Meeting report

Cell-cycle regulation

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A report on the 'cell-cycle regulation' minisymposium at the 41st Annual Meeting of the American Society for Cell Biology, Washington DC, USA, 8-12 December 2001.

At the annual meeting of the American Society for Cell Biology, several symposia and minisymposia were dedicated to the coordination of events during the cell cycle. The 'cell-cycle regulation' minisymposium, and indeed the meeting as a whole, seemed particularly charged, given the recent announcement that Leland Hartwell, Paul Nurse and Tim Hunt were recipients of the Nobel Prize in Physiology or Medicine "for their discoveries of key regulators of the cell cycle". Thus, it was entirely fitting that Nancy Walworth (of UMDNJ-Robert Wood Medical School, Piscataway USA - one of the Chairs of the minisymposium) explained at the beginning of the session the fundamental concepts that each of these three scientists introduced, including that of the checkpoint. She used this opportunity to segue into a discussion of her most recent work on the regulation of the Chk1 protein kinase in the fission yeast, *Schizosaccharomyces pombe*. (The regulatory proteins mentioned in this article and their functions in the cell cycle are listed in Table 1.)

Checkpoints

Work in Walworth's laboratory focuses on how the cell cycle is halted in response to DNA damage, and in particular how Chk1 may contribute to the maintenance of this 'checkpoint' (from which the cell cycle cannot proceed until the damaged DNA has been repaired). Work from a variety of labs has determined that the activity of Chk1 inhibits the Cdc25 phosphatase and activates the Wee1 and Mik1 protein kinases, two actions that favor phosphorylated, and thus inactive, cyclin-dependent kinase, the engine that drives the cell cycle. Walworth's studies indicate the importance of phosphorylation for Chk1 function, which occurs in a checkpoint-dependent manner and downstream of the action of Rad3p (the fission yeast homolog of the Mec1/ATR kinase). Mutation of the phosphorylated residue of Chk1 (Ser345 →Ala) compromises the Chk1 protein's checkpoint function and phenotypically mimics the effects of a *chk1* deletion. When characterizing the phosphorylation of Chk1, Walworth's colleagues observed enhanced nuclear localization of Chk1 following damage. Walworth explained that mutation of a putative nuclear localization signal (NLS, residues 377-397) in Chk1 now blocked the Chk1 protein's phosphorylation and rendered it checkpoint-deficient, suggesting that its presence in the nucleus is necessary for function. This was confirmed when they could restore nuclear localization and checkpoint function to the *chk1* NLS-deficient mutant by the addition of a heterologous NLS, from SV40. Given that a chimera of wild-type Chk1 fused to the SV40 NLS also showed enrichment in the nucleus only following DNA damage, the suggestion was that exit of the Chk1 protein from the nucleus is regulated in response to DNA damage. This hypothesis was supported by the finding that DNA damage stimulated the association of Chk1 with the *S. pombe* 14-3-3 protein, Rad24. Mutation of the 14-3-3-binding region of Chk1 abolished Rad24 binding as well as DNA-damage-checkpoint function. This region resembles a nuclear export signal, suggesting a model in which phosphorylation of Chk1 stabilizes its interaction with Rad24 in the nucleus, thereby blocking export of Chk1; this results in accumulation of the Chk1 kinase in the nucleus, where it can carry out its checkpoint function and keep cyclin-dependent kinase inactive. The export signal is not conserved in Chk1 homologs in higher eukaryotes, indicating that this level of regulation of the DNA-damage checkpoint may not be present in these organisms.

Steve Doxsey (University of Massachusetts Medical School, Worcester, USA) was a late addition to the agenda and did not appear on the published list of speakers for the minisymposium. He made an interesting case for the existence of a 'mitotic exit network' (MEN) in mammalian cells, akin to the MEN in budding yeast and the 'septation initiation network' (SIN) in fission yeast. His story begins with the identification of two centrosomal proteins, centriolin and

Table 1

Cell-cycle regulatory proteins mentioned in this article

Name of protein	Names of homologs	Enzymatic function	Cell-cycle function
Bfa1		GTPase-activating protein	Mitotic exit
Bub2			Checkpoint component
BubR1			Spindle-assembly checkpoint component
Bub3			Spindle-assembly checkpoint component
Cdc20			Activator of the anaphase-promoting complex (APC)
Cdc25		Phosphatase	Activates cyclin-dependent kinase (Cdc2)
Cdc5		Polo-like protein kinase	Mitotic exit
Cdh1			Activator of the anaphase-promoting complex (APC)
Centriolin			Centrosome component
Chk1		Protein kinase	Checkpoint component
Emi1			Inhibitor of the anaphase-promoting complex (APC)
Ipl1		Aurora-like protein kinase	Spindle-assembly checkpoint component
Kendrin	Pericentrin*		Centrosome component
Mad2			Checkpoint component
Mik1		Protein kinase	Inactivates cyclin-dependent kinase (Cdc2)
Mps1		Protein kinase	Spindle-assembly checkpoint component
Rad24	14-3-3		DNA-damage checkpoint component
Skp1			Component of a ubiquitin ligase complex, the SCF
Tem1		Ras-related GTPase	Mitotic exit
γ tubulin			Centrosome component
Wee1		Protein kinase	Inactivates cyclin-dependent kinase (Cdc2)

* Pericentrin is a smaller isoform of kendrin.

kendrin, that are antigens in the human autoimmune condition scleroderma; kendrin is a larger isoform of the centrosomal protein pericentrin. Immunogold electron microscopy using anti-centriolin antibodies illustrates its specificity within the centrosome for the 'mother' centriole. Given that both centriolin and kendrin move to the intracellular bridge and midbody during cytokinesis, and recent reports suggest a role for the maternal centriole in cytokinesis, Doxsey and colleagues were intrigued by the possibility of centriolin's involvement in cytokinesis. They injected anti-centriolin antibodies into *Xenopus* oocytes and observed cytokinesis defects; such defects were also seen when centriolin was overexpressed in tissue-culture cells. When small interfering RNAs were used to reduce kendrin protein levels, centriolin was lost from the centriole without a concomitant loss of the centriolar proteins γ tubulin or pericentrin A; cytokinesis defects were observed, but the microtubule-nucleation activity of centrosomes isolated from these cells was not affected. These data suggest that centriolin and kendrin may be involved in a regulatory pathway to signal cytokinesis. Doxsey argued that centriolin is the homolog of the budding yeast Nud1p (which anchors components of the MEN), on the basis of its sequence similarity in one specific domain and the interaction of this domain with the checkpoint protein Bub2p, as assessed by yeast two-hybrid protein-protein interaction assay and by co-immunoprecipitation when overexpressed. He also offered the possibility that kendrin may be the homolog of the fission yeast Sid4, required for localization of Cdc11 (the fission yeast homolog of Nud1p and therefore centriolin).

Stephen Elledge (Baylor College of Medicine, Houston, USA) described recently published work from his lab elucidating the MEN of budding yeast and its regulation by the polo-like kinase Cdc5p. His studies indicate that Cdc5p is required to inactivate the GTPase-activating protein (GAP) Bfa1p. This inhibition of Bfa1p results in the activation of Tem1, a Ras-related GTPase that acts through a series of proteins identified as the MEN to accomplish the down-regulation of cyclin-dependent kinase activity and the exit from mitosis. He showed that phosphorylation of Bfa1p coincides with anaphase and this phosphorylation is abolished in a *cdc5* mutant. Elledge also suggested that this regulation of Bfa1p is important during the activation of the spindle-assembly checkpoint, which prevents cells from dividing until all chromosomes are properly attached to the mitotic spindle; cells treated with the microtubule-depolymerizing drug nocodazole do not exhibit phosphorylation of Bfa1p unless the checkpoint is abrogated, as in a strain with a deletion of the *mad2* checkpoint gene. Mad2 binds to Cdc20, an activator of the anaphase-promoting complex (APC), to inhibit APC activity and arrest cells in metaphase in

response to checkpoint activation. He mentioned data showing that the DNA-damage checkpoint also impinged upon regulation of Bfa1p but not via Cdc5p.

Sue Biggins (Fred Hutchinson Cancer Research Center, Seattle, USA) continued the focus on checkpoints with her recently published work ascribing a role for the budding yeast Aurora-like kinase, Ipl1p, in the spindle-assembly checkpoint. Initial analysis of the *ipl1* mutant identified a chromosome-segregation defect, attributed to a defect in the kinetochore that allowed kinetochore beads made in *ipl1* mutant cell extract (centromere DNA on beads is exposed to cell extract to bind the required kinetochore proteins) to maintain microtubule attachments more stably than those made in wild-type cell extract. Despite the kinetochore defect, however, *ipl1* mutants do not activate the spindle-assembly checkpoint as most kinetochore mutants do, leading Biggins to hypothesize that either the checkpoint cannot monitor the defect in *ipl1* mutants or Ipl1p itself is involved in the checkpoint. The latter model proved correct: if the checkpoint is constitutively active, as it is when the protein kinase Mps1p is overexpressed, inactivation of Ipl1p results in abrogation of the checkpoint. When the checkpoint is activated by a microtubule-depolymerizing drug, such as benomyl, loss of *IPL1* function has no effect, suggesting that Ipl1p is not involved in monitoring the attachment of kinetochores to microtubules. Instead, Ipl1p might monitor tension at the kinetochore - the pulling forces of the spindle at the kinetochore opposed by the linkage that keeps sister chromatids together. Recent reports have illustrated that the absence of a sister chromatid (in the *cdc6* deletion mutant) or the loss of linkage between sister chromatids (in the *mcd1/scc1* mutant) activates the spindle-assembly checkpoint. Biggins tested whether this activation was *IPL1* dependent - and it was, indicating that Ipl1p is required to activate the checkpoint under conditions in which there is no tension. Consistent with this role, Ipl1p localizes to kinetochores.

Promoting anaphase

Valerie Sudakin, a member of Tim Yen's laboratory at Fox Chase Cancer Center (Philadelphia, USA), offered a shift in the focus of talks to the regulation of a key cell-cycle player, the APC. The APC is a multi-subunit ubiquitin ligase that targets specific proteins for destruction during key transitions of the cell cycle; for this reason, it is itself often the target of checkpoint pathways, such as the spindle-assembly checkpoint. Sudakin chronicled the efforts that members of the Yen lab have made to understand how the proteins involved in the spindle-assembly checkpoint inhibit APC activity and effect metaphase arrest. They have identified a multi-protein complex, named the mitotic checkpoint complex (MCC), that inhibits the APC's activity *in vitro*. This complex consists of BubR1, Bub3, Mad2 (all known components of the spindle-assembly checkpoint) and Cdc20, and its inhibitory activity is 3,000-fold greater than that of Mad2 protein alone. The complex exists in interphase but can only act on the mitotic APC, suggesting an additional level of regulation that is not yet characterized. Furthermore, Yen and colleagues have data suggesting that chromosomes prolong the inhibition of the APC by the MMC, by stabilizing the interaction between the two complexes. Sudakin proposed a model in which the MCC, already formed in interphase, allows rapid inhibition of the APC until anaphase; the checkpoint maintains this inhibition if chromosomes have kinetochores that are not attached to microtubules.

Peter Jackson (Stanford University, USA) elaborated on the function of Emi1, a protein his lab has identified as a novel APC inhibitor. He and his colleagues have found that Emi1 degradation is required for APC-mediated destruction of the mitotic cyclins, A and B, to occur in *Xenopus* egg extracts. Immunodepletion of Emi1 results in early destruction of cyclin B; this phenotype can be rescued with recombinant Emi1 and a non-destructible cyclin B fragment (the Δ90 fragment) that constitutively activates cyclin-dependent kinase. The amino terminus of Emi1 confers instability; the addition of a non-destructible Emi1 to cell extract produces a mitotic arrest with high cyclin levels and an inactive APC. The Emi1 protein accomplishes its inhibition of the APC by antagonizing substrate binding, as a result of an interaction between Emi1 and the first 100 amino acids of the APC activator Cdc20. Jackson and colleagues further characterized this interaction between Cdc20 and Emi1 when they began investigating the role of Emi1 in meiotic cytostatic factor (CSF) arrest; *Xenopus* eggs maintain this metaphase II arrest as they await fertilization, and CSF arrest is Ca^{2+}-labile. Immunodepletion of Emi1 from CSF-arrested *Xenopus* egg extracts resulted in continuation of the cell cycle without Ca^{2+} addition; they could rescue this phenotype with the addition of the carboxyl terminus of the Emi1 protein, which binds Cdc20. Subsequent analysis revealed that with the addition of Ca^{2+} in a normal exit from CSF arrest, Emi1 dissociates from Cdc20 and assumes a form that has slower electrophoretic mobility. *In vitro*, Emi1 is phosphorylated by Ca^{2+}-calmodulin-dependent protein kinase type II (CaMKII), a kinase involved in the signal transduction cascade that achieves exit from CSF arrest, and this inhibits the ability of Emi1 to associate with Cdc20.

The final speaker was Nagi Ayad, a member of Marc Kirschner's laboratory at Harvard Medical School (Boston, USA). He described the characterization of a novel APC substrate that regulates mitotic exit; his story provided a beautiful illustration of multiple regulatory mechanisms acting in concert to control the primary cell-cycle engine, the cyclin-dependent kinase Cdc2. Using a small-pool cDNA expression-screening strategy in *Xenopus* egg extracts, he was interested in finding new APC substrates that were specifically targeted for destruction by APC(Cdh1), a version of the APC primarily active in G1 of the cell cycle. Cdh1 is, like Cdc20, an APC activator; in addition to conferring activity,

Cdc20 and Cdh1 confer differential substrate recognition on the APC ubiquitin ligase that must modify different proteins at different points during the cell cycle. Ayad and colleagues identified a novel protein, p66, which is expressed throughout embryogenesis in *Xenopus* and has human, mouse and *Drosophila* homologs. It is a true substrate for APC(Cdh1), because mutation of the motif that is recognized by Cdh1 (the 'KEN box') or the addition of a proteasome inhibitor and Δ90-cyclin B results in p66 stability. The human p66 is degraded at mitotic exit and associates with Skp1, a component of another ubiquitin ligase complex, the SCF (Skp1-Cul1/Cdc53-F-box); an adaptor protein, the F-box subunit, confers substrate specificity on this complex. The p66 protein contains an F-box domain, and deletion of this region abolishes its interaction with Skp1; expression of this mutant in human cells or addition of it to *Xenopus* extracts produces a mitotic block (with stabilization of the Wee1 kinase and prolonged phosphorylation of Cdc2). Wee1 and p66 associate with each other, but this association is dependent on phosphorylation of Wee1; mutation of the phosphorylation site (Ser38 →Ala) abrogates the association of Wee1 with p66 and recapitulates the p66 F-box mutant phenotype, namely stabilization of Wee1. The model proposed by Ayad and colleagues involves the destruction of p66 by APC(Cdh1) throughout the G1 phase of the cell cycle, to allow for Wee1 accumulation and Cdc2 inactivation; but APC inactivation results in p66 accumulation, Wee1 degradation and Cdc2 activation.

The driving force of the cell cycle is cyclin-dependent kinase activity. Key steps in regulating the cell cycle must therefore impinge upon the activity of cyclin-dependent kinase. The cell-cycle regulation minisymposium 18 at this year's American Society for Cell Biology conference highlighted the many and diverse mechanisms the cell employs to control its division cycle and ensure the production of genetically identical and viable progeny.