© 2015. Published by The Company of Biologists Ltd | Journal of Cell Science (2015) 128, 4035-4038 doi:10.1242/jcs.181107

MEETING REPORT

Meeting report – Getting Into and Out of Mitosis

Nunu Mchedlishvili[1,*], Katarzyna Jonak[2,*] and Adrian T Saurin[3,‡]

ABSTRACT

The Company of Biologists Workshop 'Getting Into and Out of Mitosis' was held 10–13 May 2015 at Wiston House in West Sussex, UK. The workshop brought together researchers from wide-ranging disciplines and provided a forum to discuss their latest work on the control of cell division from mitotic entry to exit. This report highlights the main topics and summarises the discussion around the key themes and questions that emerged from the meeting.

The workshop was organised by Iain Hagan (CRUK Manchester Institute, UK) and Jonathon Pines (Gurdon Institute, University of Cambridge, UK), who brought together a small interdisciplinary group of 30 researchers, including leaders in the field as well as early-career scientists. The workshop gave an excellent opportunity for researchers from diverse scientific disciplines to discuss their recent work on mitotic controls. The emphasis was very much on discussion, and the historic 16th century Wiston House provided the perfect intimate setting for the participants to exchange ideas, and deliberate over the current status of the field and the prospects for the future.

The goal of cell division is to segregate the duplicated genome equally into two daughter cells that contain identical genetic material. The term mitosis was coined by Walther Flemming in 1882 to describe this process, which he also depicted in some beautiful illustrations (Fig. 1). A picture is said to be worth a thousand words, but when Flemming sat back to contemplate these drawings he must have been struck only by questions. How many of those questions still remain unanswered today? In the eyes of the casual observer, maybe not that many – we have identified most of the core parts that drive this process and we have a general understanding of the framework that connects these parts. Perhaps all that remains is for us to delve a bit deeper and fill in the missing details. In the eyes of a seasoned veteran however, it is still not known 'when, where and how' mitosis starts! These were the words that resonated the longest after the Nobel Laureate Sir Tim Hunt (The Francis Crick Institute, London, UK) gave his opening address. In fact, they never really went away but, instead, were simply resurrected in different guises throughout the workshop by those that followed. When, where and how? Three small words that can encapsulate a thousand questions about mitosis.

When?

The question of timing is a particularly pertinent one. Each cell must decide exactly when to commit to enter mitosis before embarking on a coordinated sequence of events that culminates in the physical separation of two daughter cells during cytokinesis. How does a cell ensure all these events occur in the right order and at the correct time? The decision of when to enter mitosis seems a logical place to start.

The timing of mitotic entry varies between organisms and cell types. In stem cells and embryonic systems, such as *Drosophila melanogaster* and *Xenopus laevis* oocytes, the transition between S (DNA synthesis) and M (mitosis) phase is fast, whereas in fission yeast and mammalian somatic cells it can take up to several hours. This variation in length, in what is known as G2 phase, is likely to represent functional and morphological differences between biological systems. A common trigger for mitotic entry is a bistable switch in Cdk1 activity that is regulated by the Wee1/Cdc25 control network. As pointed out by Tim Hunt, we are still uncertain about the cues that trigger this switch and, without this knowledge, it is difficult to appreciate exactly why the length of G2 is so variable. What is clear, however, is that – in a normal cell cycle – mitosis should be initiated after DNA replication is complete. Although mechanisms have been described that could coordinate these two events, we still lack a detailed understanding of how ongoing DNA replication prevents mitotic commitment. Arne Lindqvist (Karolinska Institutet, Stockholm, Sweden) showed that mammalian somatic cells begin to elevate Cdk1 activity following the completion of S phase, which enhances production of cyclin-B and other factors required for mitosis. Interestingly, Olivier Haccard (CNRS Universite Pierre et Marie Curie, Paris, France) demonstrated that Cdk1 inhibition via Cdc6 is important to regain replication competence during meiosis in *Xenopus* oocytes. Taken together, these data suggest that mutual antagonism between DNA replication and Cdk1 activity helps to ensure that mitotic commitment and DNA replication do not occur simultaneously. When replication is complete and Cdk1 activity rises, factors that are necessary for mitosis must then be synthesised, which – as Arne Lindqvist suggested – takes time and might explain why G2 takes so long in somatic cells. Silvia Santos (MRC Imperial College London, UK) demonstrated how double-negative feedback in the cyclin-B/Cdk1 core network is important to provide this time because it creates a refractory period during which cells remain unable to respond to a mitotic trigger. If the feedback is weakened, then cells enter mitosis prematurely, which causes defects in cell division.

When cells are competent to enter mitosis, they then need to 'decide' whether they should make that commitment. This will involve sensing their external and internal environment as it may be unwise for a cell to enter mitosis in the presence of damaged DNA, or to continue to grow before dividing if nutrient supplies are limiting. Fission yeast sense the nutrient state of their environment through the TOR signalling pathway and can adjust mitotic timing and cell size accordingly. Sergio Moreno (CSIC/University of Salamanca, Spain) demonstrated that the TOR and PKA pathways monitor the nutrient state in order to influence Cdk1 activation via the greatwall (Gwl)–endosulfine–PP2A-B55 pathway. TORC1 and PKA are inhibited in low-nutrient medium, which leads to

[1]MRC Laboratory for Molecular Cell Biology, University College London, Gower Street, London WC1E 6BT, UK. [2]Laboratory of Chromosome Biology, Max Planck Institute of Biochemistry, Am Klopferspitz 18, Martinsried 82152, Germany. [3]Division of Cancer Research, Medical Research Institute, Jacqui Wood Cancer Centre, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, UK.
*These authors contributed equally to this work

‡Author for correspondence (a.saurin@dundee.ac.uk)

Journal of Cell Science (2015) 128, 4035-4038 doi:10.1242/jcs.181107

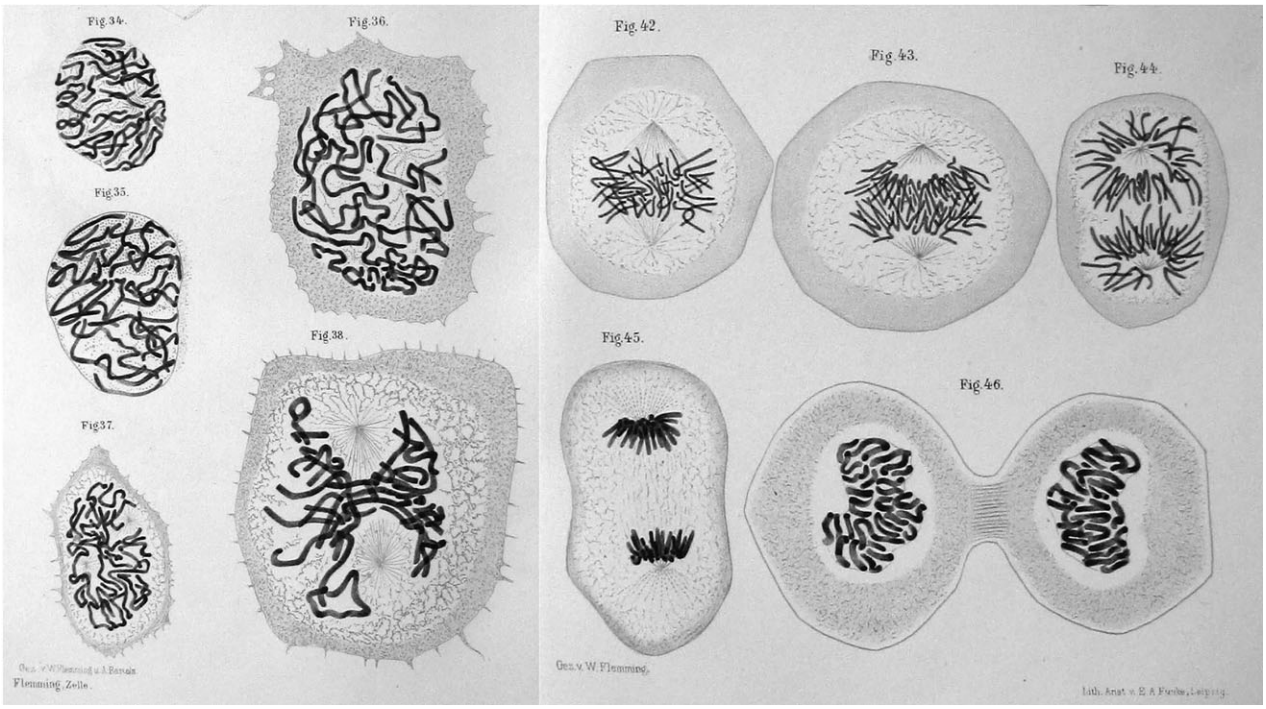

Fig. 1. Drawings of mitosis in salamander cells found in Flemming's book: Zellsubstanz, Kern und Zelltheilung, published in1882.

activation of Gwl. This, potentially, initiates an auto-activation loop, as Gwl elevates Cdk1 activity and vice versa; the net effect being the switch-like inhibition of PP2A-B55 – a phosphatase that targets Cdk1 substrates. The TOR pathway might be a conserved mechanism that helps to couple cell cycle and growth control; however, Clotilde Cadart (Matthieu Piel's group, Institut Curie, Paris, France), by using novel methods to accurately measure cell volume, showed that mitotic entry in mammalian cell lines is not coupled tightly to cell size. To paraphrase Tim Hunt, it will be important in future to determine 'whose finger (if anybody's) is on the trigger'. The Gwl–endosulfine–PP2A-B55 pathway certainly looks like a loaded gun, but whether something other than Cdk1 regulates this in mammalian cells remains unclear. It is important to point out that other kinases can operate upstream of Cdk1. For example, aurora A is necessary for translational activation of MPF during meiosis. Thomas Mayer (University of Konstanz, Konstanz, Germany) showed that aurora A becomes auto-activated in order to drive meiotic entry at the G2/M transition in meiosis I once the counteracting phosphatase PP6c is degraded by the anaphase-promoting complex (APC/C) and its co-activator Cdh1 (APC/C-Cdh1).

After mitotic entry, a biochemical signal (cyclin-B/Cdk1 activity) must be translated into a variety of structural changes that define mitosis. The structural rearrangements themselves are likely to play a big part in coordinating the timing of mitotic events. For example, Nunu Mchedlishvili (Buzz Baum's group, MRC Laboratory for Molecular Cell Biology, UCL, London, UK) showed that nuclear envelope breakdown induces reorganization of the microtubule cytoskeleton to produce more dynamic microtubules exactly when they are needed (i.e. when they encounter chromosomes). The importance of cortical contractility to drive equatorial division during cytokinesis is another well-characterised example. Ewa Paluch (MRC Laboratory for Molecular Cell Biology, UCL, London, UK) showed how, in addition to enhancing contractility at the equatorial plate, precise control of cortical contractility at the cell poles is important to stabilise a dividing cell during anaphase. When polar contractility is too high, poles can contract at the expense of one another, leading to division asymmetries and, when the instabilities are too extreme, division failure. Ewa also demonstrated that the plasma membrane itself may resist these cortical contractions to help stabilise dividing cells.

The decision of when to exit mitosis is controlled by the spindle assembly checkpoint (SAC), which inhibits the APC/C until all kinetochores are stably attached to microtubules. Once attachment is complete, the SAC is satisfied and the APC/C is fully activated, which then kick-starts a series of intrinsic timers that coordinate mitotic exit. The first is the APC/C itself, which is able to degrade substrates in a consistent order. David Morgan (University of California, San Francisco, CA) demonstrated that the timing of substrate degradation is likely to be determined by many factors, including the binding affinity of the substrate, which is conferred by degrons, such as the Cdc20-binding 'ABBA motif', and phosphorylation near the Cdc20-binding sites. He also discussed a mathematical model of a simplified APC/C two-substrate system, and presented evidence that substrate competition for the APC/C from Clb5 does not influence the timing of securin degradation. By contrast, data from Silke Hauf (Virginia Tech, Department of Biological Sciences, Blacksburg, VA), who manipulated cyclin-B and/or securin levels, led her to propose that competition for the APC/C is important to coordinate the degradation of cyclin-B and securin. How the APC/C orders substrate degradation is clearly a crucial question that remains to be resolved. It is important to note that substrate ordering is also context dependent, because APC/C-dependent degradation patterns differ upon exit from meiosis I and meiosis II in budding yeast. This was highlighted by Katarzyna Jonak (Wolfgang Zachariae's group, Max Planck Institute of Biochemistry, Martinsried, Germany), who is using mathematical modelling to investigate these differences.

Cyclin-B degradation itself provides another timer that coordinates mitotic exit, because Cdk1 substrates are dephosphorylated at

Journal of Cell Science (2015) 128, 4035-4038 doi:10.1242/jcs.181107

different times to drive different late-mitotic events. This is at least partially due to the differential kinetics of dephosphorylation but other mechanisms are likely to be layered on top of this to provide fidelity. For example, Francis Barr (University of Oxford, UK) illustrated nicely how biochemical reactions themselves provide excellent fail-safe timers to order events when seconds are critical. He showed how reactivation of PP2A-B55 upon mitotic exit provides the small window needed for chromosome segregation to finish before furrow contraction commences. He also suggested that PP2A-B55 specifically targets Cdk1 substrates, although Anna Castro (CRMB-CNRS, Montpellier, France) indicated that PP2A-B55 removes only a subset of Cdk1 phosphorylations during mitotic exit; therefore, additional phosphatases, such as PP1, are likely to be involved. The importance of PP1 in meiotic and mitotic exit was also confirmed by Tim Hunt, Thomas Mayer and Michael Goldberg (Cornell University, Ithaca, NY). Junbin Qian (Mathieu Bollen's group, University of Leuven, Belgium) showed how a key phosphatase scaffold is important to coordinate exactly when and where PP1 acts. Cdk1 inhibits association between RepoMan (CDCA2) and PP1, and aurora B drives this complex off chromatin. As soon as Cdk1 activity falls at anaphase, aurora B is lost from chromatin and RepoMan associates with PP1, which, together, cause abrupt histone dephosphorylation. Junbin also commented on how RepoMan coordinates other mitotic process because importin-β and RepoMan interact specifically at anaphase, which allows PP1-mediated dephosphorylation of importin-β to coincide with nuclear envelope reformation. These last two examples illustrate nicely the next important topic that was discussed at the workshop.

Where?

Cells are not homogenous systems and many of the signalling activities that drive mitosis are compartmentalised in the locations where they are needed. In addition, such local enrichment can be important to efficiently amplify and regulate signals. The importance of localised signalling control was discussed in several different contexts throughout the meeting. It seems appropriate to start with the important unresolved question that was highlighted by Tim Hunt during his opening address: where do the signals for mitotic entry come from? The cyclin-B/Cdk1 complex was shown to be activated first at the centrosomes in fission yeast and mammalian cells. In fission yeast, local centrosomal activation of the cyclin-B/Cdk1 complex triggers Plo1 recruitment at the spindle pole body and, as demonstrated by Iain Hagan, this plays a crucial role in the timing and execution of mitotic commitment. Furthermore, Silvia Santos showed that the phosphorylation of cyclin-B/Cdk1 initiates its relocalisation into the nucleus, which promotes further cyclin-B/Cdk1 phosphorylation in a spatial positive-feedback loop that helps to trigger rapid mitotic entry. Spatial feedback loops are likely to be important for many different mitotic signalling networks, and Bela Novak (University of Oxford, UK) illustrated their importance to control localised aurora B activity at the kinetochore.

Aurora B is well-known to require strict localisation patterns at different stages of mitosis. It forms a gradient of activity at the centromere/kinetochore during prometaphase, which is required to regulate the SAC and kinetochore-microtubule attachments. Izabela Sumara (IGBMC, Strasbourg, France) showed that aurora B interacts with microtubules in a manner that is dependent on Cul3-mediated ubiquitylation and the ubiquitin receptor protein Ubash3B. She suggested that Ubash3B ensures aurora B localisation to microtubules following their attachment to kinetochores, which might help SAC silencing. During anaphase, aurora B translocates to the spindle midzone where it establishes another gradient of activity to regulate anaphase spindle elongation and cytokinesis. Francis Barr showed how this gradient crucially depends on the kinase–phosphatase balance, with PP2A-B56γ/ε being essential to oppose aurora B activity in this region. Other clear examples of localised kinase–phosphatase scaffolds were discussed (KNL1 and RepoMan) and this is likely to be an important area for future research as we currently know very little about how kinase and phosphatase activities are spatially coordinated.

Up to now, we have illustrated how localisation confines signalling to specific regions but, often, these locally initiated signals need to spread rapidly throughout the cell. There were two very clear examples of this highlighted during the meeting, both of which were explained mechanistically. The first relates to cyclin-B/Cdk1 activation at the centrosome. It was pointed out by Jim Ferrell (Stanford University, CA), that the activation of Cdk1 at the centrosome poses a substantial problem for a *Xenopus* oocyte, which is 1.2 mm in diameter. If left to random diffusion, this active signal would take hours to propagate throughout the cytoplasm. This problem is resolved by 'trigger waves', in which active Cdk1 molecules do not simply diffuse away; instead, they diffuse and locally activate other molecules that then go on to do the same repeatedly. This continuous cycle of localised Cdk1 activation allows the signal to propagate efficiently within seconds. The second example, highlighted by Jonathon Pines, relates to the SAC and, specifically, to the production of the mitotic checkpoint complex (MCC) at unattached kinetochores, which has to spread rapidly in order to inhibit the APC/C throughout the cytoplasm. Jonathon demonstrated that the MCC itself is able to directly inhibit the APC/C–Cdc20 complex within seconds when using purified components *in vitro*. The MCC uses the D-box and second KEN-box in BubR1 to bind to their respective receptors on Cdc20, which ultimately allows the inhibitory signal to spread efficiently throughout the cytoplasm.

Re-activation of phosphatases at mitotic exit is another example that relies on spatial control. In budding yeast, Cdc14 phosphatase is kept inactive by sequestration in the nucleolus during mitosis but, upon entry into anaphase, it is released into the cytoplasm where it is activated through the Cdc fourteen early anaphase release (FEAR) pathway and the mitotic exit network (MEN). The MEN is activated only when the nucleus has been pulled into the daughter cell and a component of this network containing spindle pole bodies has entered the bud. Gislene Pereira (DKFZ/COS, Heidelberg, Germany) showed that, in the absence of the Kin4 inhibitor Lte1, Kin4 accumulates in the daughter cell and inhibits mitotic exit. She demonstrated that deletion of FEAR components, such as Spo12, rescues spindle position checkpoint (SPOC) deficiency in Kin4 mutant cells. Once mitotic exit has been initiated and chromosomes have been segregated, the cell must physically separate into two during cytokinesis. The actomyosin contractile ring, which is tethered to the membrane partly through the action of septins, plays a central role in this process. Simonetta Piatti (CRBM-CNRS, Montpellier, France) demonstrated that localised septin dynamics during cytokinesis are regulated by Rho1 GTPase and protein kinase C in budding yeast. Both are needed to stabilise the septin ring at the bud neck, which is related to the ability of PKC1 to phosphorylate Syp1 and modulate interaction with phospholipids. Understanding how distinct lipid micro-domains regulate membrane curvature and cytokinesis is likely to be an important future area of interest. Finally, Amy Gladfelter (Dartmouth College, Hanover, NH) showed how other species use compartmentalisation

Journal of Cell Science (2015) 128, 4035-4038 doi:10.1242/jcs.181107

in ever more fascinating ways. She demonstrated how the fungus *Ashbya gossypii* asymmetrically distributes mRNAs for key polarity factors by using polyQ-driven protein assemblies, a process that is required to drive symmetry-breaking events in a syncytium.

How?

The subject of 'how' immediately provokes questions about the specific mechanistic details of cell division but, here, we would like to focus only on broader generic principles that are used recurrently throughout mitosis. An excellent example is the repeated use of switch-like transitions. How are these generated in a way that is both reliable and efficient? Bela Novak pointed out that many biochemical switches use double-negative- and/or positive-feedback loops to generate bistability; the classic examples being the Wee1/Cdc25 network at mitotic entry. Bela put forward a new model for kinetochore–microtubule error correction and the SAC that was based on mutual 'activation' of aurora B kinase and cyclin-B/Cdk1, creating a bistable switch for the SAC. An important question related to efficiency is how are the switch-like signals made responsive (i.e. able to switch on and off rapidly)? At least two master regulators of mitosis are shut down quickly upon mitotic entry – APC/C and PP2A-B55 – but these then need to be rapidly reactivated at mitotic exit. It is not trivial to explain how fully inhibited complexes become reactivated so quickly. Clues as to how they achieve this were provided by Michael Goldberg and Jonathon Pines. Michael spoke about 'unfair competition' between α-endosulfine and Cdk1 substrates for PP2A-B55, in which Gwl-phosphorylated α-endosulfine is both an inhibitor and a substrate of PP2A-B55. This provides a constant source of inhibitor during M phase (when Gwl is active) that can be rapidly inactivated by PP2A-B55 itself upon mitotic exit (when Gwl activity is lost). The situation is similar for APC/C inhibition by the MCC. APC/C activity is thought to induce constitutive MCC turnover owing to Cdc20 autoubiquitylation, which allows rapid exit from arrest when MCC generation from kinetochores is shut down at metaphase. Jonathon mentioned that this MCC turnover is APC15 dependent and requires a second Cdc20 molecule because overexpressing a form of Mad2 that sequesters free Cdc20 prevents Cdc20 turnover. This implies that a pool of APC/C–Cdc20 is preserved to permit rapid mitotic exit – but how this is achieved accurately in the face of declining MCC production during prometaphase remains unclear. Jonathon also showed how the APC/C is reactivated within one minute following microtubule attachment to the last kinetochore. This exquisite responsiveness not only relies on fast APC/C reactivation but also on rapid silencing of the kinetochore SAC signal. Work from Adrian Saurin (University of Dundee, UK) and Ulrike Gruneberg (Sir William Dunn School of Pathology, University of Oxford, UK) demonstrated how this occurs. They both showed that PP2A-B56 recruitment to BubR1 is needed for rapid silencing of the kinetochore SAC signal following inhibition of Mps1. Adrian additionally showed this is dependent on PP1 kinetochore recruitment, which – he proposed – allows a aurora B to put the brakes on SAC silencing until the correct time (i.e. when correct kinetochore–microtubule attachments are formed). It was pointed out during the discussion that, although it is easy to conceptualise how one kinetochore–microtubule interaction extinguishes the localised SAC signal in yeast, the situation is far from clear in mammalian cells because they use in excess of 20 microtubules for each kinetochore. Is the SAC signal still extinguished in a switch-like manner or do individual microtubule attachment sites behave autonomously to cause more graded reductions?

Another generic question that was raised during the discussion was how phosphatases achieve specificity. We have already discussed the importance of substrate colocalisation, but do they also have catalytic substrate preferences? Francis Barr performed an unbiased phospho-proteomic screen to search for potential PP2A-B55 substrates. The rationale was that these substrates should dephosphorylate quicker upon mitotic exit following Gwl depletion and slower if PP2A-B55 has been knocked down. This yielded ∼200 putative substrates, of which a high proportion contained a nuclear localisation signal (i.e. a basic patch) juxtaposed to the phosphorylated residue. Francis pointed out that PP2A-B55 has an acidic mouth that might be important for targeting this particular subset of substrates. It was also mentioned during the discussion that substrate specificity is often determined by the regulatory domains. This was highlighted nicely by Junbin Qian, who demonstrated that PP1 dephosphorylates many histone H3 targets non-selectively but, when bound to RepoMan, specifically targets Thr residue 3 of histone H3.

Two talks touched on the important mechanistic questions of how the APC/C recognises and ubiquitylates its substrates efficiently. David Barford (MRC Laboratory of Molecular Biology, Cambridge, UK) provided a wonderful overview of the APC/C and MCC structures, which highlighted some important new findings. The co-activator C-box binds to a pocket on APC8, which is crucial for APC/C activity because it rotates the platform in a way that repositions the APC2–APC11 catalytic module, thereby promoting binding of the E2–ubiquitin conjugate close to the substrate recognition sites on the co-activator and APC10. David Morgan also showed how substrate binding increases catalytic efficiency, possibly as a result of enhanced E2–ubiquitin affinity or repositioning of the E2–ubiquitin conjugate towards the substrate. Together, these findings demonstrate beautifully how the APC/C primes its own activity through binding to co-activators but holds back until a substrate has bound before it unleashes activity at exactly the right time.

Concluding remarks

The workshop concluded with a crystal-ball-gazing discussion chaired by Jim Ferrell. The idea was to centralise ideas about the important questions that remain to be addressed in the field. It was a thoroughly enjoyable session and raised a variety of questions that laid the foundations for this article. The young scientists within the audience were, undoubtedly, left thinking that these questions could also lay the foundations for a long and exciting career studying mitosis.

Acknowledgements

The authors thank Dr Steffen Dietzel for the photographs of Flemming's book and The Company of Biologists for organising and funding this workshop. It was particularly important for early-career scientists to be able to meet and discuss topics at length with leaders in the field. Special thanks go to Nicky Le Blond, who did a fantastic job of organising and coordinating the meeting – there simply wasn't a when, where or how question that she couldn't answer!