EMBO Gold Medal Review 2003

Boveri revisited

Anthony A Hyman*

MPI für Molekulare Zellbiologie und Genetik, MPI-CBG, Dresden, Germany

In memoriam Laura Alice Hyman

The EMBO Journal (2005) 24, 1104–1110. doi:10.1038/sj.emboj.7600583; Published online 10 March 2005
Subject Categories: cell & tissue architecture; cell cycle
Keywords: C. elegans; kinetochore; microtubules; mitosis; RNA interference

When I was about 14 years old, I conceived the idea that I should become a doctor. Partially I think this was because the only other option offered by my school (William Ellis) career advisor was to join the Navy. As my school career progressed, it became increasingly clear that I did not have the academic ability necessary to become a doctor, finally culminating in such a bad set of A levels that I left school without much idea of what to do. Fortunately one of my neighbors was Peter Medawar, who suggested that I start to work at UCL in London. (Interestingly, Peter Medawar had also encouraged Freeman (2002) early in his career). For this I received the princely sum of 30 pounds sterling per week to make up the media for tissue culture. After a few months Terry Preston suggested my first ever experiment, to look at the effects of ionic strength on the rate of movement of a protozoan. I trace my decision to be a scientist back to the following incident. Every Friday evening the technicians would go out for a beer. One Friday evening I was looking down the microscope. As they departed for the pub I looked at the microscope looked at them and then stayed to finish the experiment. Clearly this must have made an impression on Terry, because he suggested that I apply as an undergraduate.

It was a great stroke of luck that I ended up at the Department of Zoology at UCL, which was at that point chaired by Ave Mitchison. This was the department of Abercrombie, and his appreciation of cell biology still infiltrated the place (Abercrombie et al., 1970). My interest was captivated. Here were small machines such as the protein translocators microtubules and actin filaments, but no one knew how they worked. How did a protein get across a membrane? How did a cell move? The most important single piece of education I received was a 100-page literature review I wrote on the T-cell receptor. This was before the receptor had been identified, and involved sorting through hundreds of papers supporting different models on antigen recognition in the context of the MHC, an idea proposed by Zinkernagel and Doherty. I was supervised in this task by Nick Crispe, a graduate student, who spent many months with me discussing the issues. Nick more than anyone else helped me to understand the excitement of science. I would wait anxiously every month for the next issue of JEM, hoping for some answers to questions that we would discuss.

My father, who had suffered from a number of idiotic managers in industry, encouraged me to think of an academic career. That summer I went to the US to work at the Roche institute at Nutley, NJ, now sadly departed, with Jim Morgan. Roche ran an undergraduate student program, which was excellent, and introduced me to bench work. At the end of my stay, Jim suggested that I should do my PhD with someone called Sulston at the LMB on Caenorhabditis elegans, a name and an organism and an institution I had never heard of. Luckily the first edition of molecular biology of the cell had just come out, with a section on C. elegans. PhD entrance in those days was a matter of a phone call, which I duly made to John. His only question to me was 'why C. elegans', an exam I passed by nervously answering that the lineage was invariant. When I came up to Cambridge I had to find a subject for my PhD. Little did I realize how little my cell biology undergraduate education had prepared me for the LMB. Surrounded by molecular biology, start and stop codons about which I only had a vague understanding, I ended up in John White’s office. He talked to me about the cytoskeleton and polarity; finally I felt a sense of familiarity and asked to work on the project. John explained to me that polarity was interesting and that I should ‘look into it’. John practiced the old English tradition of ‘corridor supervision’. How is it going, he would ask when passing in the corridor? Fine I would say, to which he would reply: ‘Good, carry on then’, a phrase my students will recognize. After a couple of months in the lab, John called me down to his office and told me that the postdocs (Andy Fire, Jim Priess and Cynthia Kenyon) had suggested that I be thrown out. When I enquired why, he replied they thought I was too lazy. Well this came as somewhat of a surprise to me since I had been working 9–7, 5–6 days a week, which I assumed to be adequate. It was true that I was getting little done but I blamed that on my inexperience. John said he would support me but suggested I stay in the lab until the postdocs left. I assumed they had been going home at say 8.00. So the next evening, equipped with my sandwiches, I decided to stay on but as 8.00 disappeared into 9 o’clock and midnight approached there seemed to be no sign of going home. I staggered out of the lab at one in the morning, leaving Cynthia and Jim still talking about C. elegans development, realizing that science would be difficult.

After a few months of working on an obscure problem in a corner of the LMB, I went to the University library and

*Corresponding author. MPI für Molekulare Zellbiologie und Genetik, MPI-CBG, Plattenhauer Strasse 108, Dresden 01307, Germany. Tel.: +49 351 210 1700; E-mail: hyman@mpicbg.de

Received: 15 October 2004; accepted: 21 January 2005; published online: 10 March 2005
Jim Priess was studying the cytoskeleton in embryonic blastomeres. Centrosomes were defined by the position of the mitotic spindle (Rappaport and Rappaport, 1974). It was thought of any cell are determined by the position of the mitotic spindle on the same axis as its mother (Hyman, 1989). From John I learned the necessity of looking for an under-studied problem and developing and applying suitable technology to its solution, a perfect introduction to science. In fact, as part of the tearoom atmosphere of the LMB, much of the discussions were devoted to technology development and how to apply it to one’s problem. It was a privilege to do a PhD at the LMB. There were few students and although you were nominally associated with a lab, you were completely free to do as you wanted as long as you submitted your thesis within 3 years of starting.

We were interested in understanding how the cell induced rotation of the centrosome-nuclear complex in the P blastomeres. Using laser ablation, we were able to map the sites of force generation to the interaction between the centrosome and a region of the cortex that lay next to the contact of the two blastomeres. Since the process was microtubule dependent, it seemed logical to conclude that interaction between centrosome-nucleated microtubules and the cortex somehow drove the rotation process (Hyman, 1989). Our knowledge of microtubules at that time made it difficult to understand how microtubules from only one centrosome could interact with the cortical site and how this capture could lead to rotation. They were seen as rather dull structures that grew and shrank at a leisurely pace. During my PhD, Tim Mitchison and Marc Kirshner published their classic papers on dynamic instability of microtubules (Mitchison and Kirschner, 1984). In essence, dynamic instability showed two things. Firstly, microtubules could interconvert between phases of slow growth and rapid shrinking, driven by GTP hydrolysis. As a consequence, different microtubules could grow and shrink in the same population. Secondly, the transition between growing and shrinking is stochastic. You can estimate that a given microtubule has, for instance, a 50% chance of interconverting between growing and shrinking, but never predict exactly when this event would happen. Mitchison and Kirschner went on to develop a search and capture model for how centrosomes could attach to a chromosome during mitosis (Kirschner and Mitchison, 1986). Microtubules would grow out from a centrosome. If they attached to a chromosome, they would be ‘captured’. If not they would undergo catastrophe and shrink back to the centrosome. I remember what a bomb shell that was; here for the first time was a mechanism by which cells could form cytoskeletal structures. We applied these ideas to form a model of centrosome–nucleus rotation in C. elegans blastomeres. Microtubules would grow from each centrosome under dynamic instability conditions until a microtubule would be captured by the cortical site. If the cortical site contained a force-generating molecule, the microtubule would allow torque to be generated between the cortical site and the centrosome, rotating the centrosome-nuclear complex (Figure 2).

The next step in my career was to think of a postdoctoral position. Although I had greatly enjoyed C. elegans cytology, I wanted to train in aspects of biochemistry. In particular I felt that the next stage in analyzing complex in vivo problems was to isolate them into subproblems using in vitro assays. Mitchison and Kirshner had established in vitro assays to look at the interaction between kinetochores and microtubules. Kinetochores are those structures on mitotic chromo-

---

**Figure 1** A C. elegans embryo going through the first cell division. Tubulin is in green, DNA in blue.
some structures: a kinetochore would be similar to a cortical site. (B) A microtubule at random is captured by the cortical site. The interaction of the microtubule with the cortical site generates torque, either through motor proteins or through depolymerization-coupled movement. This reorients the centrosome-nuclear complex.

Figure 2 A potential mechanism to reorientate the centrosomes in the P1 blastomere of the *C. elegans* embryo. (A) Microtubules nucleated from both centrosomes are undergoing dynamic instability. A cortical site that can capture microtubule is built on the cortex. (B) A microtubule at random is captured by the cortical site. The interaction of the microtubule with the cortical site generates torque, either through motor proteins or through depolymerization-coupled movement. This reorients the centrosome-nuclear complex.

The interaction of the microtubule with the cortical site generates torque, either through motor proteins or through depolymerization-coupled movement. This reorients the centrosome-nuclear complex.

The interaction of the microtubule with the cortical site generates torque, either through motor proteins or through depolymerization-coupled movement. This reorients the centrosome-nuclear complex.

...to the interaction between microtubules and kinetochores. In particular, we wanted to record these interactions using the nascent field of time-lapse video microscopy. By isolating chromosomes from cells, and adding rhodamine-labeled microtubules stabilized with taxol, we were able to watch kinetochore capture of these microtubules (Hyman and Mitchison, 1991). Addition of ATP triggered these microtubules to move along the kinetochore, demonstrating the presence of active microtubule-based motors on kinetochores. Microtubules have a structural polarity with a fast-growing plus end and a slow-growing minus end. In *vivo*, the plus ends are located at kinetochores and the minus ends are located at centrosomes. Microtubule-based motors were known to move with different polarities: some are minus-end directed while others are plus-end directed. Therefore, a minus-end-directed motor and a kinetochore would move a chromosome toward the spindle pole. We set up means of looking at microtubule polarity in our *in vitro* assays and from these methods we were able to show that microtubule-based movement on kinetochores could either be plus- or minus-end directed. This movement was determined by the phosphorylation state of the kinetochores. Thus we demonstrated that, in principle, a kinetochore contained the necessary motors to determine its own position on a mitotic spindle (Figure 3). At the same time, together with a student Lisa Belmont, we set up assays to look at microtubule dynamics, both *in vitro* and *in Xenopus* egg extracts (Belmont *et al*., 1990). From these experiments, we showed that the dynamics of microtubule ends in *Xenopus* was modulated during the transition from interphase to mitosis, providing a powerful assay with which to dissect out the proteins required for microtubule dynamics in *vivo*. Just as I was finishing in Tim’s lab, I started to work on kinetochore assembly in extracts from *Saccharomyces cerevisiae*, together with John Carbon’s laboratory (Hyman *et al*., 1992). The power of yeast genetics suggested that it should be possible to identify the factors responsible for kinetochore binding to microtubules and was the beginning of my re-entry into genetic analysis, which I had left with the *C. elegans* project.

If I look back at my training, I was lucky to be at LMB and UCSF. Both had superb intellectual atmospheres, but were interested in different problems. UCSF believed in quantitative cell biology in the same way LMB believed in molecular genetics. My research program has always been based on a fusion of the ideas that came from these two different viewpoints.

After 4 years at UCSF, I was going back to the UK for Christmas when Tim suggested that I go and visit Kai Simons, who ran the cell biology program at the EMBL in Heidelberg. While I was there, I met Eric Karsenti, who was doing pioneering work on mechanisms of meiotic spindle assembly (Karsenti *et al*., 1984). Kai Simons offered me a job, and at that time the NIH was going through one of its bouts of funding crisis. It seemed too good an opportunity to turn down and off I went to Heidelberg. The way in which a lab gets started depends entirely on the people who are prepared to put their trust in a young untired investigator of (in my case) 29 years. I was fortunate to have a great starting lab: my postdocs, Michael Glotzer from San Francisco, Rita Taba from Vienna, Fedor Severin from Moscow, my technician Tony Ashford from London, and my two French students, Regis Tournebize and Ingrid Sasoos. In addition, I was joined by Peter Sorger while he set up his own lab at MIT. Peter and I had been students at the LMB and postdocs at UCSF together, and had decided to attack the problem of kinetochore assembly in *S. cerevisiae* together. That year the weather was...
perfect. I used to walk from the lab to Eric Karsenti’s house, where he and his partner Caterine made me very welcome for supper: we would discuss spindle assembly and play the saxophone late into the night. He also taught me how to make foie gras. The key issue is the rate at which the water boils.

While he was in Marc Kirschner’s lab, Eric and John Newport had shown that in meiotic oocytes, spindles could apparently assemble around naked DNA that had been injected into the egg (Karsenti et al., 1984). This suggested that meiotic spindles did not require centrosomes in order to be bipolar. This was at odds with the prevailing view that the bipolarity of a spindle could only be determined by its two centrosomes. We thought that this would be a good starting point to pursue issues of cytoskeletal organization.

In a classic piece of EMBL collaboration, Rebecca Heald showed that chromatin beads, developed in Peter Becker’s lab, when added to Xenopus extracts made beautiful bipolar spindles, in the absence of centrosomes (Heald et al., 1996). This suggested that a driving force for spindle assembly in meiosis was the self-organization of the cytoskeleton by microtubule-based motors (see Figure 4) Karsenti and Vernos, 2001). Therefore there seem to be two possible mechanisms for spindle bipolarity. One mechanism does without centrosomes, as seen in meiotic spindles and plants. The other mechanism uses both centrosomes. In the future, it will be interesting to determine the extent to which these two assembly pathways are used in different systems (Heald et al, 1997). Soon after arriving at EMBL, I started to work on control of microtubule dynamics, a problem that has occupied my laboratory since then. Using Xenopus extracts to study microtubule dynamics, Regis Tournebize showed that microtubule stability depends on the opposed interaction of stabilizing and destabilizing factors (Figure 5). The stabilizing factors are part of a conserved family of proteins of which the first member was XMAP-215. We were able to take this to the next stage and reconstitute the dynamic behavior of
microtubules in vitro using proteins expressed in baculovirus (Kinoshita et al., 2001). We also investigated how GTP hydrolysis destabilizes microtubules. Working together with Dick Wade from Grenoble, and Thomas Mueller-Reichert, Denis Chretien and Isabelle Arnal in Heidelberg, we showed that GTP hydrolysis increases the curvature of the protofilaments, thus destabilizing the microtubule lattice (Hyman et al., 1995; Muller-Reichert et al., 1998).

If you had asked anyone what the major problem in cell division was even 5 years ago, most people would have told you it was identification of the molecules required for building the cell division machinery. This had posed innumerable difficulties. Biochemistry was hampered by the fact that most cell division organelles are small in number (1–2 centrosomes per cell, for instance) and their structure was cell cycle dependent. In contrast, a ribosome is extremely abundant, and invariant in its structure throughout the cell cycle. Genetics was hampered by the fact that most mutants in cell division proteins are cell lethal. Thus, although we had many beautiful assays for mitosis, both in vivo and in vitro, we were having a hard time in the field reducing this to molecular understanding.

Just as I had left UCSF, Peter Sorger and I had developed assays to study yeast kinetochore assembly using the yeast S. cerevisiae. Kim Nasmyth was just round the corner in Vienna, and over the years taught me the basics of analysis of cell physiology using genetics. Our work with Fedor Severin on spindle assembly in yeast (Severin et al., 2001) suggested to me that similar methods could be brought to attack the problem of cell division in C. elegans. At that point, Pierre Gönçzy joined my lab, and was keen on setting up C. elegans as a system to study spindle positioning and microtubule dynamics. I had not worked on C. elegans for 7 years and my initial enthusiasm paled as Pierre looked over my shoulder as I tried to remember how to move worms around and showed a complete blank when discussing this and that cross. So in order to get started, we called up Heinke and Ralph Schnabel, with whom I had been together at the LMB, and asked them about getting hold of some wild-type worms, and help with culture conditions. To my surprise, they offered us a complete mutant collection on chromosome III, beautifully mapped and characterized. Pierre then proceeded to screen all of these mutants and showed that when analyzed in large numbers, it was possible to define certain phenotypic classes of mutants in different cell division processes. He was able to define particular mutants that affected spindle position (Gönçzy et al., 1999, #14).

We also came back to the work of my PhD to study the biophysics of spindle positioning, working at the one-cell stage. How does a spindle move eccentrically within the cell prior to division? This eccentric movement sets up an asymmetrically placed cleavage furrow, thus defining an unequal cleavage in the cell. At this point, Stephan Grill joined the lab as a joint student with Ernst Stelzer’s lab. By applying techniques for laser ablation developed by Aist and Berns in the 1980s (Aist and Berns, 1981), we demonstrated that the force is greater on the posterior than the anterior spindle pole and that the PAR proteins determine this asymmetry (Figure 4). By collaboration with Joe Howard, we used biophysical methods to demonstrate more precisely that this was due to a two-fold increase in the number of force generation elements on the posterior of the cortex (Grill et al., 2003) (Figure 6).

However, the difficulty of cloning the genes meant that very few new molecules were coming on line. At this time, two...
things changed in my scientific life. First, Kai Simons proposed that together with Wieland Huttner and Marino Zerial, we should build a new Max Planck institute for molecular cell biology in Dresden, part of the old DDR. Joe Howard from Seattle and our scientific administrator Ivan Baines, from NIH, later joined us and were crucial for this endeavor (Huttner, 2001). Moving to Dresden was a hard decision, since I was very happy at EMBL. However, I was intrigued by the challenge of building something new. Furthermore, I thought it would be interesting to build, in one institute, facilities that would allow all the tools of modern molecular cell biology to bear on the problems of cell division. In fact building the new institute, which opened in the beginning of 2001, has been a great experience and I especially enjoyed being part of a team focused on a common goal. The second thing that changed in my scientific life was the discovery of RNA interference (RNAi) by Andy Fire (one of the postdocs who had despaired of me in Cambridge) and Craig Mello (Fire et al., 1998). It became immediately clear that one could use RNAi for genome-wide screening for genes required for cell division. C. elegans had a number of advantages for such a screen. Firstly the first cell division can easily be followed by simple transmitted light. Secondly, the structure of the gonad meant that one could ameliorate the problem of protein stability (see Figure 7). Thirdly, at that time, it was one of the few organisms with a sequenced genome.

Taking account of these advantages, Pierre Gönczy and Chris Echeverri initiated a genome-wide screen for cell division proteins (Gönczy et al., 2000). Karen Oegema, Arshad Desai and my technicians Sonja Rybina and Matthew Kirkham, together with my student Eva Hannak, developed a set of assays that have allowed for the first time a genome-wide analysis of the assembly of key mitotic organelles such as centrosomes and kinetochores (Desai et al., 2003; Kirkham et al., 2003). What have we learned from genome-wide screening? One of the key issues of course is that we have bypassed the laborious step of cloning all the genes. However, I think one obtains one extra level of understanding by looking at a genome-wide data set. In conventional genetics, you clone a mutant, giving you one piece of a puzzle. But how big is the puzzle? If it is 10 pieces, you are well along the way. If it is 100 pieces, you have a long way to go. Genome-wide screens describe the rough size of the puzzle. This allows one to design experiments based on the complexity of the problem. There is indeed a big change in biology taking place using high-throughput screens, because it allows genetics to take place on a scale that was previously not possible. This I call ‘the industrial revolution of genetics’. Similar to the way that the movement of production from a cottage industry to the factory in the 18th century standardized the production of goods in the industrial age, the industrial revolution of genetics allows the standardization of mutagenesis, screening and phenotype interpretation, with all its associated advantages.

When should these large-scale operations take place? My take on it is that it is worth doing them when the technique can as easily be performed in large scale as in individual labs. Small research labs should get on with solving problems in biological mechanism. Whether this should be carried out commercially or in large-scale academic operations is a different issue. While chromosome III was screened in my lab, the whole genome was screened in a biotech company Cenix Bioscience, founded by Chris Echeverri, Pierre Gönczy and myself, with Chris taking the lead personally as CEO, which has ascertained the role of each gene in the first cell division in C. elegans. This monumental task involved taking 43 000 movies (Soenichsen et al., in press). There were two reasons we decided to do this commercially. The first thing is that a large-scale screening operation and individual experiments are not compatible in the same lab. The scientists trying to eke out results from mechanism-directed experiments feel depressed by the amount of data coming out from the screens. The scientists running the screens feel depressed that they are not doing hypothesis-driven research. The second reason was the amount of money that the private sector can bring into a problem. However, one thing is clear: genomics has not changed the basic way science is done, rather it has removed one of the bottlenecks. Individual scientists still have to work on individual problems, just as they have always done.

Acknowledgements

I thank my father RA Hyman for his encouragement, Suzanne Eaton for her constant support and the more recent help of Luke and Max. That is what makes it all worthwhile. I would also like to thank my colleagues in Cambridge, San Francisco, Heidelberg and Dresden for the stimulating environment they have created. In particular I would like to thank for their encouragement, Richard Corfield at the secondary school and Sean Munroe as a PhD student.
References


Anthony A Hyman