Land of the Rising Chlamy
A Report of the 11th International Meeting on the Cell and Molecular Biology of *Chlamydomonas* held in Kobe, Japan, from May 11–15, 2004

The 11th International meeting on the Cell and Molecular Biology of *Chlamydomonas* was held at the International Conference Center on Port Island in Kobe, Japan, from May 11–15, 2004. This conference was superbly organized by Drs Yoshihiro Matsuda, Ritsu Kamiya and Hideya Fukuzawa and ran amazingly smoothly thanks in no small part to the efforts of many students who made a major contribution. A large number of excellent talks and posters were presented on a wide array of topics in *Chlamydomonas* biology and we apologize up-front to those colleagues whose work is not specifically mentioned in this brief report. In addition to the fantastic science, participants were also treated to wonderful food, record-breaking rainfall as well as excursions to see the ancient castle at Himeji or to learn the intricate art of sake brewing.

As is usual for *Chlamydomonas* meetings, the sharing of new methods and resources was the skeleton of the conference; the flesh was the exciting results that many participants brought along. This conference is quite unique in that by touching virtually every aspect of the biology of *Chlamydomonas*, it gives you a real sense of the organism. This is especially important for investigators who are new to *Chlamydomonas*: when you work with it, grow it, mate it for genetic analysis, freeze it, etc... whatever your subject of interest, you realize the complexity of its behavior, and the need to master every aspect of its biology. We dearly missed Lib Harris, detained by other duties. She manages the ChlamyDB database at Duke University (http://www.biology.duke.edu/chlamydb/), and knows more about *Chlamydomonas* than anyone.
else. But we heard from P. Lefebvre that the Culture Collection will be progressively transferred from Duke to Minnesota. The community will also have to cope with the upcoming departure of Chuck Hauser who has done an outstanding job at managing the *Chlamydomonas* Genome Project database (http://www.biology.duke.edu/chlamy_genome/).

Of course, because of the very diversity of the meeting, every participant knows that whatever his/her particular subject (except perhaps for flagellar motility/assembly; see below), there will be only a few communications that will deal with it specifically. A *Chlamydomonas* meeting is not where you will get a comprehensive picture of, say, chloroplast biogenesis, circadian rhythm, or nitrogen metabolism. Still, it is a must if you want to hear about the latest techniques in gene cloning, decide which RNAi strategy will work best for you, or start a collaboration in proteomics …

**Genes and Genetics, Genomes and Genomics**

As can be expected, the newly-released draft version of the *Chlamydomonas reinhardtii* nuclear genome has attracted much attention (http://genome.jgi-psf.org/ chlre2/chlre2.home.html). As Pete Lefebvre (University of Minnesota) reminded us during the final discussion, the Joint Genome Institute (JGI) has never received a specific *Chlamydomonas* grant, and we owe it to the insight of Dan Rokhsar that they chose to sequence this important (and, by the way, difficult) genome. Countless presentations demonstrated how the availability of the genome makes it so much easier to identify, clone, and mutate our genes of interest, and JGI was repeatedly thanked. It is now an important task for the *Chlamydomonas* community to complete the annotation of the genome. At an initial “jamboree” held at the JGI in December 2003, a team of annotaters had been trained to evaluate and annotate the gene models that have been generated by JGI. But the task is immense and more help is needed. During the meeting, several new annotaters were trained, and all the participants were invited to participate by communicating with the annotation team (www.chlamydomonas.info). Annotation needs soon will spread to *Volvox carteri*, which we learnt is also being sequenced by JGI.

Many thanks are due also to the Kazusa Institute, for not only sequencing a large number of *C. reinhardtii* ESTs, but also for distributing the cDNA clones. We heard from H. Fukuzawa (Kyoto University) that they have sequenced more ESTs, and will soon release an assembly of their own (http://www.kazusa.or.jp/en/plant/chlamy/EST/). It will certainly be a useful complement to the new “ACEGs” (Assembly of Contiguous ESTs based on Genome) that the *Chlamydomonas* Genome Project has put together (presented by O. Vallon). This assembly differs from the previous ones in that it makes use of the genome data to correct sequencing errors in the ESTs. Undoubtedly, EST assembly will not be sufficient to cover the transcriptome, and a full-length cDNA sequencing project will have to be launched.

In the meantime, the *Chlamydomonas* transcriptome is beginning to yield its secrets, not only in terms of sequence, but also of regulation. While Japanese labs use the Kazusa macroarray (10,368 cDNA inserts arrayed on a filter), US and European labs use the glass slide microarray produced at the Carnegie Institution (3,079 PCR products, 4 replicas, http://aracyc.stanford.edu/~jshrager/lab/chlamyarray/), as a way to identify genes induced or repressed under certain conditions. Most of these studies compare the behavior of wildtype and mutant strains affected in a regulatory gene. Among interesting results shown at the meeting, let us mention the elegant work presented by H. Fukuzawa, dissecting the regulatory network that governs the response to low CO$_2$. While CCM1 (Carbon Concentrating Mechanism 1) controls many genes directly, it also acts through a negative regulator HCRR, which is still to be cloned, that in turn controls multiple genes via the CCM2-LCR1 (Low CO$_2$-stress Response 1) cascade. Needless to say, light is also a major player in this regulation, as many of these genes are not induced in the dark. Jun Abe (Kobe University) and T. Oyamada (Okayama University of Science) presented their collections of NSG (Nitrogen Starvation Gene) and ZEG (Zygote Expressed Gene) genes, respectively. The 33 ZEG genes can be placed into 3 categories depending on the dependence of their induction upon the mating signal, actual cell fusion, or protein synthesis during zygote formation. Note that Japanese genomics does not limit itself to *Chlamydomonas*: H. Sekimoto (University of Tokyo) has described a wonderful study of *Closterium peracerosum-strigosum-littorale* complex, a heterothallic charophycean alga, combining biochemistry, EST sequencing and microarray analysis to identify not only the sex-specific pheromones of each mating type, but also many of the target genes (http://foj.c.u-tokyo.ac.jp/closterium/). Using the Stanford microarray, J. Moseley (Carnegie Institution, Stanford) has studied phosphorus starvation, showing that *PSR1* controls not only the genes involved in phosphorous metabolism, but...
also genes that belong to the general stress response (for example AOX1, PTOX1 coding for alternative oxidases). Beat Fisher (R. Eggen's lab, Swiss Federal Institute) has identified the glutathione peroxidase gene GPXH as the main gene induced by both type I and type II photosensitizers, most likely via production of $H_2O_2$. Chung-Soon Im’s (Carnegie Institution) study of blue light regulation of GSA (glutamate-1-semialdehyde aminotransferase) and LHC (light-harvesting complex) genes points to a role not only of blue light and phototropin, but also of an elusive red-light photoreceptor (no phytochrome has yet been found in the genome sequence). Interestingly, red light also influences the swimming pattern, as demonstrated by K. Josef in K. Foster’s lab (Syracuse University).

Careful gene-by-gene studies also were presented. For example Dick Sayre (Ohio State University) described FEA1, also known as the iron-binding protein H43. Using heterologous complementation in yeast, he showed that FEA1 is specific for Fe and probably acts like a siderophore. The mechanism of import is still enigmatic, but a nucleotide-binding domain may mediate iron translocation directly through the membrane. Angela Falciatore (University of Geneva) presented the curious case of the FLP gene, involved in regulation of the heme/chlorophyll biosynthetic pathway. It gives rise by alternative splicing to 2 FLU-Like Proteins differing by 12 residues. These proteins reside in the thylakoid membrane, and while both are induced by light treatment, expression of the longer form is transient. This form is the one that binds most strongly to Glu-tRNA reductase. Although both forms complement the Arabidopsis mutant, it is clear that Chlamydomonas has adapted this process for specific purposes. Remember that Chlamydomonas is able to synthesize chlorophyll in the dark, which most higher plants are too shy to do.

The response to sulfur deprivation also inspired several studies. A. Hemschemeier, from T. Happe’s lab (Ruhr Universität Bochum), showed that sulfur deprivation under microaerobic conditions leads to accumulation of formate, ethanol and acetate. Pyruvate formate lyase, pyruvate decarboxylase and a dual-function alcohol- and acetaldehyde-dehydrogenase are induced, together with the HydA gene for hydrogenase; thus, Chlamydomonas is really well equipped for fermentative processes, a feature well known to those working in the field of photosynthetic state transitions. But beware of the limitations of studies of gene transcription if you want to describe adaptive processes: K. Sugimoto (Tokyo University of Pharmacy and Life Sciences), from the lab of M. Tsuzuki, showed that sulfur-deprivation leads to an increase in transcription of SQD1 coding for sulfo-quinovose synthase, the first enzyme in the synthesis of sulfoquinovosyl diacylglycerol (SQDG). However, the rate of SQDG synthesis remains constant during starvation. Furthermore, the level of SQDG dropped sharply, as a result of increased degradation. Once again, Chlamydomonas almost tricked us …

Blue-light signaling and its main photoreceptor phototropin were the subject of many posters and presentations. Christoph Beck (University of Freiburg) showed that phototropin is partly localized in the flagellar axoneme. However, it is unclear how this relates to the known role of the photoreceptor in various stages of the cell cycle, or during chemotaxis towards ammonium (E. Ermilova, St. Petersburg University). Peter Hegemann (University of Regensburg) dissected for us the catalytic mechanism of the formation of the cysteine-adduct in the LOV1 domain of phototropin. Of course, his lab also continues to study green-light perception, mediated by the light-gated ion channels channelrhodopsins 1 and 2. While CHR1 is specific for protons, CHR2 supports large permeability to several cations. In addition, the chlamyopsin family continues to grow, with bacterial type COP5, 6 and 7 possibly involved in signal transduction via two-component systems.

In the thylakoid membrane, high light also has effects on the composition of the light harvesting proteins of photosystem II, and CP29 seems necessary for recovery after photoinhibition (J. Minagawa, Hokkaido University). Assembly and modulation of the light harvesting complex received much attention. Jon Nield (Imperial College London) showed 3D-reconstructions of the PSI and PSII supercomplexes with the antenna. Compared with pea PSI, the Chlamydomonas complex appears endowed with additional densities on the side opposite to that where higher plant LHCl binds: it is tempting to attribute these to the LHCa2 and 9 proteins that E. Stauber, from M. Hippler’s lab (Friedrich-Schiller University Jena; now University of Pennsylvania), see strongly depleted upon Fe-starvation. Under these conditions, a new N-terminally-truncated form of LHCa3 appears, possibly leading to a novel pattern of chlorophyll-binding and a lower antenna size. Interestingly the mature form of Lhca3 is the only Lhca protein that is completely degraded under iron-deficiency, whereas the other Lhca are affected to various degrees. Assembly of the PSI reaction center itself is an assisted process. PsaF is the major interaction partner of Ycf4, the chloroplast-encoded PSI assembly factor which Y. Takahashi (Okayama University) has engineered into a TAP-tagged version. In addition, PSI biogenesis requires
When it comes to modifying chloroplast genes, nobody beats *Chlamydomonas*, nobody. Dick Sayre used chloroplast transformation to study asymmetry in PSII electron transfer: a D1-L210H mutant, binding chlorophyll instead of pheophytin in the inactive branch, shows no charge separation, no P680 circulardichroism signal and increased antenna 77K fluorescence, suggesting a redistribution of excited state equilibrium between the chromophores. Surprisingly, M. Hamilton from P. Nixon’s lab (Imperial College London) showed that a H23C mutant in the α subunit of cytochrome b559 was still able to evolve oxygen: the haem is not necessary for PSII function, although it seems important for the PSII repair mechanism.

A great many presentations at the meeting made use of mass spectrometry, in particular MS-MS, to identify proteins in complex or purified preparations. Maria Mittag’s lab (Friedrich-Schiller University, Jena) thus isolated a complex of two RNA-binding proteins corresponding to their CHLAMY1 activity involved in circadian translational regulation of genes, as well as proteins with circadian oscillations. Mats Erickson’s laboratory (Umeå University) used more classical 2D gels to identify proteins modulated during iron deficiency. Full-fledged proteomics studies were presented, such as the dissection of the 58 proteins of chloroplast ribosomes, by K Yamaguchi (Nagasaki University). This includes several plastid-specific ribosomal proteins, among which is a new PSRP7 with two S1 domains. The labs of M. Hippler (University of Pennsylvania) and N. Dencher (Darmstadt University of Technology) are busy with thylakoid proteins. While the former has perfected the use of IEF-SDS 2D electrophoresis for the study of membrane proteins, the latter uses Blue native PAGE to study the complexes and supercomplexes of the membrane. The Hippler lab has developed a program, GenomicPeptide Finder, which allows de novo sequencing of peptides from MS/MS spectra and direct comparison with the genome sequence. The program has the potential to identify peptides that are split by an intron and thereby facilitates gene identification as well as gene annotation. But this year, the proteomics of *Chlamydomonas* trans-splicing of three chloroplast-encoded *psaA* transcripts, and M. Goldschmidt-Clermont (University of Geneva) presented new developments in their study of RAA1. Surprisingly, a mutant with a truncation in RAA1 is impaired in the splicing not only of exons 2 and 3, but also of exons 1 and 2. The protein presents a new type of 38-amino acid repeat, distinct from the classical TPR and PPR repeats, and is found in 2 complexes, one of which also contains RAA2.

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...easily, the sporangial autolysin does not belong to this family: it is a serine protease, released in the periplasm upon activation, but perhaps initially associated with the flagella. This localization would allow for a specific action on the interior of the sporangial wall, thus avoiding degradation of the daughter cell wall.

With more than 40 members, there is no shortage of DnaJ domain proteins, the co-chaperones of the HSP70 system. Michael Schroda (University of Freiburg) is going after those of the chloroplast, and one of them, CDJ2, is found to interact with VIPP1, a multimeric protein shuttle between the thylakoid and the envelope. HSP70B is probably involved in the functional cycle of this protein which may have a role in lipid transfer or in protein translocation through the thylakoid. A DnaJ-like protein (RSP16) has also turned up in the flagellum as an integral part of the radial spokes (Pinfen Yang, Marquette University), suggesting an involvement in both assembly and maintenance of this unusually stable complex.

In Volvox carteri, GlsA, an important regulator of development as it governs the pattern of asymmetric division in the embryo, is also a DnaJ protein. Steve Miller (University of Maryland Baltimore County) showed that it pulls down the cytosolic HSP70A, which happens to be the only protein thus far found to be asymmetrically located in the embryo; at the 32-cell stage, it is more abundant in the anterior 16 blastomeres, which undergo asymmetric division. GlsA also contains SANT domains which may carry out deacetylation of tubulin or histones. However, inhibitors of tubulin deacetylation do not mimic the gIsA mutation. Another famous Volvox gene is invA, involved in inversion of the embryo. It was shown by Ichiro Nishii (Riken Institute) to be a new member of the kinesin family, dragging the cell through the cytoplasmic bridges at inversion. Its homolog IAR1 rescues the function, showing that the unicellular Chlamydomonas is preadapted to multicellularity.

Is Chlamydomonas reinhardtii the ancestor of Volvox carteri, or is it the reverse? Neither, would reply T. Pröschold (University of Cologne), but they definitely are so close that they should be brought together into the same genus. He scared the audience to death when he described how the rules of taxonomy could lead to renaming Chlamydomonas reinhardtii to something like Chlamydomonas pulvisculus or Oogonium reinhardtii. Fortunately, there is an alternative, to use reinhardtii as the type species for the genus Chlamydomonas. This of course would drive C. moewusii to another genus, like Chlorococcum, in accordance with the enormous evolutionary distance long recognized between the two classical strains of Chlamydomonology. That night, after many beers at the bar "L’Estacade" on the 2nd floor of the Portopia hotel, some of us managed to convince Pröschold that a renaming was in order, and the name Estacadiella draftii was coined for our favorite organism, in honor of the liquid that was feeding our inspiration. It is to be feared that this decision will not have survived the dissipation of the morning mist ...

Mutants, mutants, mutants: Chlamydomonas people never have enough. RNAi, initially introduced by M. Fuhrmann (University of Regensburg), is now routine, at least in certain labs. Susan Dutcher used it to silence some 29 genes, generally using constructs with the first coding exon and following intron. But silencing of the RNAi transgene itself is sometimes a problem, as exemplified by K. F. Lechtreck (University of Cologne) in an elegant study of centrin: when the construct was placed under the nitrate inducible promoter of NIA1, its expression was invariably followed by its silencing, never to be recovered even when the promoter was switched off. Karin van Dijk may have the solution to that problem. She presented the new tools generated by the Cerutti laboratory (University of Nebraska), in particular an improvement of the RNAi technology that can be described as "double RNAi": an inverted repeat of a MAA7 fragment (coding for tryptophan synthase (j) is placed in tandem with one for the target gene. Continuous application of 5-fluoroorindole selection insures that MAA7 is still silenced, hence also the target gene. Seven genes have been downregulated with this strategy, and the vector is already circulating among Chlamydomonas laboratories. In their study of gene silencing, they also use a two-hybrid library, and a new selectable TAP-tag where ble (encoding resistance to zeocin) is sandwiched between the tag and the target. This vector has been used to isolate a MUT11-complex with histone methyltransferase activity.

With the entry into the genomics era, new approaches can be considered for direct and reverse genetics. Insertional tagging remains the method of choice for cloning genes on the basis of phenotype. Emilio Fernandez (University of Cordoba) described a "small" library of 22,000 mutants tagged with the AphVIII gene, which confers paromycin resistance; from these he has picked –150 with defects in nitrogen metabolism and regulation (using a NIA1-ARS construct as a sensor of nitrogen signalling) for sequencing of flanking regions via a modified TAIL-PCR. Could this evolve into a resource for the community, with systematic sequencing of FSTs (Flanking Sequence Tags)? For point mutations, though,
you may have to turn to the TILLING project that M. Kobayashi (University of California Berkeley) described for us. A consortium of laboratories has teamed up with the *Arabidopsis* Tilling Project in Seattle (http://tilling.fhcrc.org:9366/) for a pilot screen with promising results. The aim is to screen a heavily-mutated population for a mutation in your gene of interest, via PCR and Cel-l detection of mismatches. Ah, if only, if only ... If only gene targeting was possible, *Chlamydomonas* would finally live up to its nickname of “Green Yeast” (Goodenough 1992). We know that it occurs in the cell, no one would survive without it. But it has proved extremely difficult to harness for site-directed mutagenesis, because the frequency of homologous recombination is extremely low compared to that of random insertions. At this meeting, B. Zorin from P. Hege mann’s lab showed a dramatic increase in the ratio of homologous to non-homologous recombination, by using single-stranded DNA. For the moment, it is only the classical positive selection strategy: starting with a transformant carrying a truncated non functional *aphVIII(Δ)* transgene, you restore resistance to paromomycin by transformation with a plasmid carrying the missing *aphVIII* fragment. But with a 50/10 ratio of homologous/heterologous recombinants, hopes are rising ... Will B. Zorin be the Pure of Heart that brings us the Holy Grail?

**Flagella and Basal Bodies**

In Kobe, it again became clear that *Chlamydomonas* is the premier model organism for analysis of flagellar assembly and motility. The advantages of this system include easy classical/molecular genetics, a sequenced genome, the ability to purify biochemically useful amounts of integral flagellar components, and the availability of multiple methods for measuring flagellar beat parameters. In addition as mentioned above, this was the meeting where the *Chlamydomonas* flagellum joined the proteomics revolution, and George Witman (University of Massachusetts Medical School) described the progress made by his laboratory in defining most of the components of the flagellum. In her presentation, Susan Dutcher (Washington University) described the use of comparative genomics to identify proteins shared by *Chlamydomonas* and humans but not by *Arabidopsis*, which lacks both flagella and basal bodies. Dutcher also continued this analysis by using RNAi to reduce expression of a subset of these proteins not upregulated following deflagellation as candidates for basal body components. These analyses and data presented in a poster by Eric Johnson (Witman lab) identified a series of *Chlamydomonas* flagellar and basal body polypeptides that are homologues of proteins involved in causing a wide array of human symptoms including Bardet-Biedl syndrome, kidney dysfunction, developmental abnormalities, hydrocephaly and retinal degeneration. These observations further reinforce the importance of studying basic biology in *Chlamydomonas*, as the findings can be of direct relevance to understanding of human disease states.

Starting at the basal body, three presentations from Susan Dutcher (Washington University), Carolyn Silflow (University of Minnesota) and Masafumi Hirono (University of Tokyo) described the molecular characterization of novel components of this enigmatic organelle that are required for assembly of flagella. Mutations in these genes result in a partial or complete flagella-less or “bald” phenotype. Karl-Ferdinand Lechtrekk (University of Cologne) described the use of GFP-tagged proteins to probe the dynamics of striated fibers associated with the basal bodies. Near the flagellar transition zone, Moe Mahjoub and Lynne Quarmby (Simon Fraser University) reported on the localization of Fa2p that is required for flagellar autotomy. Intriguingly, they observed that Fa2p is present at the base of the excised flagellum (i.e. on the axonemal side of the excision machinery) and also identified a weak signal associated with the basal body region, suggesting that it may also be associated with that organelle.

In the conference keynote address, we heard from Pete Lefebvre (University of Minnesota) about the progress being made in his lab to define the molecular mechanisms involved in the control of flagellar length. Four mutant loci that result in a long flagella phenotype have been identified, and Nedra Wilson from the Lefebvre lab described her analysis of LF4p which is a MAP kinase localized in the flagellum. Intriguingly, other LF proteins are located in the cytoplasm, and it will be fascinating to work out how they control flagellar length and perhaps also aspects of intraflagellar transport (IFT). Junmin Pan from the Snell lab (University of Texas Southwestern Medical Center) described an aurora kinase that appears to function in signaling flagellar disassembly.

The IFT phenomenon, which was first identified in *Chlamydomonas* in 1993 by the Rosenbaum group, continues to be a really hot topic. This process is required for protein assembly at the distal flagellar tip and involves both antero- and retrograde transport along doublet microtubules powered by heterotrimeric kinesin and the 1b cytoplasmic dynein isoform. At the meeting, we heard in talks from Doug Cole (University of Idaho) and Michael Chapman (Witman lab, University of Massachusetts Medical...
School, and also in several poster presentations, about the roles of various IFT particle components in this fascinating process. For example, Chapman demonstrated that the IFT20 protein is required for correct assembly of the flagellar transition zone and may also function in membrane protein trafficking. Ben Lucker and Doug Cole presented a poster describing the progress being made in defining the molecular architecture of the particles that are trafficked along the flagellum, and with Ursula Goodenough (Washington University) also showed that IFT proteins become redistributed during gametogenesis.

Significant progress is also being made in defining the molecular composition of integral axonemal structures required for intraflagellar signaling. For example, Matthew Wargo from Elizabeth Smith’s laboratory (Dartmouth College) presented evidence for a series of calmodulin-binding proteins within the central pair microtubule complex. As this structure is thought to initiate Ca\(^{2+}\)-mediated control of waveform, this study may lead to the key players in initiating the signaling cascade. We learnt in a poster from Beth Mitchell (LeMoyne College) and colleagues that the central pair complex anchors enzymes involved in energy metabolism including both glycolysis and nucleotide phosphotransfer reactions. They suggest that this structure acts as a scaffold for a flagellar ATP-generating system. Furthermore, in a technical tour-de-force, David Mitchell (Upstate Medical Center, NY) showed electron microscopic studies that revealed the twist of the central pair microtubules and defined their orientation with respect to the plane of flagellar bending. Mitchell demonstrated that the radials spokes are not required for twisting to occur and indeed that central pair rotation is likely driven by bend propagation.

Much progress was also reported on composition and assembly of the radial spokes that act as a mechanochemical transducer of signals from the central pair complex and result in regulation of inner and outer arm dynein function. For example, it previously had been reported that Radial Spoke Protein 3 (RSP3), which is located at the base of the radial spoke, is a protein kinase A (PKA) anchoring protein (AKAP) in vitro. Winfield Sale (Emory University School of Medicine) now reports that site-directed mutagenesis of the PKA-binding domain of RSP3 results in an immotile phenotype in about one-half of the mutated cells, suggesting that mislocalized and/or unregulated PKA impairs flagellar motility. Perhaps related to this, Pinfen Yang reported that RSP11, encoded at the pf25 locus, contains an RIIa domain similar to that through which the PKA regulatory subunit (RIIa) binds to AKAPs. This raises the intriguing question of whether RSP3 and RSP11 interact via the PKA-binding domain of the former and the RIIa-binding domain of the latter. Nucleotide metabolism and calmodulin-binding also arose in radial spokes when Stephen King (University of Connecticut Health Center) described an integral calmodulin-regulated nucleoside diphosphate kinase within the spoke stalk. Thus a recurring theme in this meeting was the identification of proteins potentially controlled in a Ca\(^{2+}\)/calmodulin dependent manner and which consequently (based on the Ca\(^{2+}\)-binding affinity of calmodulin) are likely involved in Ca\(^{2+}\)-dependent waveform conversion. A poster from Dennis Diener and Joel Rosenbaum (Yale University) described how radial spoke assembly begins in the cell body and how subcomplexes are transported by IFT before final assembly occurs within the flagellum itself. Takahiro Kohno from the Kamiya lab demonstrated that electroporation with recombinant proteins can be used to rescue radial spoke defects. It was also very gratifying to see that the *Chlamydomonas* meeting now attracts scientists working in organisms other than green algae, and a poster was presented by Yukoh Satoh and Kazuo Inaba (Shimoda Marine Research Center, University of Tsukuba) on radial spoke components within sperm flagella from the chordate *Ciona* and the identification of homologues in *Chlamydomonas*.

Intriguing new information on the inner and outer dynein arm motors was also presented in Kobe. For example, Haru-aki Yanagisawa and Ritsu Kamiya (University of Tokyo) reported the identification of the long-sought *Chlamydomonas* tektin. Surprisingly, this *Chlamydomonas* protein was not present in the Sarkosyl-resistant protofilament ribbons that derive from axonemal doublet microtubules as it is in other organisms. Rather, they found that *Chlamydomonas* tektin acts as the attachment site for specific inner dynein arms. In another poster presented by Yumi Watanabe from the Kamiya lab, we learnt from incorporation of fluorescent actin by electroporation and subsequent bleaching that this inner arm component undergoes turnover even in the absence of treadmilling. Triscia Hendrikson from the Sale lab (Emory University School of Medicine) presented a poster characterizing the IC138 intermediate chain that is involved in the phosphoregulation of inner arm 11. An intriguing poster presented by Hitoshi Sakakibara (Kansai Advanced Research Center) provided evidence that the conformation of the two motor units in this dynein is altered following phosphorylation. Two posters presented by Toshiki Yagi (Kamiya lab, University of Tokyo) and Kazuhiro Oiwa (Kansai Advanced Research Center) focused on the
inner arm dynein c which is a single headed motor that moves in a processive manner along microtubules and is located at the base of the S2 radial spoke.

Within the outer dynein arm system, Maureen Wirschell and George Witman (University of Massachusetts Medical School) described a novel complex defined by the products of the ODA5 and ODA10 genes that anchors a flagellar adenylate kinase near the outer arm, ensuring that both high-energy phosphate bonds of ATP are readily available to the dynein motor. Ken-ichi Wakabayashi (University of Connecticut Health Center) described the use of sulfhydryl modification and 2-D electrophoresis to analyze the changes in redox state that occur within one component of the outer arm docking complex. Linda DiBella (University of Connecticut Health Center) reported on two light chains that are present in inner and outer dynein arms and which interact with components required for both assembly and regulation.

Exciting new advances and observations were reported on analysis of flagellar beating. In two presentations from Ken Foster’s laboratory (Syracuse University), we heard about a new microscope system that allows the beat parameters of individual flagella to be monitored for extended periods of time. This system has great potential to investigate the precise changes that occur under various environmental conditions or in different mutant backgrounds. Kenjiro Yoshimura (University of Tsukuba) reminded us that Chlamydomonas exhibits gravitactic behavior that likely requires a signal transduction system, and Noriko Ueki from the Kamiya lab (University of Tokyo) described a new mutant that failed to show Ca2+-dependent flagellar dominance. She demonstrated that neither this phenomenon nor inner arm I1 – previously implicated in waveform control – are absolutely required for phototactic behavior, indicating that there is an alternate mechanism and structure for generating phototaxis. A poster from the Kamiya lab presented by Susumu Aoyama revealed that outer doublet microtubules in splayed axonemes undergo a cyclic association/dissociation. Intriguingly, dissociation did not require bending of the outer doublet pair, leading them to propose that curvature does not control dynein-microtubule interactions.

In conclusion, the Kobe meeting will be remembered in Chlamydomonas history not just for the exotic locale and superb organization, but also for the fantastic science presented that clearly keeps the green yeast at the forefront of research in many very different fields. As a model organism, it should attract those demanding simple biochemistry and easy genetic analysis in the same packaging, plus direct relevance to “higher” organisms. Despite the old saying that “the grass is always greener on the other side …”, in the case of Chlamydomonas it is just as green as it needs to be, and what’s more it moves!!! Are you ready for the Invasion of the Swimming Plants?

References


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