From cells on up

Symposium on Cell Biology of Development and Disease

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A star is born: the Temasek Life Science Laboratory (TLL) in Singapore hosted its first annual symposium between 14 and 15 February 2003, entitled 'Cell Biology of Development and Disease'. The TLL is Singapore's newest biological research institute, established in 2002 by Temasek Holdings, the investment holding arm of the Singapore government. This short but intense meeting brought together speakers from Asia, Europe and the USA to discuss the function of cells, both in isolation and in the broader contexts of development and pathogenesis.

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Introduction

The Temasek Life Science Laboratory (TLL) aims to create a vibrant and interactive intellectual community by placing young scientists on an equal footing with established group leaders and by promoting collaborations between all its researchers. This unusual and stimulating meeting combined the three main areas of research at the TLL: cell biology, developmental biology and pathogenesis. Although these separate areas are usually represented by their own separate meetings, this symposium proved that, in fact, these topics fit together extremely well. This is because the cell provides a unifying principle that connects development and disease with more basic molecular biological processes.

Expounding the cell-cycle sutra

The first session, 'Cytoskeleton and polarity', opened with a keynote address by M. Bornens of the Institut Curie in Paris, France. His talk raised the philosophical issue of individuation—how a daughter cell becomes a separate individual that is distinct from its parent cell. Bornens focused on the centriole as a determinant of

cell individuation, which is logical given that these structures undergo a discrete duplication preceding each cell division. Importantly, when a centriole duplicates, the daughter centriole that forms is functionally and structurally distinct from the original mother centriole. Both mother and daughter centrioles are able to nucleate microtubules during the G1 phase, but these are retained by the mother centriole, whereas the daughter releases its microtubules into the cytoplasm, where they can be used as 'seeds' for the formation of complex cytosolic arrays at the leading edge of the cell. An explanation for this difference may be that the protein ninein, which is involved in microtubule anchorage, is associated with the mother, but not the daughter, centriole. Interestingly, the daughter centriole shows highly motile behaviour and localizes to sites that are distant from the mother. This differential positioning might have a direct role in cytokinesis; completion of cleavage to release the daughter cell does not occur until the mother centriole moves to the midbody, which suggests that it induces the last cleavage step to produce two distinct cells (Piel *et al.*, 2001).

Two related talks focused on spindle orientation relative to the cleavage furrow. S. Oliferenko (TLL, Singapore) discussed the problem of spindle alignment in a simple organism, the fission yeast *Schizosaccharomyces pombe*. Oliferenko and co-workers have previously shown that in *S. pombe*, metaphase spindles are orientated with respect to the medial actomyosin ring by astral microtubules (Oliferenko & Balasubramanian, 2002). She presented new evidence that these astral microtubules serve as a monitoring component of a spindle orientation checkpoint, which delays the metaphase-to-anaphase transition until the spindle poles are properly orientated with respect to the long axis of the cell. Astral microtubules might sense proper alignment either by generating tension on the spindle or by providing a path for regulatory molecules to be transferred from the actomyosin ring to the spindle poles by minus-end-directed microtubule motors.

In cells that divide asymmetrically to produce two types of daughter cell, the spindle must be orientated not just with respect to the cleavage furrow site, but also with respect to the inherent asymmetry of the mother cell before division. During *Drosophila* development, neuroblasts divide asymmetrically into two daughter cells of unequal size. F.W. Yu of the Institute of Molecular and Cellular Biology (IMCB, Singapore) presented data showing that the mitotic spindle has a crucial role in the generation of this asymmetry. Yu showed that not only is the spindle itself asymmetrical, but also it is

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displaced towards the smaller daughter cell. This displacement is controlled by the apical complex, which is a set of conserved cell polarity marker proteins located asymmetrically in the cell cortex, through the regulation of astral microtubule growth (Cai *et al.*, 2003). These results indicate that spindle morphology responds to polarity cues located on the cortex.

A session on the cell cycle was chaired by S. Hsu of the National University of Singapore (NUS, Singapore), who provided a thought-provoking view of the cell cycle based on motifs from Zen brush paintings. By comparing the two alternating cell-cycle steps of replication and division to a circle drawn with two brush strokes, Hsu moved away from the Cartesian dualism of Bornens' distinction between mother and daughter centrioles, to a more holistic view of the cell cycle as a single integrated process.

The idea that apparently separate aspects of cell division are connected is clearly supported by the studies of checkpoints that monitor the process. Most work on cell-cycle checkpoints has focused on DNA replication and chromosome segregation, but a talk by M. Balasubramanian (TLL, Singapore) suggested that this genome-centric view does not explain everything. Balasubramanian presented evidence of a cytokinesis checkpoint in *S. pombe* that monitors the division of the cytoplasm as well as the chromosomes (Liu *et al.*, 2000). This checkpoint was discovered through the analysis of a temperature-sensitive mutant allele of the *Cps1* gene, which encodes a 1,3- β glucan synthase subunit involved in the synthesis of the cell wall. Cells that were unable to assemble a septum correctly in the previous cell cycle are delayed in the G2-M transition. Under these circumstances, the cell division apparatus (the actomyosin ring) is maintained

in the interphase cells (Fig. 1) through a checkpoint pathway involving the Cdc14-like phosphatase Clp1; the loss of Clp1 bypasses the cytokinesis checkpoint and prevents the retention of the actomyosin ring, which allows cell division to resume. The loss of this checkpoint is lethal when actin ring or septum assembly is compromised.

U. Surana (IMCB, Singapore) and M. Yamamoto (Tokyo, Japan) provided interesting insights into the mitotic and meiotic cell cycles, respectively. Yamamoto described his laboratory's research on Mei2, a key protein in the promotion of both pre-meiotic DNA synthesis and the first stage of meiotic cell division (meiosis I) in *S. pombe*. Under mitotic conditions, Mei2 is rendered inactive through phosphorylation by the Pat1 kinase, but during meiosis, Pat1 is inactivated and unphosphorylated Mei2 accumulates. Yamamoto has previously shown that Mei2 localizes to a nuclear dot structure during meiotic prophase. Interestingly, a specific RNA, meiRNA, is required for the formation of this dot, and experiments using LacO as a marker for chromosomal position have shown that the dot associates with the *sme2* locus, which encodes meiRNA. Formation of the dot is prevented by the 14-3-3 protein Rad24, which antagonizes the interaction between meiRNA and phosphorylated Mei2, possibly by masking the RNA-binding motifs on Mei2 (Sato *et al.*, 2002). The Mei2–meiRNA interaction thus provides an interesting example of a protein–RNA interaction that takes place at a specific nuclear location and is an important regulator of the cell cycle.

One theme to emerge from the meeting is that many aspects of cell behaviour are monitored by checkpoint pathways that arrest the cell cycle in response to adverse events. Indeed, one must be careful in interpreting the phenotypic effect of mutations, because the observed phenotype could be a non-specific, indirect consequence of checkpoint activation. Fortunately, because many distinct checkpoints share common effectors, they can often be bypassed using known mutations, which allows us to distinguish the direct effects of a given mutation from such indirect effects caused by checkpoint activation. For example, the ability of *clp1* mutants in *S. pombe* to restore cell-cycle progression in cell-wall-synthesis mutants showed that the arrest was due to a checkpoint and not necessarily a consequence of impeded septum formation. So, although the prevalence of checkpoints means we have to consider cellular processes more carefully, it also provides us with many useful standard checkpoint-bypass mutations.

Another cautionary note was sounded during the poster session. It is obvious that a mechanical process such as cytokinesis requires cytoskeletal components and molecular motors. However, posters by V. Wachtler, M. Mishra and H. Wang, all from the Balasubramanian laboratory, showed that cytokinesis in *S. pombe* also relies on sterol metabolism, protein chaperones and the secretory pathway (for example, see Wachtler *et al.*, 2003). The involvement of these 'house-keeping' functions in cytokinesis shows that we need to keep an open mind about what is involved in a process of interest, even if it seems mechanical. To continue with the Zen philosophy motif raised by Hsu, the interconnectedness between mechanical, regulatory and metabolic aspects of cell division is reminiscent of the Buddhist concept of *pratitya samutpada* (dependent co-origination), which states that everything in the universe depends on the existence of everything else. Traditional Buddhist scriptures illustrate this concept with metaphors, such as bundles of reeds supporting one another or collections of iridescent pearls reflecting each other's image, but cell biology might present an even more concrete example of this mutual dependence.

Fig. 1 | Cytokinesis checkpoint in *Schizosaccharomyces pombe*. Actomyosin rings visualized in mutant cells that are defective in septum cell wall synthesis in division. When septation is impeded, the cells arrest with interphase nuclei and a persistent actomyosin ring, reflecting a checkpoint that prevents termination of cytokinesis or progression through the next cell cycle. (Figure kindly provided by M. Balasubramanian.)

From cells to organisms

Another topic addressed at this meeting was the role of cells in developmental biology. The talks in the 'Developmental mechanisms' session were primarily aimed at the cell biology of embryonic development, and most used zebrafish because this model system provides a unique combination of optical clarity and powerful genetics, and is well represented in the research that takes place in Singapore, where this tropical fish is a native species.

A beautiful illustration that combined the imaging and genetics of zebrafish development was provided by L. Solnica-Krezel (Nashville, TN, USA), who studies cell movements during gastrulation by using mutants that are specifically affected in this process but that are unaltered in their cell-fate specification (for example, see Marlow *et al.*, 2002). Solnica-Krezel described the involvement of the Wnt-signalling pathway in the regulation of various cell movements, such as convergence and extension during gastrulation and the movement of one set of cells under another (subduction) during posterior body formation. In both cases, Wnt-pathway mutants impeded the physical behaviour of the cells without affecting their developmentally programmed cell fate. In a similar presentation, S. Fong from V. Korzh's group (IMCB, Singapore), showed that Wnt signalling is necessary for cell migration in the neuroectoderm, but is not needed for the specification of neural cell fates.

K. Sampath (TLL, Singapore) described her experiments using zebrafish to understand the induction of the floor plate, which is a group of cells that are responsible for patterning of the neural tube. Using a conditional allele of the transforming growth factor- β (TGF- β) family member, cyclops, a key regulatory molecule in this process, her group has shown that floor plate induction takes place early in embryonic development, during gastrulation. This is yet another reminder of Lewis Wolpert's famous aphorism: "It is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life."

Axon formation is an example of the importance of cell structure changes during development. S. Jesuthasan (TLL, Singapore) showed that zebrafish mutants can be used to analyse the topographic mapping of retinal ganglion cells. For example, his group has characterized and cloned one such mutant, *esrom*, in which axons branch excessively in the optic tectum.

A. Ephrussi (Heidelberg, Germany) spoke about the localization of *oskar* RNA in *Drosophila* oocytes. Transcripts that encode the oskar protein, which is required for assembly of the posterior pole plasm, are translationally repressed until they are localized to the posterior of oocytes. Ephrussi's group has recently shown that two translational repressors of the heterogeneous nuclear ribonucleoprotein (hnRNP) family, hrp48 and Y14, as well as the exon-junction protein RNA-binding motif protein 8 (Rbm8), are required for *oskar* RNA localization. These results indicate that translation and messenger RNA localization are directly coupled.

Y.J. Jiang (IMCB, Singapore) presented experiments in which zebrafish are used to show that the ubiquitin-proteasome pathway is involved in the cyclical activity and synchronization of the segmentation clock, a fascinating biological oscillator that drives the periodic formation of somites in vertebrate embryos. It is notable that biochemical oscillator pathways in individual cells can drive the formation of large-scale structures, such as somites, during development. This continued the theme from the Wnt-pathway talks that signalling pathways that control cell behaviour are equally important for development as those that determine cell fate.

Fig. 2 | Pathogenic cell specialization in *Magnaporthe grisea*. The large swollen cell is the appressorium, which is a specialized attack cell that generates strong turgor pressure due to the overproduction of glycerol. This pressure is vented against the cell wall of the host plant, rupturing the wall and allowing entry of the fungus. Melanin production due to peroxisomal activity is crucial for maintenance of this turgor. (Figure kindly provided by N. Naqvi.)

When cells turn against us

Pathogenic disease is a war fought by cells and, in the evolutionary arms race of host-pathogen interactions, pathogens have become specialized in amazing ways. N. Naqvi (TLL, Singapore) opened the 'Pathogenesis' section of the meeting with a glimpse into the cellular mechanisms of invasion of the rice blast fungus *Magnaporthe grisea*. This pathogen constructs a highly specialized cell called an appressorium, which builds up immense internal pressure that can suddenly be vented to rupture the wall of a plant cell (Fig. 2). During formation of the appressorium, peroxisomes produce a large quantity of melanin, which contributes to the high turgor pressure. After punching a hole in its victim's outer defences, the fungus develops hyphae that grow into the host cell to consume it from within. These specialized hyphae contain a modified peroxisome called a woronin body (WB). Thus, peroxisomes have been implicated in two stages of infection, but are they necessary for this process? When Naqvi's group screened for insertional mutants that affect various stages of the infection process, two of the resulting mutants, *Pex6* and *Hex1*, affected peroxisome biogenesis. These mutants have misshapen appressoria, which might decrease the impact of the initial invasion. Both also arrested at the infection stage, possibly due to impairment of WB function. Given the importance of reactive oxygen intermediates, such as hydrogen peroxide in plant-host-defence pathways, these results suggest that peroxisomal enzymes in the WB, such as the peroxide-degrading enzyme catalase, might help the fungus to avoid host defences. In any case, this is a striking illustration of the many roles that a single organelle can have in invasive growth.

Pathogens with the temerity to infect humans not only have to overcome our immune systems, but also have to face man-made drugs that are specifically designed to kill them. But many pathogens have acquired drug resistance using surprisingly simple methods. For example, mycobacteria that cause tuberculosis (TB)

can become resistant to drugs by entering a dormant state in response to hypoxia, an environmental condition found in tissues that are distant from the lungs. These bacteria may then resurface in the lungs of the patient after treatment. T. Dick (IMCB, Singapore) set out to find mycobacterial targets for dormancy-specific drugs by identifying gene products that are upregulated during dormancy. One of these is a transcription factor, dormancy survival regulator (DosR), which is upregulated as soon as the bacterium enters into dormancy, suggesting that it may be a key regulator of this state. A knockout strain that lacks *dosR* fails to induce the other hypoxia-specific genes, and these cells die under hypoxic conditions rather than entering the dormant state (Boon & Dick, 2002). DosR and its effectors might therefore provide potential drug targets that prevent TB-inducing mycobacteria from entering dormancy.

Perspectives

This symposium reflected the current trend of re-integrating biology, by recognizing that the fields of cell biology, developmental biology and pathogenesis, which are often treated as separate subjects or even consigned to distinct academic departments, share common interests. This commonality is the basis of the TLL, and the success of this symposium suggests that the TLL will be similarly successful in helping Singapore to continue its growth in basic research.

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REFERENCES

- Boon, C. & Dick, T. (2002) *Mycobacterium bovis* BCG response regulator essential for hypoxic dormancy. *J. Bacteriol.*, **184**, 6760–6767.
- Cai, Y., Yu, F., Lin, S., Chia, W. & Yang, X. (2003) Apical complex genes control mitotic spindle geometry and relative size of daughter cells in *Drosophila* neuroblast and pl asymmetric divisions. *Cell*, **112**, 51–62.
- Liu, J., Wang, H. & Balasubramanian, M.K. (2000) A checkpoint that monitors cytokinesis in *Schizosaccharomyces pombe*. *J. Cell Sci.*, **113**, 1223–1230.
- Marlow, F., Topczewski, J., Sepich, D. & Solnica-Krezel, L. (2002) Zebrafish Rho kinase 2 acts downstream of Wnt11 to mediate cell polarity and effective convergence and extension movements. *Curr. Biol.*, **12**, 876–884.
- Oliferenko, S. & Balasubramanian, M.K. (2002) Astral microtubules monitor metaphase spindle alignment in fission yeast. *Nature Cell Biol.*, **4**, 816–820.
- Piel, M., Nordberg, J., Euteneuer, U. & Bornens, M. (2001) Centrosome-dependent exit of cytokinesis in animal cells. *Science*, **291**, 1550–1553.
- Sato, M., Watanabe, Y., Akiyoshi, Y. & Yamamoto, M. (2002) 14-3-3 protein interferes with the binding of RNA to the phosphorylated form of fission yeast meiotic regulator Mei2p. *Curr. Biol.*, **12**, 141–145.
- Wachtler, V., Rajagopalan, S. & Balasubramanian, M.K. (2003) Sterol-rich plasma membrane domains in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.*, **116**, 867–874.