Inhibition of Cyclin-dependent Kinase 1 Induces Cytokinesis without Chromosome Segregation in an ECT2 and MgcRacGAP-dependent Manner*

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Cleavage furrow formation marks the onset of cell division during early anaphase. The small GTPase RhoA and its regulators ECT2 and MgcRacGAP have been implicated in furrow formation in mammalian cells, but the signaling upstream of these molecules remains unclear. We now show that the inhibition of cyclin-dependent kinase (Cdk1) is sufficient to initiate cytokinesis. When mitotically synchronized cells were treated with the Cdk-specific inhibitor BMI-1026, the initiation of cytokinesis was precociously before chromosomal separation. Cytokinesis was also induced by the Cdk1-specific inhibitor purvalanol A but not by Cdk2/Cdk5- or Cdk4-specific inhibitors. Consistent with initiation of precocious cytokinesis by Cdk1 inhibition, introduction of anti-Cdk1 monoclonal antibody resulted in cells with aberrant nuclei. Depolymerization of mitotic spindles by nocodazole inhibited BMI-1026-induced precocious cytokinesis. However, in the presence of a low concentration of nocodazole, BMI-1026 induced excessive membrane blebbing, which appeared to be caused by formation of ectopic cleavage furrows. Depletion of ECT2 or MgcRacGAP by RNA interference abolished both of the phenotypes (precocious furrowing after nocodazole release and excessive blebbing in the presence of nocodazole). RNA interference of RhoA or expression of dominant-negative RhoA efficiently reduced both phenotypes. RhoA was localized at the cleavage furrow or at the necks of blebs. We propose that Cdk1 inactivation is sufficient to activate a signaling pathway leading to cytokinesis, which emanates from mitotic spindles and is regulated by ECT2, MgcRacGAP, and RhoA. Chemical induction of cytokinesis will be a valuable tool to study the initiation mechanism of cytokinesis.

One of the dynamic mitotic processes, anaphase, is also the beginning of cytokinesis. During anaphase, two sister chromatids separate and move to spindle poles, and the cell elongates, as do the pole-to-pole spindles. Simultaneously, a cleavage furrow starts to form in the equatorial plane. Positioning of the cleavage furrow is induced by signals from the mitotic spindle during early anaphase. The molecular mechanism of cleavage furrow positioning and its regulation remains largely unknown. Recent data suggest that a signal emanating from the central spindle is critical to initiate cytokinesis (1). It has been reported that the small GTPase RhoA accumulates at the cleavage furrow (2). ECT2, a guanine nucleotide exchange factor for the Rho family GTPases, is essential for cytokinesis (3, 4) as well as epithelial cell polarity (5). ECT2 is regulated by an autoinhibitory mechanism as well as nuclear sequestration in interphase (6). A mutation in the pebble gene, the Drosophila orthologue of human ECT2, inhibits cytokinesis in fly embryos (7). Pebble interacts with RacGAP50C, the Drosophila orthologue of human MgcRacGAP, which connects the contractile ring to cortical microtubules at the site of furrow ingression (8). MgcRacGAP, a GTPase-activating protein for the Rho GTPases, is also involved in cytokinesis (9, 10). HeLa cells depleted of MgcRacGAP using siRNA2 do not assemble well developed central spindles and fail to complete cell division (11). Binucleated blastomeres containing chromatid bridges were observed at early embryonic stages of MgcRacGAP-null mice (12). Therefore, MgcRacGAP is required for both mitosis and cytokinesis. Inhibition of Cyclin-dependent Kinase 1 Induces Cytokinesis without Chromosome Segregation in an ECT2 and MgcRacGAP-dependent Manner*

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mitotic cells by BMI-1026. We also found that RhoA and two Rho regulators, ECT2 and MgcRacGAP, are essential to furrow induction induced by BMI-1026. These observations suggest that inhibition of Cdk1 activity is sufficient to induce cytokinesis, which is regulated through ECT2, MgcRacGAP, and RhoA.

EXPERIMENTAL PROCEDURES

Cell Culture and Synchronization—The HeLa cell line and its derivative, which expresses GFP-histone H2B, were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 100 units/ml penicillin G plus 100 μg/ml streptomycin (Invitrogen). Synchronization of the cell cycle was carried out by double thymidine block (21). In brief, cells were arrested for 14 h with 2.5 mM thymidine (Sigma) followed by an 8-h release. After the second thymidine treatment, cells were released into fresh medium containing 40 ng/ml of nocodazole (Sigma) to arrest the cells at prometaphase. In some experiments, mitotic cells were collected by the knock-off (mechanical disruption) procedure after nocodazole treatment at a concentration of 40 ng/ml for 3–6 h. The remaining adherent cells were used as interphase cells. BMI-1026 has been described (20). Purvalanol A, PNU112455A, and Cdk4 inhibitor were from Calbiochem, San Diego, CA.

Western Blot Analysis—Cell lysates were clarified by centrifugation (14,000 × g, 20 min, 4 °C), and the protein concentration was determined using the BCA assay kit (Pierce). Samples were separated and analyzed on 6 or 8–16% gradient SDS-PAGE gels (Invitrogen) and then probed with the primary antibody for 1–6 h at room temperature, and incubated with the secondary antibody for 1 h at room temperature. Bound antibody was detected using horseradish peroxidase-conjugated anti-rabbit and anti-mouse IgG. The signals were detected using ECL chemiluminescence reagents (Amersham Biosciences).

Immunocytochemistry—Cells grown on coverslips were fixed with 100% methanol for 20 min, permeabilized for 10 min with 0.1% Triton X-100 in PBS, and then washed with PBS. Samples were incubated with PBS containing 2% serum for 1 h and then with a rabbit anti-survivin antibody (Abcam) and a mouse anti-γ-tubulin antibody (Sigma) for 1 h. Samples were then treated with FITC-conjugated anti-rabbit IgG, Alexa 488-conjugated anti-mouse IgG (Molecular Probe, Eugene, OR) and DAPI (Sigma). Images were observed with the Zeiss Axiosvert S-100 inverted microscope equipped with an environmental chamber. Time-lapse images were captured at 3-min intervals by the Photometric digital camera controlled by Openlab software.

RESULTS

Cdk1 Inhibition Precociously Induces Cleavage Furrow Ingression in Mitotic Cells—To test the effects of the Cdk1 inhibition on cytokinesis, HeLa cells expressing GFP-histone H2B were arrested at prometaphase by nocodazole, released from the arrest, and then treated with the Cdk inhibitor BMI-1026. The morphological changes were recorded by time-lapse microscopy (Fig. 1A). Surprisingly, BMI-1026 potently induced cleavage furrow formation. Furrow ingression was observed at an earlier time point in BMI-1026-treated cells (10–15 min after release from nocodazole) than untreated cells (90 min after release from nocodazole). Moreover, although untreated cells started cytokinesis after chromosome separation, furrow ingression was initiated before chromosome separation in BMI-1026-treated cells. This phenotype, induced by BMI-1026 after nocodazole release, was very similar to the Cut phenotype (23). However, a population of BMI-1026-treated cells did not undergo abscission, and the emerging daughter cells subsequently merged to generate cells with aberrant nuclei. As BMI-1026 specifically inhibits mitotic Cdk but not other mitotic kinases such as Plk1 and Aurora A (20), it is likely that Cdk1 inhibition induces cytokinesis initiation. To confirm that BMI-1026 induces cytokinesis by inhibiting the Cdk1/cyclin B complex, we tested other Cdk inhibitors. Purvalanol A displays a narrow specificity to Cdk1/cyclin B (IC50 = 4 nM) compared with that to Cdk1/cyclin A (IC50 = 70 nM), Cdk2/cyclin E (IC50 = 35 nM), and Cdk5/p35 (IC50 = 75 nM). As shown in Fig. 1B, Purvalanol A induced furrow ingression in mitotic cells as BMI-1026 did. In contrast, the Cdk2/Cdk5-specific inhibitor PNU112455A or
Cdk4 inhibitor did not display this activity. These results suggest that Cdk1 inhibition is sufficient to induce cleavage furrow ingression.

We also utilized another method to inhibit Cdk1. We introduced affinity-purified anti-Cdk1 monoclonal antibody to asynchronous HeLa cells by Provecin. Consistent with the requirement of Cdk1 for the entry to M phase, the appearance of mitotic cells was strongly inhibited by anti-Cdk1 (Fig. 2B), suggesting that anti-Cdk1 effectively inhibited Cdk1 in these cells. A population of anti-Cdk1-transfected cells should enter M phase before Cdk1 was effectively inhibited. The inhibition of Cdk1 by anti-Cdk1 antibody after M phase entry might induce cleavage furrow ingression before chromosome separation as BMI-1026 did. Although control IgG-transfected cells generate cells with single nuclei, anti-Cdk1-introduced cells frequently contained multiple nuclei of different sizes (Fig. 2). These results support that inhibition of Cdk1 after M phase entry induces precocious cleavage furrow ingression before chromosome separation. Efficient generation of multinucleate cells by anti-Cdk1 also support the notion that abscission did not occur frequently after cleavage furrow ingression induced by Cdk1 inhibition. All of these results suggest that Cdk1 inhibition is sufficient to induce cleavage furrow ingression in mitotic cells.

Normal Mitotic Spindles Are Required for Furrowing Induced by Cdk1 Inhibition—There is accumulating evidence that cytokinesis is induced by signals emanating from overlapping mitotic spindles (1). To test whether spindles are required for furrow ingression induced by BMI-1026, we performed similar assays in the presence of the microtubule depolymerizing agent nocodazole. HeLa cells were arrested at prometaphase by nocodazole (40 ng/ml), released from the drug for 15 min, and then treated with 200 nM BMI-1026 or vehicle alone (control) at time 0. Cell morphology was recorded by time-lapse video microscopy. Representative frames are shown. Numbers indicate time after BMI-1026 addition (min). Arrows denote dividing cells. B, HeLa cells were treated with 40 ng/ml nocodazole for 6 h. Cells were released in normal culture medium for 15 min, and then the drugs indicated were added to the medium (BMI1026, 0.2 μM; Purvalanol A, 15 μM; Cdk2/5 inhibitor, 400 μM and Cdk4 inhibitor, 100 μM). Cell morphology was monitored with time-lapse microscopy 45 min after the drug addition. The percentages of the cells that underwent cell division are shown. The experiments were carried out three times, and at least 40 cells were counted in each experiment.
consistent with a previous report that BMI-1026 causes precocious mitotic exit in U2OS cells (20). Next, we examined the effects of partial depolymerization of mitotic spindles on induction of furrow ingestion by BMI-1026. In the presence of a low concentration of nocodazole (40 ng/ml), BMI-1026 induced excessive membrane blebbing in most of the cells (Fig. 3A, middle panels). Strong tubulin staining was observed in these blebs (see Fig. 7), but survivin was not concentrated in the blebs (data not shown). To test the possibility that incomplete depolymerization of mitotic spindles is responsible for this phenotype induced by BMI-1026, the microtubule stabilizing agent taxol was utilized in place of nocodazole. As shown in Fig. 3A, BMI-1026 also potently induced membrane blebbing in the presence of taxol. Localization of tubulin, but not survivin, was evident on the blebs induced by BMI-1026 in the presence of taxol (Fig. 3B, see also Fig. 7). However, survivin was concentrated at the structures, which could be identified as the midzone created by abnormal cytokinesis (Fig. 3B). We further tested the localization of two Rho regulators, ECT2 and MgcRacGAP, which are known to localize at the midbody during cytokinesis (4, 9). These proteins were localized at structures that were similar to the midbody, although cytokinesis was abnormal in these cells (Fig. 3B). Therefore, BMI-1026 appeared to induce both excessive blebbing and furrowing in the presence of taxol. Presumably, furrowing was initiated at the sites where microtubule bundling was evident and thus chromosomal passenger proteins and Rho regulators accumulated. Taken together, these results suggest that perturbation of normal mitotic spindles, but not their complete disappearance, is responsible for excessive membrane blebbing induced by BMI-1026.

ECT2 and MgcRacGAP Are Required for BMI-1026-induced Precocious Furrowing and Excessive Blebbing—It has been shown that two regulators of Rho GTPases, ECT2 and MgcRacGAP, are involved in cytokinesis (4, 9). We first tested whether cleavage furrow ingression induced by BMI-1026 after release from nocodazole is affected by knockdown of ECT2 or MgcRacGAP. HeLa cells expressing GFP-histone H2B were transfected with siRNAs for ECT2 or MgcRacGAP, arrested with nocodazole, released to fresh medium, and then treated with BMI-1026. These siRNAs efficiently knocked down ECT2 and MgcRacGAP as detected by immunoblotting (Fig. 4A). Whereas BMI-1026 induced precocious cleavage furrow ingression in 97% of control siRNA-transfected cells (n = 100), most of ECT2- or MgcRacGAP-siRNA-transfected cells spread without furrowing within 80 min after BMI-1026 treatment (Fig. 4B). Only a small population of ECT2-siRNA-transfected cells (10%) or MgcRacGAP-siRNA-transfected cells (16%) exhibited furrowing (n = 100). These results suggest that ECT2 and MgcRacGAP are required for BMI-1026-induced cytokinesis.

We next tested whether ECT2 and MgcRacGAP are involved in BMI-1026-induced membrane blebbing in the presence of nocodazole. HeLa cells expressing GFP-histone H2B were transfected with siRNA for ECT2 or MgcRacGAP, arrested with nocodazole (40 ng/ml), and then treated with BMI-1026. Whereas most of the control siRNA-treated cells (98%, n = 100) exhibited excessive blebbing (Fig. 4C, left, 30 min) and then spread (Fig. 4C, left, 72 min), most of ECT2-siRNA- or MgcRacGAP-siRNA transfectedants did not show this phenotype (Fig. 4C, middle and right). Only a minor fraction of ECT2-siRNA-transfected cells (5%) and MgcRacGAP siRNA-transfected cells (17%) showed excessive blebbing (n = 100). These results suggest that ECT2 and MgcRacGAP are required for the excessive blebbing induced by BMI-1026.

RhoA Knockdown Reduces BMI-1026-induced Precocious Furrowing and Membrane Blebbing—It has been reported that Rho GTPases play a critical role in cell division (24, 25). To test which Rho GTPase is responsible for furrow ingestion induced by BMI-1026, we knocked down representative Rho GTPases by RNAi (Fig. 5A). We found that knockdown of RhoA affected precocious cytokinesis initiation induced by BMI-1026 after nocodazole release, whereas knockdown of Rac1 or Cdc42 did not display significant effects (Fig. 5B). The effect of RhoA knockdown on furrowing was evident 15 min after BMI-1026 addition, but the number of cells with ingestion increased at later time points. Thus, RhoA depletion did not prevent BMI-1026-induced furrowing, but effectively delayed it. As the RhoA was efficiently depleted by siRNA transfection (Fig. 5A), weak inhibition of furrowing in RhoA siRNA-transfected cells might be attributed to compensatory mechanisms by other Rho GTPases. In contrast, the knockdown levels of Cdc42 and Rac1 were lower than that of RhoA. It is possible that residual levels of these GTPases are sufficient to induce cytokinesis.

We also investigated which Rho GTPase is responsible for excessive membrane blebbing induced by BMI-1026 in the presence of a low concentration of nocodazole. Although knockdown of Cdc42 or Rac1 did not exhibit significant effects on BMI-1026-induced blebbing, most of the cells transfected with RhoA siRNA spread without exhibiting excessive membrane blebbing (Fig. 6A). Therefore, RhoA appeared to play a role in BMI-1026-induced excessive blebbing in the presence of a low concentration of nocodazole. Although RhoA knockdown did not completely inhibit excessive blebbing, it efficiently delayed it (Fig. 6B).
Other Rho GTPases might compensate for this activity when RhoA is knocked down. As the knockdown levels of Cdc42 and Rac1 were lower than RhoA, it is also possible that Cdc42 or Rac1 plays some role in excessive blebbing.

It has been reported that RhoA is localized to the cleavage furrow during cytokinesis (24, 25). When mitotically arrested cells were released from nocodazole and then treated with BMI-1026, RhoA was concentrated at regions corresponding to the cleavage furrow and then at the midbody (Fig. 7, left panels). When BMI-1026 was added in the presence of a low concentration of nocodazole, RhoA was localized at the necks of blebs (Fig. 7, middle). BMI-1026-treated cells in the presence of taxol exhibited similar localization of RhoA (Fig. 7, right). Taken together, these results further support that the RhoA signaling pathway is involved in BMI-1026-induced precocious furrowing as well as excessive blebbing.

We also examined whether the Rho effector kinase, ROCK, contributes to BMI-1026-induced precocious cytokinesis in the presence of nocodazole, using the ROCK-specific inhibitor Y-27632 (26). However,
we could not observe any significant effect of Y-27632 on BMI-1026-induced furrowing at a final concentration of 100 μM, although cytoskeletal reorganization of Swiss 3T3 cells was completely inhibited at this concentration (data not shown). These results suggest that ROCK is not a critical regulator of BMI-1026-induced precocious cytokinesis.

**DISCUSSION**

Normal cytokinesis is properly coordinated by the spindle function to ensure accurate segregation of chromosomes. We found that treatment of mitotic cells by the Cdk inhibitor BMI-1026 or purvalanol A potently initiates cytokinesis, but without chromosome separation. The effects of the Cdk1 inhibitors on mitotic cells are surprising in two ways. First, the initiation of cytokinesis was induced chemically. Second, BMI-1026 treatment effectively uncoupled cytokinesis from chromosome separation. Similar to normal cytokinesis, two Rho regulators, ECT2 and MgcRacGAP, were required for BMI-1026-induced cytokinesis. We also found that RhoA is involved in BMI-1026-induced cytokinesis. We found that abscission is not always accompanied by BMI-1026-induced cytokinesis. As BMI-1026 causes cytokinesis before the completion of chromosome separation, the central spindles were not well developed at the time of furrowing. It has been shown that the signals for cleavage furrow formation emanate from overlapping mitotic spindles between the separating chromosomes (1). Thus, the initiation of the central spindle assembly that occurs after release from nocodazole may induce furrowing. Presumably, enough signals could not accumulate to induce abscission because of the lack of well developed central spindles in BMI-1026-treated cells.

BMI-1026 has been shown to specifically inhibit Cdns both in vitro and in vivo (20). Selective inhibition of Cdk1 among the mitotic kinases in vivo has been demonstrated by induction of a potent G2/M arrest, mitotic catastrophe, and precocious mitotic exit. The Cdk1-specific inhibitor purvalanol A also induced a similar phenotype as BMI-1026, but Cdk2/5-specific and Cdk4-specific inhibitors did not show these phenotypes. Moreover, the introduction of an anti-Cdk1 antibody resulted in the generation of aberrant nuclei, a phenotype expected from

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**FIGURE 6. RhoA is involved in BMI-1026-induced membrane blebbing.** A, HeLa cells transfected with the indicated siRNA were cultured for 24 h and then arrested by nocodazole (40 ng/ml) treatment. Cells were treated with BMI-1026 in the presence of nocodazole and subjected to time-lapse video microscopy to observe excessive blebbing. Representative frames of the movies are shown. Numbers indicate minutes after BMI-1026 treatment. B, quantitation of cells with excessive membrane blebbing.

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**FIGURE 6. RhoA is involved in BMI-1026-induced membrane blebbing.** A, HeLa cells transfected with the indicated siRNA were cultured for 24 h and then arrested by nocodazole (40 ng/ml) treatment. Cells were treated with BMI-1026 in the presence of nocodazole and subjected to time-lapse video microscopy to observe excessive blebbing. Representative frames of the movies are shown. Numbers indicate minutes after BMI-1026 treatment. B, quantitation of cells with excessive membrane blebbing.
FIGURE 7. RhoA accumulates at BMI-1026-induced cleavage furrow, midbody, and the necks of blebs. HeLa cells arrested with 40 ng/ml nocodazole for 6 h were released into fresh medium and then treated with 200 nM BMI-1026 (left). HeLa cells arrested with 40 ng/ml nocodazole (middle) or 50 nM taxol (right) were treated with 200 nM BMI-1026. Cells were fixed with 10% trichloroacetic acid, stained with anti-RhoA antibody, and then examined by fluorescence microscopy.

When mitotically arrested cells were treated with BMI-1026 in the presence of a high concentration of nocodazole, cells spread without initiation of cytokinesis. Therefore, mitotic spindles might be required for BMI-1026-induced cytokinesis. The induction of cell spreading by BMI-1026 was consistent with the previous report that BMI-1026 induces mitotic exit in U2OS cells (20). We also found that BMI-1026 induced excessive membrane blebbing in the presence of a low concentration of nocodazole. Although most spindles were disrupted and de-localized in cells treated with a low concentration of nocodazole, these abnormal spindles were probably sufficient to initiate furrow formation. Thus, the blebs observed in cells in the presence of a low concentration of nocodazole might represent ectopic cleavage furrows. As excessive blebbing is accompanied by abnormal cytokinesis (28–30), it is also possible that these blebs might represent cortical activity that was located along the cleavage furrow in normal cytokinesis but was mis-localized and amplified by partial disruption of mitotic spindles in cells treated with BMI-1026 in the presence of a low concentration of nocodazole. As BMI-1026-induced excessive blebbing was inhibited by knockdown of ECT2, MgcRacGAP, or RhoA, bleb formation appeared to be regulated by the Rho signaling pathway, similar to furrow ingression.

We showed that knockdown of RhoA, but not Rac1 or Cdc42, effectively delayed BMI-1026-induced cytokinesis. RhoA was localized at the cleavage furrow and the necks of the blebs induced by BMI-1026. These results suggest that RhoA is involved in cytokinesis. However, despite the high knockdown level of RhoA, RhoA siRNA could not completely inhibit BMI-1026-induced cytokinesis. Therefore, it is possible that other Rho GTPases closely related to RhoA, such as Rhob and RhoC, are also involved in cytokinesis. It has been shown that RhoA is activated during cytokinesis, and this activation is inhibited by dominant-negative Y-26732 (21). MgcRacGAP, which is a GAP for Rac1 and Cdc42, is converted to a RhoGAP by phosphorylation by Aurora B in M phase and regulates cytokinesis (10). We showed that knockdown of ECT2 or MgcRacGAP efficiently inhibited BMI-1026-induced cytokinesis. Therefore, the Rho signaling pathway regulated by ECT2 and MgcRacGAP might be critical to regulate cytokinesis. As the ROCK inhibitor Y-26732 did not affect BMI-1026-induced cleavage furrow ingression, other Rho effectors such as citron kinase might play a critical role in cytokinesis.

We propose the following model for the precocious induction of cytokinesis by BMI-1026 (Fig. 8). The mitotic checkpoint regulates both chromosome separation and cleavage furrow formation by inhibiting anaphase-promoting complex/cyclosome, which degrades and cyclin B through ubiquitin-mediated proteolysis. When cells are synchronized at prometaphase by nocodazole, the mitotic checkpoint is activated, and the anaphase-promoting complex/cyclosome becomes inactive for securin and cyclin B degradation. When the checkpoint is silenced by release from nocodazole, the anaphase-promoting complex/cyclosome is activated, and then cyclin B and securin are degraded. This signaling in turn activates two pathways that regulate cell division. Securin degradation results in cohesion inactivation, which initiates chromosome separation and central spindle formation. Silencing of the mitotic checkpoint also activates another pathway toward cytokinesis. Cyclin B is degraded by active anaphase-promoting complex cyclosome,
and then cleavage furrow ingestion is induced. It has been reported that expression of nondestructable forms of cyclins inhibits cytokinesis, indicating that Cdk1/cyclinB negatively regulates cytokinesis (18, 19). Recently, it has been shown that Cdk1 also negatively regulates central spindle formation by phosphorylating MKLP1 (27). Therefore, Cdk1 inhibition by BMI-1026 should also stimulate central spindle formation. In *Drosophila* cells, the Pavarotti/RacGAP50C (*Drosophila* MKLP1/MgcRacGAP) complex accumulates at the central spindle, and the association of Pebble (*Drosophila* ECT2) with the complex activates the Rho signaling pathway to initiate cytokinesis (8). BMI-1026 treatment of prometaphase cells inhibits Cdk1 before cyclin B and secures destruction to induce central spindle formation without chromosome separation. Although the central spindle may not be well established without chromosome separation, this may be sufficient to establish the cleavage plane. In *Drosophila* embryos, Cdk1 negatively regulates the Rho signaling that emanates from the central spindle (19). BMI-1026 thus precociously induces cleavage furrow ingestion by removing the negative regulation. The critical cytokinesis regulator that is regulated by Cdk1/cyclin B may be ECT2, as suggested by genetic work in *Drosophila* (19). When cells were treated with BMI-1026 in the presence of nocodazole, no signal for cytokinesis emanates because of the lack of the central spindle, and, therefore, cells exited mitosis without cytokinesis. However, when overlapping spindles were partially retained in the presence of a low concentration of nocodazole, the signal for cytokinesis emanating from these partial spindles induces ectopic cleavage furrows, which are observed as excessive membrane blebbing.

We have utilized the Cdk1 inhibitor BMI-1026 to analyze the initiation of cytokinesis. Although a number of Cdk inhibitors have been identified and characterized, many of them exhibit relatively broad specificities and therefore inhibit multiple biological functions. BMI-1026 is a unique small molecule inhibitor that shows a narrow specificity to Cdk1 (20). As Cdk1 is also required to enter mitosis, analysis of M phase exit alone is difficult using other methods such as expression of dominant-negative-Cdk1 and Cdk1 depletion by RNAi. In addition to other small molecule inhibitors, such as blebbistatin (31), monastrol (32), Aurora kinase inhibitors (33, 34), and Y-26732 (26), BMI-1026 will be useful for further studies to clarify the signaling pathways that regulate cell division. As cytokinesis is a spatially and temporally regulated event, chemical induction of cytokinesis by BMI-1026 will be useful for analysis of cytokinesis at the molecular level.

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