Cross Talk between NDR Kinase Pathways Coordinates Cytokinesis with Cell Separation in *Schizosaccharomyces pombe*

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NDR (nuclear Dbf-2-related) kinases constitute key regulatory nodes in signaling networks that control multiple biological processes such as growth, proliferation, mitotic exit, morphogenesis, and apoptosis. Two NDR pathways called the septation initiation network (SIN) and the morphogenesis Orb6 network (MOR) exist in the fission yeast *Schizosaccharomyces pombe*. The SIN promotes cytokinesis, and the MOR drives cell separation at the end of cytokinesis and polarized growth during interphase. We showed previously that cross talk exists between these two pathways, with the SIN inhibiting the MOR during cytokinesis through phosphorylation of the MOR component Nak1 by the SIN Sid2 kinase. The reason for this inhibition remained unclear. We show here that failure to inhibit MOR signaling during cytokinesis results in cell lysis at the site of septum formation. Time-lapse analysis revealed that MOR signaling during cytokinesis causes cells to prematurely initiate septum degradation/cell separation. The cell lysis phenotype is due to premature initiation of cell separation because it can be rescued by mutations in genes required for cell separation/septum degradation. We also shed further light on how the SIN inhibits the MOR. Sid2 phosphorylation of the MOR proteins Sog2 and Nak1 is required to prevent cell lysis during cytokinesis. Together, these results show that SIN inhibition of the MOR enforces proper temporal ordering of cytokinetic events.

NDR signaling network analogous to the hippo pathway in mammalian cells and the mitotic exit network (MEN) in the budding yeast *Saccharomyces cerevisiae* (1–4). SIN signaling is essential for actomyosin ring assembly and constriction as well as for septum formation. The SIN also regulates mitotic entry, spindle checkpoint inactivation, spindle elongation, telophase nuclear positioning, and inhibition of polarized growth during cytokinesis (5–9). Recent work has begun to identify targets of the SIN, which include cytoskeletal and signaling proteins (5, 8, 10–13). SIN signaling is under precise temporal control; the SIN is activated in the anaphase of mitosis and inactivated upon completion of actomyosin ring constriction and septum formation (14). Regulation of other cell cycle signaling networks by the SIN may be important for properly coordinating cell cycle events. For example, the SIN inhibits a second conserved NDR kinase pathway called the morphogenesis Orb6 network (MOR) (9), which is analogous to the RAM (regulation of Ace2 and morphogenesis) network in budding yeast (3). The MOR normally drives septum degradation and cell separation following actomyosin ring constriction as well as polarized growth during the interphase by promoting localization of the actin cytoskeleton to cell tips (15). In mitosis, the SIN and other proteins cause actin to reorganize at the cell division site for cytokinesis. The SIN blocks polarized cell growth during cytokinesis by inhibiting the MOR pathway. Constitutive activation of the MOR pathway allows cells to perform polarized growth even when the SIN is active and impairs cell viability when the cytokinetic machinery is compromised (9). Several issues regarding SIN inhibition of the MOR remain. For example, it is unclear why MOR activity is toxic during cytokinesis. In addition, how the SIN inhibits the MOR is not fully explained. MOR pathway proteins Nak1 kinase and its Sog2 binding partner were identified as two targets of the SIN Sid2 effector kinase (12), with Sid2 phosphorylation of Nak1 contributing to SIN inhibition of the MOR during cytokinesis. The function of Sid2 phosphorylation of Sog2 was not determined. However, disruption of Sid2 phosphorylation of Nak1 bypasses the effects of the SIN on MOR signaling only partially, suggesting that the SIN may act on additional targets to fully inhibit the MOR.

Here we show that SIN inhibition of the MOR requires Sid2 phosphorylation of both Nak1 and Sog2. In addition, we find that failure of the SIN to inhibit the MOR causes severe lysis defects during cytokinesis. The cell lysis defects occur because improper MOR signaling prematurely triggers the cell separation program. Taking the data together, this report reveals the importance of coordinating SIN and MOR activities during cytokinesis.

**MATERIALS AND METHODS**

Yeast strains and growth conditions. *S. pombe* strains used in this study are listed in Table 1. General yeast techniques and manipulations were carried out as previously described (16). Cells were grown either in YE (yeast extract) medium or in Edinburgh minimal medium (EMM) with appropriate supplements. In the case of cells displaying a lethal cell lysis phenotype, 1.2 M sorbitol was added to the medium as needed. All strains were cultured at 25°C except for temperature-sensitive strains, which were grown either in YE (yeast extract) medium or in Edinburgh minimal medium (EMM) with appropriate supplements. In the case of cells displaying a lethal cell lysis phenotype, 1.2 M sorbitol was added to the medium as needed. All strains were cultured at 25°C except for temperature-sensitive strains, which were grown either at the permissive temperature of 25°C or at the restrictive temperature of 36°C as indicated. *S. pombe* transformations were carried out using either a lithium acetate method (17) or electroporation (18). DNA was prepared from bacteria and isolated from agarose gels using QIagen kits.

**Microscopy/time-lapse analysis.** Cells were either fixed in methanol or visualized live. DNA was visualized by staining with DAPI (4',6'-diamidino-2-phenylindole) (Sigma-Aldrich) at 2 μg/ml. Time-lapse analy-
sis to visualize lysis in myo2-YFP+Nak1-Mor2 or rlc1-GFP+Nak1-Mor2 cells was done using a Nikon Eclipse E600 fluorescence microscope coupled to a cooled charge-coupled-device camera (ORCA-ER; Hamamatsu, Bridgewater, NJ). Image processing and analysis were carried out with IPLab Spectrum software (Signal Analytics, Vienna, VA) and ImageJ software (National Institutes of Health). A log-phase cell suspension (2 × 10^6 cells/mL) was placed on a microscope slide between a 2% YE agar slab and a coverslip, sealed with Valap, and imaged for 30 min to 2 h. To follow the dynamics of the actomyosin ring and septum, time-lapse movies were produced from frames taken every 2 min with a 50× oil objective (Nikon) (numerical aperture [NA], 0.9). Time-lapse movies to visualize ring dynamics in LifeAct-GFP pcp1-mcherry cells growing in medium containing sorbitol were acquired using an inverted microscope (TE 2000-E; Nikon) equipped with a spinning disk confocal system (CSU10B; So Lamar Technology Group) utilizing MetaMorph software. A time-lapse Z-series of images was captured using a 60× Plan Apo oil objective (Nikon) (NA, 1.4) and a camera (MGI EMCCD; Rolera). Cellular actin (Lifeact-green fluorescent protein [LifeAct-GFP]), spindles, microtubules, and cell septa (differential interference contrast [DIC]) were simultaneously monitored by collecting a Z-stack of nine images (0.5 μM wide) every 2.5 min for about 180 to 250 min. Time-lapse images were processed using Velocity three-dimensional (3D) image analysis software (PerkinElmer) and ImageJ software (National Institutes of Health). Fluorescence intensity measurements (average intensity) for LifeAct-GFP at the actomyosin ring and cell tips were performed using ImageJ software (National Institutes of Health) and Microsoft Excel.

### RESULTS

Unregulated MOR signaling causes cell lysis during cytokinesis.

We previously showed that a fusion between two MOR pathway proteins, the Nak1 kinase and the Mor2 scaffold protein (Nak1-Mor2), could potently bypass the block in polarized growth imposed by constitutive SIN signaling (12). We also noticed that expression of the Nak1-Mor2 fusion is highly toxic in wild-type cells. The only way we could generate cells expressing the fusion protein was by expressing it from an attenuated version of the thiamine-repressible mnt1 promoter (mnt41) and maintaining the cells in the presence of thiamine on plates containing 1.2 M sorbitol. Induction of Nak1-Mor2 expression in wild-type cells caused a high frequency of cell lysis in septating wild-type cells but not in cells where actomyosin ring assembly was blocked using the cdc3–124 profilin mutation (12), suggesting a lysis defect specific to cytokinesis and not interphase. Furthermore, this defect could be rescued by addition of an osmotic stabilizer (1.2 M sorbitol) to the culture medium (Fig. 1). The rescue of cell lysis in septating cells by addition of 1.2 M sorbitol suggested that the Nak1-Mor2-expressing cells might have a cytokinesis-specific cell wall defect, as the lysis phenotype of cell wall mutants can typically be rescued by osmotic stabilizers. To determine the precise stage of cytokinesis/septation during which lysis occurs, we performed time-lapse analyses on cells expressing the Nak1-Mor2 fusion and a marker for the actomyosin ring (either yellow fluorescent protein-Myo2 [YFP-Myo2] or Rlc1-GFP) (Fig. 2A). In separate time-lapse experiments, septum formation was also followed using DIC (Fig. 2B). Cytokinesis in S. pombe normally proceeds through an orderly series of events (19). The actomyosin ring forms in early mitosis; then, the SIN becomes active in anaphase and causes the primary septum to form behind the constricting actomyosin ring. Completion of ring constriction and septum formation coincides with SIN inactivation. Once the primary septum is completed, the cell forms a new cell wall behind the septum and secretes enzymes to degrade the septum and bring about cell separation. The MOR pathway is required (post-SIN inactivation) for septum degradation and cell separation (20), although the mechanism is not

### Table 1: Fission yeast strains used in this study

| Strain     | Genotype                                    | Source                     |
|------------|---------------------------------------------|----------------------------|
| YDM105     | ade6-210 leu1-32 ura4-D18h                 | Laboratory stock           |
| YDM3440    | cdc3-124 cdc16-116 ade6-M21X ura4-D18 leu1-32 h | Laboratory stock           |
| YDM4210    | orb6-25 ade6-216 leu1-32 ura4-D18 h       | Laboratory stock           |
| YDM5740    | kanMX6-Pmyo2-mYFP-ura4-2 ade6-M210 leu1-32 ura4-D18 h | Jian-Qiu Wu               |
| YDM4223    | rlc1-GFP::ura4-4 cdc7-GFP::ura4-4 leu1-32 h | Laboratory stock           |
| YDM2028    | acg2Δ::kanR ura4-D18 ade6-216 leu1-32 h    | Carlos Vasquez             |
| YDM2801    | agn1Δ::ura4-4 D18 ade6-32 h                | Carlos Vasquez             |
| YDM2342    | mid2Δ::ura4-4 his3-D1 leu1-32 D18 ade6-M21X h | Goold laboratory          |
| YDM6296    | cdc3-124 cdc16-116 nmt1-sog2-5A h          | Laboratory stock           |
| YDM6318    | cdc3-124 cdc16-116 nmt1-sog2-5A::leu1-1 h  | Laboratory stock           |
| YDM6303    | cdc3-124 cdc16-116 nmt1-sog2-5A::leu1-1 + pDUAL-GFH41c-Nak1-5A (ura4+) ura4-D18 leu1-32 ade6-210 h | Laboratory stock           |
| YDM6307    | cdc3-124 cdc16-116 + pDUAL-GFH41c-Nak1-5A (ura4+) ura4-D18 leu1-32 ade6-210 h | Laboratory stock           |
| YDM6320    | cdc3-124 cdc16-116 nmt1-sog2-5A::leu1-1 + pDUAL-GFH41c-Nak1-5A (ura4+) ura4-D18 leu1-32 ade6-210 h | Laboratory stock           |
| YDM6301    | nmt1-sog2-5A::leu1-1 + pDUAL-GFH41c-Nak1-5A (ura4+) ura4-D18 leu1-32 ade6-210 h | Laboratory stock           |
| YDM6334    | nmt1-sog2-5A::leu1-1 + pDUAL-GFH41c-Nak1 (ura4+) ura4-D18 leu1-32 ade6-210 h | Laboratory stock           |
| YDM6336    | nmt1-sog2-5A::leu1-1 + pDUAL-GFH41c-Nak1-5A ura4-D18 ade6-210 h | Laboratory stock           |
| YDM6223    | pAct-LifeAct-GFP::lea1-1 ade6-210 ura4-D18 leu1-32 lys1 h | Balasubramanian laboratory |
| YDM6263    | pAct-LifeAct-GFP::lea1-1 + pRep41-Nak1-Mor2 ade6-210 ura4-D18 leu1-32 h | Laboratory stock           |
FIG 1 Inability to inhibit the MOR during late mitosis results in growth defects due to cell lysis during cytokinesis. (A) Representative images of wild-type (WT) cells expressing either vector or the Nak1-Mor2 fusion for 19 h at 25°C in medium lacking thiamine to induce expression of the fusion protein. White arrows indicate the point of lysis in the cell middle and expulsion of cytoplasmic material. (B) Quantitation of the lysis defect in cells, grown as described in the figure panel, is represented as the percentage of lysed cells. 

Our time-lapse data showed that cells lysed only during cytokinesis and that lysis was occurring at various points during cytokinesis (Fig. 2). Cell lysis was observed after ring assembly but before completion of constriction (11 of 14 cells), as well as after completion of ring constriction during cell separation (3 of 14 cells) (Fig. 2A). Consistent with these results, time-lapse analysis of septum formation in Nak1-Mor2-expressing cells using DIC microscopy showed that cell lysis occurred both in cells that were forming or had just completed septum formation (6 of 10 cells) and in cells that had completed septum formation and were undergoing cell separation (4 of 10 cells) (Fig. 2B). It is not clear why the timing of cell lysis during cytokinesis is somewhat variable. Possible explanations include stochastic variations in the delivery or activation of cell wall lytic enzymes or the fact that the Nak1-Mor2 fusion is carried episomally and therefore varies in copy number between cells. Therefore, inappropriate MOR activity causes cell lysis during cytokinesis, but cell lysis does not seem to be restricted to just one phase of cytokinesis.

MOR signaling during cytokinesis causes cell lysis by triggering premature cell separation. Previous studies showed that the SIN and MOR promote distinct actin polarity programs, with the SIN driving actomyosin ring assembly and the MOR directing actin to the cell tips for polarized growth. Constitutive SIN signaling triggers actomyosin ring assembly (21) and inactivates the MOR to block polarization of actin to the cell tips and cell elongation (9). Simultaneous SIN and MOR activation results in cells attempting to perform both cytokinesis and polarized growth, with actin at both the cell middle and the cell tips (9). Thus, one reason for the toxicity of MOR activity during cytokinesis could be competition for actin and other cytoskeletal components. To test whether the Nak1-Mor2 fusion might interfere with actin organization during cytokinesis in wild-type cells, we examined actin organization in Nak1-Mor2-expressing (and control) cells undergoing cytokinesis using GFP-tagged Lifeact to monitor actin organization (Fig. 3). (Note that cells were grown in the presence of 1.2 M sorbitol to avoid cell lysis.) Consistent with our observations described above (Fig. 2A), actomyosin rings in Nak1-Mor2-expressing cells appeared normal compared to those in wild-type cells (Fig. 3A to C). However, when we measured the ratio of Lifeact-GFP fluorescence at the actomyosin ring to that in the rest of the cell (Fig. 3D), we observed significantly more actin outside the ring (mostly at cell tips) in cells expressing the Nak1-Mor2 fusion. It was unclear whether the dual ring/cell tip actin localization observed in Nak1-Mor2-expressing cells could interfere with actomyosin ring function or could account for the cell lysis phenotype. To examine the effect of Nak1-Mor2 expression on the timing of the phases of cytokinesis, we performed time-lapse analyses of Nak1-Mor2-expressing (and control) cells using GFP-tagged Lifeact to monitor actin organization. Cells were cultured in media containing the osmotic stabilizer sorbitol to prevent cell lysis. In order to follow the timing of the cytokinetic process closely, we also monitored the mcherry-tagged SPB Pcp1 marker.

The timing of most events seen with wild-type cells was similar to that seen with Nak1-Mor2-expressing cells, with the times required for ring formation as well as the durations of ring constriction not showing significant differences. Nak1-Mor2-expressing cells showed a slight but significant increase in the time between completion of actomyosin ring assembly and initiation of ring constriction. Most strikingly, Nak1-Mor2-expressing cells showed a very significant acceleration of cell separation compared to control cells (Fig. 3C). We concluded that early cell separation in cells expressing the Nak1-Mor2 fusion protein indicates premature activation of the cell separation process in these cells. Premature initiation of septum degradation and cell separation likely accounts for the cell lysis observed when Nak1-Mor2 cells are grown in media without sorbitol. Inappropriate cell wall degradation before or during septum assembly may cause weaknesses in the cell wall/septum that cause cell lysis. Taken together, these results demonstrate that inability to restrain MOR signaling during cytokinesis disrupts the normal order of cytokinetic events, leading to precocious initiation of cell separation/septum degradation.

MOR-induced cell lysis depends on genes required for cell separation. Because the MOR is normally required to bring about cell separation through degradation of the primary septum following completion of ring constriction, we hypothesized that the cell lysis phenotype induced by Nak1-Mor2 expression might be
caused by MOR-dependent degradation of the primary septa before the new cell wall has been properly assembled at the cell division site. If this is the case, then deletion of the enzymes that degrade the primary septa and are required for cell separation should rescue the cell lysis phenotype. Consistent with this idea, deletion of the Ace2 transcription factor, which is required for cell cycle-dependent transcription of genes required for cell separation (22), or deletion of two of its targets involved in cell separation (Agn1 and Mid2) (23–25) rescues the cell lysis and growth defect caused by the Nak1-Mor2 fusion (Fig. 4A to C). These results suggest that Ace2 functions downstream of or parallel to the MOR pathway for cell separation. Consistent with this notion, the cell separation defect in the orb6–25 MOR mutant seen at a restrictive temperature is rescued by ectopic expression of Ace2 or its target Mid2, as indicated by a decrease in the percentage of binucleate orb6–25 cells with septa after arrest at the restrictive temperature (Fig. 4D). Taken together, these results suggest that premature initiation of cell separation caused by ectopic MOR signaling results in cell lysis. Therefore, SIN inhibition of the MOR likely functions to prevent cells from undergoing cell separation before completion of cytokinesis.

**SIN inhibition of the MOR occurs in part through phosphorylation of Sog2.** Our experiments using the Nak1-Mor2 fusion suggest that failure of the SIN to inhibit the MOR during cytokinesis results in cell lysis. However, the Nak1-Mor2 fusion might not perfectly recapitulate loss of SIN inhibition of the MOR because, although the fusion is resistant to SIN inhibition, it also triggers aberrantly high levels of MOR activity compared to wild-type cell results (12). We previously showed that disruption of Sid2 phosphorylation of Nak1, using the nak1–5A mutant, partially relieves SIN inhibition of the MOR. Interestingly, Nak1-5A expression did not cause a cell lysis phenotype or completely bypass SIN inhibition, suggesting that the SIN might interfere with MOR activity through additional targets. One such target is the Nak1-associated essential Sog2 protein (26), which we showed is also phosphorylated by Sid2 (12). To test this idea, we attempted to create a nonphosphorylatable form of Sog2. Sog2 contains 11 potential Sid2 phosphorylation sites (RXXS motifs), three of which (S421, S449, and S665) were identified as phosphorylated in vivo using mass spectrometry in our earlier study (12). Two additional RXXS sites (S312 and S657) were identified as phosphorylated using mass spectrometric analysis in an independent study (27). All 5 sites were mutated to alanine to create a Sog2-5A mutant (Fig. 5A). To test whether Sog2-5A can bypass SIN inhibition of polarized growth, we expressed it in cells with constitutive SIN signaling (i.e., cdc16–116 cdc3–124 cells, where the cdc16–116 mutation is used to constitutively activate the SIN and the cdc3–124 mutation blocks septum formation and avoids nonspecific effects...
FIG 3 Cells with unregulated MOR signaling separate prematurely. (A and B) Cells carrying the vector control (A) or the Nak1-Mor2 expression plasmid (B) were grown in medium lacking thiamine for 19 h at 25°C to induce expression of the Nak1-Mor2 fusion. The medium also contained sorbitol (osmotic stabilizer) to prevent cell lysis. Cells were monitored by fluorescence and DIC time-lapse microscopy for 2 to 3 h. Images taken at 7.5-min intervals from the time-lapse movie are shown. The actomyosin (AM) ring dynamics were followed by monitoring the GFP-tagged Lifeact peptide that binds actin. SPB separation was monitored using the Pcp1 mcherry-tagged SPB marker. DIC movies of the cells to observe formation of the division septum were simultaneously filmed and are also included in the panels. (C) Graphs representing quantification of distance between SPBs, time taken to complete the indicated cytokinetic phases, and time between different cytokinetic phases (in red) are shown. The time at which SPBs initiated separation was assigned as time zero. The cytokinetic process was monitored from start (T0) to finish in at least 17 cells carrying the vector and 11 cells expressing the Nak1-Mor2 fusion. Statistical analysis using unpaired t tests was done to compare the times required for completion of different cytokinetic phases (in red). These analyses revealed that the time between the appearance of the actomyosin ring and the initiation of ring constriction differed significantly (***) between cells carrying the vector control (left graph) and those expressing the Nak1-Mor2 fusion (right graph). Also, the differences in time between completion of ring constriction and initiation of cell separation were extremely
on cell growth triggered by ectopic septation upon SIN activation). Expression of Sog2-5A alone was insufficient to bypass the polarized growth arrest implemented upon SIN activation and resembled results observed in vector control cells (Fig. 5B and C). Expression of a Nak1-5A mutant showed a significant increase in cell length over time as shown previously (12). Interestingly, coexpression of both Sog2-5A and Nak1-5A (in cdc16–116 cdc3–124 cells) caused heightened cell elongation not seen upon coexpression of wild-type Sog2 with the Nak1-5A mutant (Fig. 5B and C). In addition, expression of Nak1-5A and Sog2-5A in wild-type cells also caused a cell lysis phenotype similar to, but not quite as strong as, that observed when Nak1-Mor2 is expressed (Fig. 5D and E). This phenotype is not observed in Nak1-5A Sog2-wt or Nak1-wt Sog2-5A-expressing cells (Fig. 5E), suggesting that Sid2 phosphorylation of both Nak1 and Sog2 is important to prevent premature cell separation and cell lysis during cytokinesis.

**DISCUSSION**

This report shows how SIN inhibition of the MOR pathway is required for orderly execution of late mitotic events. In a normal cell cycle, SIN signaling during the anaphase and telophase drives actomyosin ring assembly, constriction, and septum formation while at the same time inhibiting MOR-mediated cell separation and polarized growth. Completion of actomyosin ring constriction and septum formation events triggers SIN inactivation (14), allowing the MOR to become active and initiate cell separation and interphase polarity. Interestingly, SIN mutants at semipermissive temperature have a cell lysis phenotype during cytokinesis identical to that of cells with constitutive MOR signaling (28). The lysis phenotype in SIN mutants at a semipermissive temperature can be rescued by mutations in the MOR pathway (9), consistent with incomplete or weakened septa in SIN mutants being sensitive to premature septum degradation caused by a compromised inhibition of the MOR. MOR signaling during cytokinesis also directs some actin away from the actomyosin ring toward cell tips, which might be expected to cause defects in actomyosin ring function. Surprisingly, with the exception of a slight delay in initiation of actomyosin ring constriction, MOR activity during cytokinesis did not cause major problems in actomyosin ring assembly or statistically significant (****) between cells expressing the vector (left graph) and those expressing the Nak1-Mor2 fusion (right graph) (**, P = 0.0065; ****, P < 0.0001). (D) Unregulated MOR can direct actin away from the cell middle during cytokinesis. Average actin fluorescence intensities were measured at the cell middle (the medial ring) and at the cell ends (the regions to the right and left of the medial ring) using Image J software (FIJI). Cells from the time-lapse movies shown in Fig. 3 were measured at the time of actomyosin ring assembly. A graph indicating the ratio of fluorescence measured at cell middle (ring) to that at the cell ends is shown. Statistical analysis done using unpaired t tests shows that the differences in ratios between the cells expressing the vector (pREP41) and those expressing the Nak1-Mor2 fusion were very statistically significant (P = 0.0015).
constriction, although it is possible that defects might be more pronounced under conditions where cytokinesis is already compromised.

Conceptually similar regulation of the budding yeast RAM pathway (the MOR equivalent) has been implicated in coordinating late cytokinetic events. As with the MOR, the RAM pathway promotes septum degradation/cell separation and cell polarity. How the RAM pathway promotes cell separation has been well worked out. The RAM promotes transcriptional and translational control of cell separation genes through regulation of the Ace2 transcription factor and the Ssd1 RNA binding protein (3). How the MOR drives cell separation is not known,

FIG 5 SIN inhibition of polarized growth occurs in part through phosphorylation of Sog2. (A) Diagram of Sog2. The five Sid2 phosphorylation sites (RXXS) mutated to alanine (indicated by the letter A) are indicated. (B and C) Coexpression of Sog2-5A and Nak1-5A enhances bypass of SIN inhibition of cell elongation. cdc16–116 cdc3–124 cells with the indicated plasmids were grown at 25°C in the absence of thiamine for 19 h (to induce expression of the indicated proteins) and then shifted to 36°C to activate the SIN. Samples were collected every 3 h. (B) Images representing the indicated cells at the 0-h and 9-h time points are shown. Cells were stained with DAPI and visualized by a combination of fluorescence and DIC microscopy. (C) Cell lengths of 50 cells were measured for each time point. Error bars denote SD values obtained from the average cell lengths measured from three separate experiments. Statistical analysis using unpaired t tests (asterisks) showed that the differences in cell lengths between cells expressing the Nak1-5A mutant and cells coexpressing both Nak1-5A and Sog2-5A were statistically significant (P < 0.0001). The differences in cell lengths between cells coexpressing Nak1-5A and Sog2-5A and the ones coexpressing Nak1-5A and wild-type Sog2 were also statistically significant (P < 0.0001). (D) Representative images of wild-type cells coexpressing the Sog2-5A and Nak1-5A proteins after 19 h (at 25°C) in medium lacking thiamine to induce expression of the proteins are shown. White arrows indicate the point of lysis in the cell middle. (E) Representative images of colonies of wild-type cells coexpressing the indicated Sog2 and Nak1 proteins after 36 h (at 25°C) on agar plates lacking thiamine to induce expression of the indicated proteins. Black arrows indicate areas with lysed cells.
although our data suggest that it could act upstream of or in parallel to the Ace2 transcription factor. The RAM network is under precise regulatory control, with mitotic Cdk1 activity working through multiple mechanisms to prevent premature RAM activation and cell separation (3, 29). Whether the MEN acts, like the SIN, to inhibit RAM activity until cytokinesis is complete is not known.

We previously described how the SIN inhibits the MOR in part through Sid2 kinase phosphorylation of Nak1, which inhibits the interaction between Nak1 and the Mor2 MOR scaffold protein (12). Here we identified an additional mechanism of MOR inhibition by the SIN through Sid2 phosphorylation of Sog2. Although removal of Sid2 phosphorylation sites in Sog2 (Sog2-5A) does not have an obvious phenotype on its own, it is capable of enhancing the ability of Nak1-5A to induce polarized growth in the presence of SIN signaling. In addition, cells expressing Sog2-5A and Nak1-5A together display a cell lysis phenotype not observed when either single mutant is expressed. The lysis phenotype is similar to, but not as strong as, that of Nak1-Mor2-expressing cells. The weaker phenotype of Sog2-5A Nak1-5A cells compared to Nak1-Mor2 cells could be attributed to incomplete identification of Sid2 phosphorylation sites on these or other proteins. It is also possible that the strong phenotype of Nak1-Mor2 cells occurs because this construct causes hyperactivation of the Orb6 downstream kinase (12). In future studies, it will be important to determine the mechanism by which Sid2 phosphorylation of Sog2 regulates MOR signaling. Taken together, these results further our knowledge of cross-regulation between NDR kinase pathways in fission yeast and how this regulation orders the final steps of cytokinesis. This work also raises the intriguing possibility that similar cross talk between homologous mammalian Ndr kinase pathways may also exist.

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