the cytoplasm during mitosis. At telophase, the fragments coalesce at the spindle poles/daughter cell centrosomes and the Golgi then reform. Conversely, Noemi Cabrera-Poch (Imperial Cancer Research Fund), using a similar approach, presented evidence demonstrating that during prophase the Golgi membranes fragment into large particles that interact first with the separating centrosomes and then with the astral microtubules. The Golgi fragments appear to be bound to microtubules, but the Golgi membranes do not move and disperse relative to the underlying cytoskeletal elements. During the discussion, it was mentioned that cell type or differences in the subcellular localization of the differing fusion proteins being used as reporter molecules by the two groups might account for these discrepancies. The laboratories involved agreed to share their cell lines and reagents to attempt to resolve these conflicting data.

Gary Borisy (University of Wisconsin) presented real-time videos of microtubule dynamics, and he focused attention on events occurring at the minus end of microtubules. Microtubules are directional polymers, and while subunits can add to both ends *in vitro*, the process is most likely to be different *in vivo*. Many microtubules in cells have their minus ends embedded in the centrosome and thus are presumed to be unable to exchange subunits, while the plus ends, distal to the centrosome and hence free, continually add and lose subunits by events referred to collectively as dynamic instability. Experimental conditions, as well as normal cellular events, can generate

cellular microtubules with both ends free and this has led Borisy to study what he refers to as the turnover paradox. Sometimes, non-centrosomal microtubules in cells lose subunits from the minus end faster than they gain them from the plus end and hence depolymerize completely. In other examples, however, free microtubules in the cytoplasm can experience a net addition of subunits to the plus end that is exactly balanced by a net loss of subunits from the minus end, a situation known as treadmilling. The data presented demonstrated that the stability of a free microtubule appears to be a function of several factors, among them the presence of a centrosome and the position of the microtubule in the cell relative to the nucleus and the cell periphery. These observations have led to the conclusion that microtubules which are released from the centrosome, by either random breakage or an active severing process, have either a stabilizing factor attached to the minus end or an associated factor that promotes treadmilling. In the absence of either type of factor, the microtubule is unstable and will eventually disappear.

Motor enzymes of the cytoskeleton and their control

The interaction of the motor proteins dynein and kinesin with microtubules (the substrate for motility) and membranes (the cargo for motility), and the manner in which the association with cargo and the interaction with cytoskeletal elements are regulated, was the focus of a number of presentations in various sessions. Of particular interest were presentations addressing the regulation of these motor proteins and their involvement in certain human maladies. Working with *Neurospora*, Stephan Seiler (University of München) reported that key C-terminal residues in the kinesin heavy chain are essential for function in this organism. A mutant lacking 14 residues in the C-terminus ($\Delta 787-800$) fails to rescue the null. These 14 residues are required for cargo binding and are conserved in all kinesins; hence, the kinesin heavy chain probably binds directly to its cargo. Moreover, other residues in the globular domain (RIAKPLR) appear to be involved in folding of the molecule. The current hypothesis suggests that inactive kinesin is folded essentially in half, with the head domain interacting directly with the tail. Cargo binding dissociates this head-tail interaction, unfolding the protein and revealing sites for further controlling modifications such as phosphorylations, etc.

KIF3A/B-KAP3 is a heterotrimeric kinesin that is expressed in neurons and other cells. Like the founding member of this class of motors, conventional kinesin, KIF3A/B is a plus-end-directed microtubule motor, and the KAP3 subunit binds cargo. Immunofluorescence reveals a punctate staining pattern that is distinct from synaptic vesicles. Nobutaka Hirokawa and collaborators (University of Tokyo) have generated a knock-out mouse for the 3B subunit. KIP3B^{-/-} mice are embryonic lethal, 50% of the embryos display situs inversus and the embryos are often missing cilia or at best have cilia that lack the central pair microtubules (i.e. the axonemes are 9 + 0). Situs inversus is the condition where the major body organs (i.e. heart and liver) come to lie on the opposite side of the body axis to where they are normally positioned. Ciliary beating

is assumed to be responsible for normal body plan asymmetry, and in its absence the organs position randomly (hence 50% situs inversus). However, the cause of situs inversus in humans has not yet been firmly established. The model that Hirokawa proposed suggests that the KIF3A/B complex normally transports axonemal material required for ciliogenesis. Cilia form and then their normal beat process sets up a right-to-left flow in the nodal area of the developing embryo. The right-to-left flow in the node in turn sets up a left-to-right gradient of an as yet unidentified morphogen, which is responsible for controlling left-right asymmetry during development. The function of the KIF3A/B that is localized to those punctate structures in neurons of the adult nervous system, however, remains unclear.

Richard Vallee (University of Massachusetts Medical School) considered the problem of how dynein and its interaction with cargo are regulated. A dynein-related defect is suspected in lissencephaly, a human brain disorder characterized pathologically by a smooth, not convoluted, cortex. The *LIS-1* gene is responsible for this defect, and *LIS-1* has homology to the *Aspergillus nudF* and budding yeast *PAC1* genes. Both *nudF* and *PAC1* mutants show nuclear positioning defects after mitosis. The phenotype is very similar to the phenotype of *nudA* and *dyn1*, which are dynein heavy chain mutants in *Neurospora* and budding yeast, respectively. In normal neuronal progenitor cells, the nucleus migrates up and down the cell as a function of cell cycle time (i.e. similar to fungal nuclear migrations that are defective in *nudA* cells). Cytoplasmic dynein co-immunoprecipitates with LIS-1 when LIS-1 is expressed in mammalian tissue culture cells, indicating an interaction between dynein and LIS-1. Overexpression of LIS-1 resulted in mitotic defects, yet did not dissociate dynein from dynactin (a multimeric complex required for dynein to have normal activity). Rather, dynein and dynactin remain associated and localize normally to the kinetochore. Hence, the interaction of LIS-1 with dynein presumably occurs via a novel mechanism.

In a related presentation, Giorgos Diamantopoulos (University of Geneva) presented data on the subcellular localization of the microtubule binding protein CLIP 170, which binds to microtubule plus ends. These are the sites of subunit exchange and are where endocytic vesicles first encounter and presumably associate with microtubules. Moreover, the sites on the microtubules occupied by CLIP 170 are probably different from the sites to which other microtubule-associated proteins (MAPs) bind. For example, CLIP 170 does not compete with dynein, MAP 2c or MAP 4 for binding to microtubules, e.g. overexpression of MAP 2c causes MAP 4 to detach from microtubules yet has no effect on the binding of CLIP 170. It is likely that CLIP 170 binds to the GTP cap, i.e. to those subunits at the microtubule plus end that have not yet hydrolyzed their bound GTP to GDP. Interestingly, Vallee reported that dynactin binds to the plus ends of microtubules as well, and overexpression of LIS-1 disrupts this localization of dynactin. Holly Goodson (University of Geneva) addressed the co-localization of CLIP 170 and dynactin at microtubule plus ends. Overexpression of either the p150 or p50 components of the dynactin complex has no effect on CLIP 170 binding to microtubule plus ends; thus, it is likely that CLIP 170 targets the plus end

of microtubules independently of dynactin. However, overexpression of CLIP 170 does affect dynactin distribution: both the Arp1 and p150 components of dynactin become concentrated at sites of CLIP 170 overexpression. Thus, CLIP 170 and dynein may interact functionally, perhaps by either modulating dynein function or directing the dynein–dynactin complex to the microtubule plus end.

George Bloom (University of Texas Southwest Medical Center) addressed the problem of how Golgi membranes move between the Golgi apparatus and the subcellular compartments to which the Golgi sends and from which it receives membranes. Kinesin moves membranes from the Golgi to the endoplasmic reticulum (ER), and a kinesin antibody can inhibit this process. Conversely, dynein moves membranes from the ER to the Golgi, and these membranes also carry kinesin but the kinesin is inactive. A reconstituted *in vitro* system comprised of Golgi membranes, microtubules and an ATP-regenerating system allowed analysis of the kinesin-driven motility, assayed by video microscopy. The motility of vesicles and membrane tubules in this *in vitro* system can be blocked by GTP- γ -S and aluminum fluoride, two known G-protein activators. Moreover, constitutively active forms of cdc42 and Rac, members of the Rho family of GTPases, block membrane-tubule motility, but not vesicle motility. The data suggest, therefore, that cdc42 and Rac are involved in the control of microtubule plus-end-directed transport of membranes away from the Golgi apparatus.

The actin-based component of the cytoskeleton is also involved in membrane vesicle transport, and several presentations summarized recent data on myosin-based motility. Kathryn Miller and colleagues (Washington University) addressed the role of myosin VI in membrane transport and protein localization. Immunofluorescence of early *Drosophila* embryos reveals that myosin VI is localized to punctate structures which move in an actin-dependent manner. A role for myosin VI in membrane remodeling is suggested, for example, in the formation of the syncytial blastoderm and in the process of individualization that occurs during sperm maturation in *Drosophila* (whereby an initially shared membrane is resolved into individual membranes surrounding each mature sperm). Moreover, myosin VI and an associated polypeptide (D-CLIP-190) localize to the posterior pole in embryos. Cytochalasin D disperses these proteins (as well as other posterior markers such as oskar) from the posterior pole; microtubule-disrupting drugs have no effect on this localization as it is maintained in their presence (although these proteins do require intact microtubules for transport to the posterior pole initially). Folma Buss and coworkers (University of Cambridge) reported that myosin VI is associated with the Golgi complex in NRK cells. Using GFP-tagged myosin VI deletion constructs, these workers identified C-terminal residues of myosin VI as containing information required for binding to Golgi membranes. Another myosin (myosin II) also binds to Golgi membranes, as reported by David Burgess (Boston College); *in vitro* reconstitution shows that the binding of myosin II is saturable, suggesting a finite number of receptors. Furthermore, phosphorylation of residues in the rod domain of myosin II produces a myosin II that will no longer bind to Golgi membranes, suggesting a means

of controlling the interaction of myosin II with the membrane.

In budding yeast, *myo2-66* is a myosin V mutant. Work reported by Lois Weisman (University of Iowa) showed that *myo2p* has a mutation in the membrane-binding domain of myosin V. Consequently, cells carrying this mutation are defective in vacuole inheritance. The yeast vacuole is moved by *myo2p*, cargo binding occurs in the tail domain and two binding regions have been identified: one for the vacuole (which is non-essential) and another for an essential, yet to be identified cargo. This system offers an excellent opportunity, via suppressor analysis etc., to identify the vacuolar receptors for myosin V.

Vesicle coat proteins

In sessions that addressed the identity and characterization of the surface components of the vesicles being moved by these cytoskeletal systems and motor proteins, interesting related data were presented. For example, Frances Brodsky (University of California, San Francisco) reported the crystal structure of the portion of the clathrin heavy chain that controls self-assembly (the hub domain), solved to 2.6 Å resolution. This portion of clathrin is composed of a 10-helix unit composed of two and a half repeats (2 + 4 + 4); two such units occur in the region crystallized, and sequence analysis indicates that a total of seven such repeats occur in a single leg of a clathrin triskelion. The hub domain, when combined with recombinant proteins representing the remainder of the clathrin heavy chain, forms a self-assembling system that was used to determine the order of triskelion assembly, which also revealed the need for specific adaptor proteins that are important for assembly and function of the triskelion. Finally, sequence analysis was used to demonstrate that several yeast vacuolar sorting mutants have fewer but similar repeats, suggesting the likely structural defect in these mutations as well.

With respect to adaptor proteins (APs), four have been identified in mammals. Margaret (Scottie) Robinson (University of Cambridge) presented an analysis of AP function: AP1, 2 and 3 are associated with clathrin-coated vesicles of the trans Golgi network (TGN), while AP1, 2 and 4 are associated with clathrin-coated vesicles of the plasma membrane. Interestingly, several mouse AP3 mutants have phenotypes similar to those of patients with the human genetic disorder Hermansky–Pudlak syndrome. Different AP-3 subunits (there are four per AP, but each AP has a different complement) are mutated in mocha and pearl mice. These mouse mutants not only have aberrant coat color, but also, as in the human disorder, have problems with lysosomes and related organelles (melanosomes and platelet dense granules). The movement of proteins from the TGN to the cell surface in polarized MDCK cells was studied by Enrique Rodriguez-Boulant (Cornell University) who followed the movement of the apical surface protein p75 tagged with GFP. Microinjection of function-blocking kinesin antibodies (but not antibodies to dynein) demonstrated that kinesin is required to move vesicles from the TGN to the cell apex in these cells. By injection of mutant forms of dynamin, a role for dynamin in the release of p75 vesicles from the TGN could also be inferred.

Secretory and endocytic pathways

Two vesicular coat protein complexes function in membrane traffic in the secretory pathway, and these were discussed by Rainer Pepperkok (EMBL-Heidelberg). In yeast, the COP-II complex is involved in the movement of vesicles from the ER to the Golgi; conversely, COP-I retrieves membrane from the Golgi and returns it to the ER. In mammals, the process is more complex as an additional compartment, the intermediate compartment (IC), functions at this ER–Golgi boundary. Using GFP-tagged membrane compartment markers and fluorescently labeled COP-I, it could be determined that directional transport between the IC and Golgi requires COP-I, but not COP-II, while the analogous path from the IC to the ER requires COP-II.

In photoreceptor cells, rhodopsin is synthesized in the inner segment (IS) and transported from the Golgi in the IS to the outer segment (OS), where the new rhodopsin molecules replenish those lost naturally by shedding from the tip of the OS. The IS and OS are joined by a structure called the connecting cilium, which is derived from the 9 + 0 primary cilium. Data presented by Uwe Wolfrum (University of Mainz) demonstrated that rhodopsin-containing vesicles are transported from the Golgi to the connecting cilium in a dynein-dependent process that requires the protein Tetex-1, a dynein light chain that binds both to dynein heavy chain and to opsin. Transport in the connecting cilium from the IS to the OS, however, requires myosin VIIa, which is localized to the connecting cilium membrane. It is hypothesized that transport of rhodopsin-containing membrane plaques occurs through the connecting cilium membrane via an interaction of myosin VIIa with actin filaments that are assumed to lie outside the outer doublets between the ciliary axoneme and the membrane. Note that defects in the myosin VIIa gene cause one form of Usher syndrome in humans. Usher syndrome patients have combined defects in vision and hearing.

Viki Allen (University of Manchester) studies vesicle cycling between the ER and Golgi. She and her collaborators have localized, via immunofluorescence, the dynein heavy chain to Golgi membranes and the intermediate compartment; dynein seems to cycle between these compartments and the ER as well. The motor molecules probably stay bound to these various membrane compartments throughout the process of membrane cycling from the Golgi to the ER and back again. Thus, the direction of movement is probably not controlled by the association of motors with the membrane, but rather controlled by the activity of the motor enzymes. The reverse direction of movement (i.e. plus-end-directed) is not likely to occur via conventional kinesin, but rather by a slightly larger form of kinesin (heavy chain mol. wt = 130 000). The activity of this protein is blocked by one kinesin antibody (H1) but not by another (SUK4), suggesting that the 130 000 mol. wt component is a novel form of kinesin.

Several presentations directly linked data from endocytotic mechanisms to cytoskeletal function. Regis Kelly (University of California, San Francisco) discussed in part the structure of the membrane at the synapse. He presented an interesting model in which synaptic vesicles on the inside of the synaptic membrane are positioned coincident

with glutamate receptors on the outside of the membrane, and the endocytic machinery lies between the synaptic vesicle clusters. The basic unit of structure *en face*, therefore, is a disk of endocytotic machinery surrounded by an annulus of exocytotic machinery.

Studying the exocytotic and endocytotic events that occur at the synapse, Pietro De Camilli (Yale University) demonstrated that a minimum of four proteins—clathrin, AP2, AP180 and synaptotagmin—will function on artificial membranes composed of liposomes *in vitro*. *In vivo*, however, other accessory factors are required, and these include dynamin and synaptojanin (an inositol phosphatase). Synaptojanin knock-out (SJ-1) mice are born normally, but the majority die in the first day and the remainder in about a week. The mice display severe neurological impairment and have many more clathrin-coated vesicles positioned at synapses than normal mice. The data suggest that synaptojanin is required for uncoating clathrin vesicles, and in the absence of the enzyme the endocytosis required to recycle synaptic membrane and generate new synaptic vesicles is blocked, leading to the neurological deficit noted in the knock-out mice.

Regulation of membrane traffic

ADP-ribosylation factors (ARFs) are Ras-related GTPases that control membrane traffic by functioning as on/off switches depending on whether they have GTP or GDP bound, respectively. ARF1 is involved in the regulation of membrane traffic from the ER to the Golgi and plasma membrane, while ARF6 regulates the plasma membrane–endosomal recycling pathway and influences the cortical actin cytoskeleton. Julie Donaldson (NIH), studying ARF6, showed that it can be reversibly activated by aluminum fluoride, and this has led to the discovery of an upstream regulator of ARF6. The regulator is a heterotrimeric G-protein, and expression of a constitutively active mutant α subunit ($G_{\alpha q}$) mimics aluminum fluoride treatment. Either expression of the mutant or treatment with aluminum fluoride results in an accumulation of ARF6 at the plasma membrane and the production of membrane protrusions. Two key residues in ARF6 are critical for activation, either by aluminum fluoride or by overexpression of the mutant α subunit. In a related presentation, Dorothy Shafer (Washington University School of Medicine) showed that an activated mutant form of ARF6 (Q67L) induces actin assembly and vesicle movement *in vivo*. Thus, ARF6 appears to be directly involved in regulating actin assembly in the cortex; however, the identity of the particles induced to move by ARF6 is yet to be determined.

In contrast to these direct effects of ARF6 on the actin cytoskeleton, Pierre Chardin (CNRS) reported on ARF1, which, in its active form with GTP bound, binds to the membrane and recruits various coatamer complexes that serve a scaffolding function for vesicle budding. GTP hydrolysis then releases the coat complex and ARF1–GDP. GDP release from ARF is very slow but can be accelerated by GEFs (GTP-exchange factors). ARNO is a GEF specific for ARF, which has an essential invariant glutamate residue required for the GTP-exchange reaction. Interestingly, other GEFs of the Ras and Rho family of proteins do not appear to have a similarly essential

glutamate (and they also have different structures). Nucleotide exchange on ARF also requires sec7 domains, and these are conserved from yeast to mammals. Finally, ARNO can exchange GTP on ARF only in the presence of lipid vesicles, and only when ARF is myristoylated. Thus, interaction of ARF with the membrane is probably essential for its function *in vivo*.

Other small G-proteins of the Rab family are required for the membrane sorting processes that occur to maintain the identity of the various membrane-bound compartments in the cell. Two presentations addressed the function of Rab proteins in membrane trafficking. Birte Sonnichsen (EMBL) reported that 22 proteins from brain cytosol bind to a Rab5-GTP affinity column, and these can be used to stimulate a membrane fusion reaction *in vitro*. However, EEA1 (early endosomal antigen 1) is the only protein of the 22 that is required for fusion. It is believed that the Rab proteins help to shape endosomal membrane domains, making them competent for fusion or recycling. Rab6 is ubiquitous and associates with membranes of the Golgi and the TGN. Bruno Goud (Institut Curie) reported that an effector of Rab6 called Rabkinesin-6 is associated with the Golgi. The current hypothesis is that Rab6-GTP serves as an intermediary in the binding of Golgi membranes or Golgi-derived vesicles to microtubules via Rabkinesin-6, which then catalyzes movement.

Membrane trafficking, the cytoskeleton and disease

Tau is a MAP that is aggregated and hyperphosphorylated in patients with Alzheimer's disease (AD). However, whether the protein is phosphorylated and then aggregates, or vice versa, is still a matter of debate. Eva-Maria Mandelkow (Max Planck Institut) and colleagues tracked individual vesicles in living cells and showed that tau does not affect the speed of kinesin-driven transport. Rather, tau decreases the run length (i.e. increases the chance that a given vesicle will release from the microtubule) and decreases the probability that a vesicle will attach to a microtubule. The net effect is that minus-end-directed movement (i.e. via dynein) predominates, and membrane-bound organelles and vesicles accumulate near the cell center. A current hypothesis is that in the initial stages of AD, some factor induces the upregulation and hence the synthesis of tau protein, and tau binds to axonal microtubules in increasing amounts. This has two effects: the axonal microtubules are stabilized and tau inhibits the function of kinesin; thus, organelles begin to clear from the axon, accumulating in the cell body. The cell responds by increasing kinase levels, tau becomes hyperphosphorylated and releases from microtubules, which then disassemble, the axon regresses, and hyperphosphorylated aggregates of tau form.

During antigen processing, proteases are required to convert large antigens into peptides that can bind class II major histocompatibility complex (MHC) molecules. There are several candidate proteases, among them cathepsins B, L and S (Cys proteases), and cathepsins D and E (Asp proteases). Colin Watts (University of Dundee) reported on a new protease, AEP (asparaginyl endopeptidase), a Cys protease that cleaves on the C-terminal side of asparaginyl residues. The protease can be inhibited

by the tetrapeptide AENK (but not by AEQK), and N-glycosylation of the key asparaginyl residue in the substrate prevents cleavage by AEP. The enzyme AEP appears to be involved in the processing of a microbial antigen (tetanus toxin C fragment), and thus it is hypothesized that pathogens may evade immune surveillance by selectively N-glycosylating key residues to protect them from digestion by AEP.

Listeria monocytogenes was the focus of two related presentations. Because inhibitors of several cell signaling pathways prevent infection, *Listeria* must employ some host cell functions during infection. Pascale Cossart (Institut Pasteur) reported on internalins (InlA and B), bacterial proteins that mediate infection by one of two pathways. InlA is a leucine-rich repeat (LRR) protein of ~800 residues that binds to the receptor for E-cadherin in human epithelial cells. Interestingly, murine cells can not be infected via the InlA-dependent route, even though the mouse E-cadherin is 80% similar to human E-cadherin. Pro₁₆ is the key residue in human E-cadherin: if Pro₁₆ is mutated, human cells are then resistant to infection. Conversely, if residue 16 in the mouse E-cadherin is mutated to Pro, then mouse cells expressing the mutant E-cadherin can be infected by *Listeria*. InlB, also an LRR protein, is smaller (~630 residues) and allows the bacterium to gain entry into a wide variety of tissue culture cells via an as yet unidentified receptor, although the mechanism is suspected to be tyrosine kinase based. After tyrosine phosphorylation of several proteins, PI-3 kinase is activated and even InlB alone can activate PI-3 kinase. Wortmannin, which inactivates PI-3 kinase, blocks bacterial infection in cell lines in which the bacterium utilizes the InlB pathway.

Once a cell has been infected, the bacterium generates motile force to move inside the cell by polymerizing a tail of actin filaments, and for this the bacterial ActA protein is required, as reviewed by Lisa Cameron (Stanford University). *Listeria* strains lacking ActA can not move, but is ActA the only protein required? This question was answered by showing that polystyrene beads alone, if coated only with ActA, could move when placed in an actin-rich extract. Small beads (<0.5 μ m in diameter) move when a localized concentration of ActA on the bead surface is generated randomly, while large beads (2 μ m) only move if a localized concentration of ActA on the bead is generated experimentally. These data show that, if given a randomly or specifically generated nucleation site containing the appropriate amount of ActA molecules, a bundle of polymerizing actin filaments can nucleate from this site and produce a force for movement of the bead or the bacterium. In addition, the data explain why ActA on *Listeria* is restricted to one pole of the cell. For large structures such as a bacterium or a 2 μ m bead, localization of ActA is required to generate a functional nucleation domain for the assembly of the actin bundle, which is necessary for intracellular motility.

Compounds that have specific effects on the actin-based cytoskeleton also affect membrane fusion. In particular, Gareth Griffiths (EMBL) reported the effect of various agents on the fusion of phagosomes with endosomes. Fusion requires the assembly of actin bundles onto the membrane, the phosphoinositide PIP₂ and the protein ezrin (or moesin). Apparently, the actin bundles nucleated

from one organelle provide a substrate upon which other organelles can move toward it for fusion. This implies, therefore, that the moving organelles have some form of myosin on their membranes to catalyze this actin-dependent movement. Indeed, in the data presented, actin was nucleated from a phagosome with the minus end distal to the membrane, the orientation required for a plus-end-directed myosin-like motor to translocate another membrane toward the phagosome. If the phagosomes were first stripped of peripheral membrane proteins by salt extraction, the resulting membranes could no longer nucleate actin. When recombinant ezrin (or moesin) was subsequently added, nucleation of actin occurred. Thus, although not required for the actual events of membrane fusion, an actomyosin system potentiates the process *in vitro* and suggests that a similar potentiation of membrane fusion by an actin-based system could function *in vivo* as well.

Summary comments

This meeting lacked a platform presentation addressing current hypotheses of Golgi stack maturation. In one such hypothesis, movement of proteins through the Golgi stacks is thought to occur via a process of cisternal maturation as opposed to an alternative, more widely accepted model which assumes that vesicle movement from stack to stack is responsible for Golgi maturation. One poster presentation, by Lidia Bonfanti and collaborators (Consorzio Mario Negri Sud), presented evidence supporting the cisternal maturation model, but because these data had already been published at the time of this meeting (*Cell*, **95**, 993–1003, 1998), they will not be reviewed here. Of the numerous other presentations in poster format, many were also notable. A poster presentation by Helen Nilsson (University of Goteborg) and coworkers, studying pigment granule translocation on microtubules, deserves comment. Pigment granules were isolated from *Fundulus melanophores* and microtubule asters were generated in *Spisula* oocyte extracts. The pigment granules were isolated from cells that were either in the aggregated state (a dynein-dependent direction) or the dispersed state (kinesin-dependent). When combined with microtubule asters, pigment granules isolated from dispersed melanophores distributed along the lengths of the microtubules or accumulated at the plus ends. Conversely, if aggregated cells were the source of the purified pigment granules, the granules bound to the microtubules and aggregated around the centrosome of the aster. This centripetal movement was ATP-dependent and could be blocked by the anti-dynein m74-1 monoclonal antibody. Thus, one can isolate a homogeneous population of membranes that are targeted for one end or the other of the microtubule. The system will be useful for individuals interested in how motor activity, and hence the directionality of membrane movement relative to the microtubule cytoskeleton, is controlled.

Space limitations prevented the inclusion of summaries of the other fine platform and poster presentations that greeted attendees of this exceptional meeting in Santa Maria Imbaro, Italy. This type of topically focused meeting, in relaxed confines, occurs too seldom in the fast-paced world of science as it is practiced these days. The interaction between two previously disparate fields of

investigation was productive and fulfilled the initial ideal of this summer series of research conferences when they were conceived a decade or more ago. As the representative from the Education Committee that oversees these meetings for the ASCB, I think it is very unfortunate that the ASCB, EMBO and the Dudley Wright Foundation have found it necessary to cease funding these highly productive, important and stimulating summer research conferences.

Acknowledgements

I thank Conly Rieder (Wadsworth Center, Albany, NY) for helpful suggestions that made the manuscript more accessible to the non-specialist, and the reviewers of this manuscript for their constructive comments. Work in the author's laboratory has for many years been funded by the NSF (currently MCB 97-27818) and this support is greatly appreciated.