An overview of the fission yeast septation initiation network (SIN)

Andrea Krapp1 and Viesturs Simanis1

Cell Cycle Control Laboratory, Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), 155 chemin des Boveresses, 1066 Epalinges s/Lausanne, Switzerland

Abstract
The fission yeast septation initiation network, or SIN, is a signal transduction network that is required for septum formation in Schizosaccharomyces pombe. Its activity is tightly regulated through the cell cycle, to ensure proper co-ordination of mitosis and cytokinesis. SIN signalling requires three protein kinases for its function and is mediated by a ras-superfamily GTPase. We discuss the elements of the SIN and how they are regulated.

Fission yeast growth and division
A wild-type cell of the fission yeast Schizosaccharomyces pombe is a rod capped by hemispherical ends, and grows primarily by elongation at its tips. The cell divides by forming a new cell wall, or division septum, at the middle of the cell. The position of the division site is defined at the onset of mitosis [1], when the protein mid1p leaves the nucleus to form a ring underlying the cell cortex, which is thought to provide a scaffold to anchor the CAR (contractile actomyosin ring). Mid1p provides the spatial cue for the CAR, but is not required for its assembly or contraction [2,3]. The CAR is assembled in stages throughout mitosis [4]. At the end of anaphase, it contracts and is thought to guide synthesis of the multilayered division septum. Cell separation is effected by dissolution of the primary septum. Once separated, the daughter cells resume growth initially at the pre-existing (old) end. The other (new) end does not begin to elongate until S-phase has been completed and cells have attained a critical size [5]. The SIN (septation initiation network) is required for the onset of septum formation (for reviews see [6–8]).

The cell must overcome several hurdles to make certain that the outcome of cytokinesis is two viable daughter cells; first, the spatial problem of where to assemble the CAR; second, formation and contraction of the CAR must be properly co-ordinated with other cell cycle events to ensure that cytokinesis does not occur before chromosome segregation has been completed; third, contraction of the CAR must not initiate if its assembly is incomplete or defective. As we shall see, the SIN and its regulators play an important role in the co-ordination of mitosis and cytokinesis.

Keywords: cell cycle, cytokinesis, fission yeast, kinase, septation initiation network (SIN).

Abbreviations used: CAR, contractile actomyosin ring; cdc, cell division cycle; GAP, GTPase-activating protein; MEN, mitotic exit network; PP2A, protein phosphatase 2A; SIN, septation initiation network; SPB, spindle pole body.

Correspondence may be addressed to either of these authors (email andrea.krapp@epfl.ch or viesturs.simanis@epfl.ch).

Fission yeast septation initiation network

The SIN (septation initiation network) is a signal transduction network that is required for septum formation in Schizosaccharomyces pombe. Its activity is tightly regulated through the cell cycle, to ensure proper co-ordination of mitosis and cytokinesis. SIN signalling requires three protein kinases for its function and is mediated by a ras-superfamily GTPase. We discuss the elements of the SIN and how they are regulated.

What is the SIN and what happens to the cell when it doesn’t function properly?
Signal transduction by the SIN (see Figure 1) is considered to be modulated by the ras superfamily GTPase member spg1p [9]. Its nucleotide status is regulated by a bipartite GAP (GTPase-activating protein) comprising a catalytic subunit cdc (cell division cycle) 16p [10,11] and a scaffold subunit byr4p [12]. The latter juxtaposes spg1p and cdc16p to promote GTP-hydrolysis by spg1p [13,14]. To date, no clearly identifiable, essential, GEF (guanine-nucleotide-exchange factor) for spg1p has been identified in genetic screens for septation mutants. In the absence of cdc16p, byr4p prevents spg1p GTP hydrolysis and release, which may facilitate its switch to the GTP-bound form. Genetic evidence suggests that the essential effector of spg1p is the protein kinase cdc7p [9]. Spg1p is required for the localization of cdc7p in vivo, but not its activity in vitro [15,16]. The protein kinases sid1p [17] and sid2p [18], with their respective regulatory subunits cdc14p [19] and mob1p [20,21], are also required for SIN signalling. Both kinases require their regulatory subunit for maximal activity in vitro and for localization in vivo [17,20–23].

The SIN proteins assemble on a scaffold comprising cdc11p and sid4p [24,25]. The C-terminus of cdc11p interacts with sid4p, which in turn is anchored to the SPB (spindle pole body) by binding to ppc89p [26]. All three proteins have extensive regions of coiled structure, and many SIN proteins bind to the N-terminus of cdc11p, which is essential for signalling [27,28]. Loss-of-function mutants in any of the genes sid1, cdc14, sid2, mob1, sid4, cdc11, spg1, cdc7 and etd1 lead to the formation of elongated, multinucleated cells [29], the so-called septation initiation phenotype. The nuclear cycle (S-phase and mitosis) continues in the absence of cytokinesis, until cells eventually lyse. Loss-of-function mutants of either of the GAP components cdc16p and byr4p undergo multiple rounds of CAR and septum formation, without undergoing cell cleavage. Cells may have one or two nuclei, indicating that septum formation can be uncoupled from the normal order of cell cycle events by SIN activation.

Key words: cell cycle, cytokinesis, fission yeast, kinase, septation initiation network (SIN).
The main components and some regulators of the SIN

A null allele of ppc89 displays a complex phenotype [26], consistent with its proposed role as an integral structural protein of the SPB.

Contractile ring components and F-actin are seen in the medial region of SIN mutants, suggesting that the ring can assemble, at least in part, without the SIN. The SIN also appears to be required for proper assembly of the septum at the division site [30] and is required to maintain a defective CAR (see below). The protein kinase plo1p regulates the SIN, in addition to many other mitotic events [31]. Loss of plo1p affects multiple processes in mitosis and cytokinesis, and the complexity of the plo1 null mutant phenotype reflects this. However, plo1 expression shut-off strains display a septation initiation phenotype [31], and conditional plo1 alleles that show a septation initiation-like phenotype have been described, suggesting a direct role of plo1p in SIN regulation. Epistasis analysis suggests that the core SIN proteins are downstream of plo1p [32]. Increased expression of spg1, cdc7 or plo1 can induce septum formation in interphase cells [9,31,33].

Localization of SIN proteins

All of the SIN proteins associate with the SPB at some point in the mitotic cell cycle (for review, see [7]). Localization of the SIN at the SPB, which also hosts many mitotic regulators, may help in the co-ordination of mitosis and cytokinesis. The three scaffold proteins, cdc11p, sid4p and ppc89p, are observed at the SPB at all cell cycle stages [24,26,34]. Spg1p, byr4p and cdc16p associate with the SPB throughout interphase [16,35,36]. Spg1p associates with cdc7p on both SPBs from the onset of mitosis. Byr4p, but not cdc16p, associates with both SPBs early in mitosis. Once cells undergo the anaphase A-B transition, cdc7p and sid1p-cdc14p associate preferentially with the newly-created spindle pole [16,17,37], while the cdc16p-byr4p GAP reassembles on the old SPB. Whether the asymmetry plays a regulatory role or simply reflects underlying differences between the two SPBs is unclear at present. Laser ablation experiments have suggested that at least one functional SPB is required for septum formation [38]. Sid2p-mob1p associates with both SPBs throughout mitosis and also forms a double ring flanking the division septum during its synthesis [18,20,21]. This requires SIN activity, and current dogma posits this as the trigger for septation. Plo1p localizes to the SPBs from mitotic onset, and is also observed at the CAR and mitotic spindle. The SPB signal decreases in intensity throughout anaphase [39]. Etd1p [40,41] is located at the cell tips in interphase and forms part of the CAR in mitosis. It requires SIN function for localization to the CAR. A link to the SIN is revealed by the finding that cdc7p association with the SPB and sid2p-mob1p association with the CAR require etd1p. Though its role is unclear, etd1p may be a part of a SIN-CAR regulatory link. In the absence of either subunit of the GAP, the other subunit does not associate with the SPB and all the remaining SIN proteins are localized to the SPB in interphase and symmetrically during mitosis. Studies of SIN protein localization in SIN mutants, and their effects upon sid1p-cdc14p and sid2p-mob1p kinase activity, have led to the proposition that the order of function within the SIN is cdc7p/sid1p/sid2p (see Figure 1). This remains to be demonstrated biochemically. Studies of the dynamics of some of the SIN proteins at the SPB by FRAP (fluorescence recovery after photobleaching) has revealed that they turn over rapidly [27]. It remains to be determined whether the SPB plays a role as an assembly or activation site for the SIN proteins, akin to the kinetochore in the spindle assembly checkpoint.

Regulators of the SIN

In addition to the regulatory components of protein kinases mentioned above, screens for dosage or extragenic suppressors of SIN mutants have identified several genes whose products regulate SIN signalling. The Forkhead-associated RING-finger protein dma1p was identified in a screen for inhibitors of the SIN [42]. It binds to sid4p at the SPB, and may regulate plo1p association with the SPB [43]. It is also found at the CAR during anaphase; its binding partners at this location are unknown. Increased expression of dma1 produces a SIN phenotype; the null mutant is viable, but dma1p is essential to prevent septation in spindle assembly checkpoint-arrested cells. Pp2a (protein phosphatase 2A) subunits have also been identified as regulators of the SIN; par1p is a B'-regulatory subunit which seems to regulate SIN signalling. It localizes to the SPBs throughout mitosis, and also to the CAR. Its targets are unknown, but genetic evidence points to
cdc7p–spg1p, mob1p–sid2p and cdc11p as potential targets [44–46]. Cdc11p is heavily phosphorylated while the SIN is active in anaphase, which depends in part upon cdc7p and plo1p in vivo [47]. Dephosphorylation of cdc11p at the end of mitosis is less efficient in par1 mutants, consistent with genetic data linking P2A and cdc11p. Entry into mitosis requires the mitotic cdk (cyclin-dependent kinase) cdc2p–cdc13p [48], which then acts as an inhibitor of the SIN early in mitosis. Inactivation of cdc2p permits the SIN proteins to adopt the presumed active, asymmetric anaphase configuration; the relevant targets for cdk inhibition of the SIN are not known. It is interesting to note that cdc13p associates with cdc11p [27].

Loss-of-function mutations in two RNA-binding proteins, scw1p [49,50] and zfs1p [51], will rescue most conditional SIN mutants to some extent. Though a genome-wide expression analysis has identified RNAs whose stability is regulated by zfs1p [52], the relevant target RNAs for SIN regulation are unknown.

The CDC14-family phosphoprotein phosphatase flp1p (also called clp1p) is important for SIN signalling [53,54]. Most SIN mutants show a strong negative genetic interaction with a flp1 null mutant, but the relevant target is presently unknown. A regulatory feedback loop between the SIN and flp1p has been proposed to be important for checkpoint function (see below). The nuclear protein dnt1p antagonizes the SIN [55]. It shows some homology with Net1p, the nuclear inhibitor of Cdc14p in budding yeast. However, Dnt1p does not appear to be a functional counterpart of Net1p.

### The SIN in checkpoint function

Fission yeast has a checkpoint that detects defects in CAR assembly or integrity [56,57]. The defective ring persists at the cell cortex, but cells complete mitosis, disassemble the mitotic spindle and initiate the next round of S-phase. The active checkpoint prevents entry into the next mitosis. SIN proteins remain in the asymmetric, late anaphase configuration, which is thought to reflect the active state [58]. This checkpoint depends upon the phosphoprotein phosphatase flp1p, the SIN, the mitotic inhibitor wee1p, the protein kinase lsk1p and the 14–3–3 protein rad24p [56,57,59–62]. Flp1p is regulated, at least in part, by sequestration in the nucleolus. At mitotic onset, it leaves the nucleolus, associating with the CAR, kinetochores, and mitotic spindle. SIN function is required for flp1p to remain in the cytoplasm, which seems to be essential for the CAR checkpoint to function.

The budding yeast counterparts of cdc16p and byr4p, called Bub2p and Bfa1p, are implicated in a checkpoint that senses spindle orientation. Recent studies cast doubt on whether such a checkpoint exists in fission yeast [63]. However, mutants in cdc16 and dma1 [10,42] cannot maintain a mitotic arrest at the non-permissive temperature. It is unclear at present how the spindle assembly checkpoint and the SIN cross-talk.

### The SIN in the meiotic cycle

The meiotic cell cycle does not have a formal cytokinesis phase. After the two nuclear divisions, the four haploid nuclei are packaged into spores, whose synthesis starts from the SPB. The effect of inactivating some SIN components upon meiosis has been analysed. It has been found that loss of SIN function does not seem to affect the two meiotic divisions, but that it blocks spore formation, and appears to prevent proper encapsulation of the nucleus by the forespore membrane. Localization of SIN proteins shows that, in contrast with the mitotic cycle, none of them shows any asymmetry in either meiotic division; however, proteins such as cdc7p and sid1p–cdc14p do not appear on the meiotic SPBs until the second meiotic division [64]. The targets of the SIN in meiosis have not yet been identified.

### Is the SIN present in other organisms?

Direct, or functional, counterparts of most of the SIN proteins have been identified in budding yeast, where it is known as the mitotic exit network, or MEN. The only SIN proteins that do not have clear counterparts in Saccharomyces cerevisiae are cdc14p and sid1p. However, it has been suggested that in budding yeast, the cdc7p orthologue (Cdc15p) activates the sid2p orthologue (Dbf2p) directly [65]. In addition to cytokinesis, the MEN regulates cyclin degradation and exit from mitosis, which the SIN does not (for a reviews, see [6–8,66]). Some SIN proteins, such as plo1p, mob1p, and sid2p have clear counterparts in higher eukaryotes. A polo-like kinase is involved in cytokinesis (for review, see [67]) in mammalian cells. Sid2p is a member of the NDR-family of protein kinases, which function as tumour suppressors (for reviews, see [68,69]) and mob1-family proteins have also been implicated in cell division and proliferation [70–72]. Centriolin, which has a short domain in common with cdc11p, is required for abscission at the end of cytokinesis [73,74]. Counterparts of other SIN proteins are harder to identify in higher organisms, and though some of them seem to be present in plants, it is unclear whether they fulfil the same role as in fission yeast (reviewed in [75]). The ability to identify only the ‘top’ and ‘bottom’ of the SIN may reflect the differences in time between defining the division site and the initiation of cytokinesis between fission yeast and animal cells.

The Authors Journal compilation ©2008 Biochemical Society

V.S. thanks the Swiss National Science Foundation, ISREC and EPFL for financial support.

### References


13 Furge, K.A., Wong, K., Armstrong, J., Balasubramanian, M. and Albright,


26 Rosenberg, J.A., Tomlin, G.C., McDonald, W.H., Nuytinck, S., Feoktistova,


