CDK11\textsuperscript{p58} – cyclin L1\textbeta{} regulates abscission site assembly

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Rigorous spatiotemporal regulation of cell division is required to maintain genome stability. The final stage in cell division, when the cells physically separate (abscission), is tightly regulated to ensure that it occurs after cytokinetic events such as chromosome segregation. A key regulator of abscission timing is Aurora B kinase activity, which inhibits abscission and forms the major activity of the abscission checkpoint. This checkpoint prevents abscission until chromosomes have been cleared from the cytokinetic machinery. Here we demonstrate that the mitosis-specific CDK11\textsuperscript{p58} kinase specifically forms a complex with cyclin L1\textbeta{} that, in late cytokinesis, localizes to the stem body, a structure in the middle of the intercellular bridge that forms between two dividing cells. Depletion of CDK11 inhibits abscission, and rescue of this phenotype requires CDK11\textsuperscript{p58} kinase activity or inhibition of Aurora B kinase activity. Furthermore, CDK11\textsuperscript{p58} kinase activity is required for formation of endosomal sorting complex required for transport III filaments at the site of abscission. Combined, these data suggest that CDK11\textsuperscript{p58} kinase activity opposes Aurora B activity to enable abscission to proceed and result in successful completion of cytokinesis.

Progression through the different phases of the cell cycle is regulated by the sequential activity of conserved cyclin-dependent kinases (CDKs)\textsuperscript{3} bound to regulatory cyclins (1). Cytokinesis is the final stage of the cell cycle, when one cell physically divides into two. Cytokinesis is a dynamic and intricate process that proceeds through a carefully orchestrated sequence of steps and must be tightly regulated to maintain genome integrity (2). Disruption at any stage of cytokinesis results in tetraploidy and supernumerary centrosomes, a genetically unstable state (3). Surprisingly, little evidence exists of cyclin-CDK complexes that drive cytokinesis.

The first step of cytokinesis is the assembly and contraction of an actomyosin ring that drives ingestion of a membrane furrow between the separating sister chromosomes during anaphase (4). After furrow ingression, anillin–septin filaments promote the formation of an intercellular bridge (ICB) between the two daughter cells (2). At the same time, the spindle midzone is reorganized into the stem body (also called the Flemming body), an electron-dense central bulge in the middle of the ICB (5). Subsequently, as the ICB matures, septin filaments and FIP3 endosomes mediate the formation of constriction sites on either side of the stem body (2, 6). Endosomal sorting complex required for transport III (ESCRT III) is initially recruited to the stem body, and from there, ESCRT-III filaments are targeted to the abscission site, where membrane remodeling and, finally, fission occur, generating two separate daughter cells (7, 8).

The Aurora B–dependent abscission checkpoint (also called the NoCut pathway) maintains genomic integrity by coordinating abscission onset with the completion of chromosome segregation and nuclear membrane reformation (9–11). Aurora B directly phosphorylates the ESCRT-III component CHMP4C, which delays ESCRT-III filament formation and inhibits relocalization of the AAA-ATPase Vps4 to the abscission site, where membrane fission occurs (12–15). How Aurora B–dependent inhibition of cytokinesis is overcome to drive both abscission site assembly and completion of cytokinesis is not known.

The cyclin dependent kinase CDK11 (also known as PITSLRE kinase and CDC2L1) is a member of the p34Cdc2 kinase family (16) and has been implicated in a variety of cancers (17–19). Three CDK11 isoforms are expressed from one mRNA transcript: full-length CDK11\textsuperscript{p110}, CDK11\textsuperscript{p58}, and CDK11\textsuperscript{p46} (20, 21). CDK11\textsuperscript{p110} is present throughout the cell cycle and is involved in splicing (22–24). In contrast, CDK11\textsuperscript{p58} expression is restricted to G\textsubscript{s}/M by a cap-independent mechanism utilizing an internal ribosome entry site in the CDK11\textsuperscript{p110} coding sequence (20, 25), suggesting a specialized mitotic role of the p58 isoform. Finally, CDK11\textsuperscript{p46} is generated by caspase-dependent cleavage of CDK11\textsuperscript{p110} and is restricted to cells undergoing apoptosis (26). To date, roles of CDK11 in different phases of mitosis have been suggested, including sister chromatid cohesion (24, 27, 28), spindle formation (29), and cytokinesis (30–32); however, which CDK11 isoform regulates each of these diverse mitotic functions is unknown.

Here we demonstrate that the mitosis-specific CDK11\textsuperscript{p58} isoform, in conjunction with cyclin L1\textbeta{}, localizes to the stem body in the intercellular bridge. The kinase activity of this complex is required for ESCRT-III targeting to the abscission site and completion of cytokinesis.


**CDK11p58 is required for abscission**

**Results**

**CDK11p58 localizes to the stem body**

Defining the localization of cyclin–CDK complexes has been vital in understanding their role in regulating cellular processes (33, 34). To dissect the mitotic role of CDK11, we determined where endogenous CDK11 localized during cell division using an antibody that recognizes all CDK11 isoforms. During interphase, CDK11 localized to foci within the nucleus (Fig. 1A), consistent with previous studies (24, 27, 28). However, during cytokinesis, a subpopulation of CDK11 localized to the central bulge within the ICB, called the stem body (Fig. 1, A and B), as defined by colocalization with a known marker of the stem body, anillin (2).

As multiple CDK11 variants are expressed from a single transcript (20, 21, 25) (Fig. 1C), it is important to establish which variant localizes to the stem body. Therefore, we generated stable HeLa cells expressing inducible epitope-tagged CDK11p110 (HA-p110) or CDK11p58 (HA-p58). The N-terminally HA-tagged CDK11 constructs exhibited differential staining that together reproduced the localizations observed for endogenous CDK11. We found that HA-p110 localized to nuclei, whereas the HA-p58 isoform localized to the stem body (Fig. 1D), suggesting a role of the mitosis-specific CDK11p58 kinase in cytokinesis.

**CDK11p58 forms a complex with cyclin L1β**

CDKs function in complex with cyclins (1). Cyclin L1, which has three main splice variants (α, β, and γ), is the regulatory cyclin for CDK11 (35). *In vitro*, all three isoforms of CDK11 can interact with all cyclin L1 isoforms (36); however, the *in vivo* interactions and subcellular localization of each isoform are unknown. To determine which cyclin L1 isoform regulates CDK11p58, HeLa cells expressing different inducible GFP-tagged cyclin L1 isoforms were generated. GFP-cyclin L1α localized to nuclei, β to the stem body, and γ to Golgi-like structures (Fig. 2A). To determine whether localization of GFP-cyclin L1β correlated with an interaction with CDK11p58 at the stem body, HeLa cell cultures were enriched for late mitotic cells, and GFP-tagged cyclin L1 isoforms were then purified using GFP-Trap™ beads and probed for copurifying endogenous CDK11 isoforms. Consistent with the localization results, GFP-cyclin L1α preferentially copurified with CDK11p110, and GFP-cyclin L1β preferentially copurified with CDK11p58 (Fig. 2, B and C). Taken together, these data suggest that a complex of CDK11p58 and cyclin L1β localizes to the stem body during cytokinesis, implicating their site of function during mitosis.

**Depletion of CDK11 causes cytokinetic defects**

To determine the role of CDK11 in cell division, CDK11 was depleted from asynchronous cells by siRNA that targets all CDK11 isoforms. Expression of CDK11 was reduced by 80% (Fig. S1). CDK11 depletion caused an increase in binucleate cells and an increase in the number of daughter cells attached by an ICB (Fig. 3, A and B), phenotypes typical of cytokinetic failure that concur with previous studies (25, 30–32). In contrast, although previous studies have reported defects in sister chromatid cohesion (24, 27, 28), centrosome maturation (37), and spindle formation (29), we observed no defects in the early stages of mitosis or localization of CDK11 to those cellular...
structures. To determine what stage of cytokinesis is regulated by CDK11, we next analyzed the kinetics of mitotic progression. CDK11-depleted cells were arrested at prometaphase with the Eg5 inhibitor monastrol (38) and then released, and mitotic progression was followed by the emergence of cells undergoing chromosome segregation (post-anaphase onset, late mitosis) and, subsequently, formation of ICBs. In both control and CDK11 siRNA–treated cells, a peak of anaphase cells occurred 2 h after release, indicating that depletion of CDK11 did not perturb spindle formation or anaphase onset (Fig. 3C). Furthermore, under both control and CDK11 siRNA conditions, there was a peak accumulation of ICBs 4 h after release, suggesting that CDK11 is not required for ICB formation but, rather, affects ICB maturation and/or abscission site assembly. At later time points, the occurrence of ICBs in control cells dropped as cells completed cytokinesis and proceeded to the next cell cycle (Fig. 3C). In contrast, ICBs persisted longer in CDK11-depleted cells compared with cells treated with control siRNA (Fig. 3C and D). To further define how CDK11 regulates the final stages of cytokinesis, we followed cytokinetic progression in live cells upon CDK11 depletion (Movies S1 and S2). The time required to complete cytokinesis was measured from furrow ingression (anaphase) until breakage of the bundled microtubules (abscission). Cells depleted of CDK11 showed a delay in abscission, taking 75% longer for the daughter cells to physically separate from the start of furrow ingression (256.1 ± 22.5 min compared with 146.5 ± 14.9 min in control cells) (Fig. 3E).

**CDK11p58 kinase activity is required for cytokinesis**

To investigate which CDK11 isoform regulates abscission, we performed siRNA rescue experiments using HeLa cells expressing RNAi-resistant CDK11p110 or CDK11p58 (Fig. 3F). Both HA-p110 WT and HA-p58 WT rescued the cytokinetic delay and restored the number of daughter cells attached by an ICB. However, as CDK11p58 is expressed from an internal ribosome entry site in the full-length mRNA transcript (20), the HA-p110 WT construct would express both CDK11p110 and CDK11p58 isoforms. To prevent CDK11p58 translation from the HA-p110 WT transcript without affecting CDK11p110 activity, we mutated the CDK11p58 start codon (HA-p110 MA) as described previously (Fig. 1C) (25). Expression of the siRNA-resistant HA-p110 MA mutant did not rescue ICB accumulation following CDK11 siRNA (Fig. 3G), confirming that CDK11p58 per se is required for abscission. Moreover, the...
**CDK11<sup>p58</sup> is required for abscission**

Kinase activity of CDK11<sup>p58</sup> was required, as expression of an RNAi-resistant kinase-dead mutant (HA-p58<sup>DN</sup>) failed to rescue the abscission defects in CDK11-depleted cells (Fig. 3G). Therefore, the kinase activity of CDK11<sup>p58</sup> is required for completion of cytokinesis.

**CDK11<sup>p58</sup> activity is required for ESCRT-III targeting to the abscission site**

To identify specific processes downstream of CDK11, we examined the recruitment and positioning of known cytokinetic factors following CDK11 depletion. Anillin, RhoA, and...
septins, all required in the early stages of cytokinesis (2, 39), were initially recruited to the ingressing furrow and then targeted to the constriction sites and stem body during ICB formation independently of CDK11 (Fig. S2, A–C). Likewise, recruitment of the Centralspinindin complex and the chromosome passenger complex to the ICB, as visualized by MKLP1 and INCENP antibody staining, respectively, were unperturbed by CDK11 depletion (Fig. S2, D and E). These data suggest that CDK11 functions during abscission, the final stage of cytokinesis.

Assembly of the abscission site occurs after the scaffold protein anillin has left the ICB (2), when components of the ESCRT-III complex asymmetrically relocalize from the stem body to one arm of the ICB (6–8). Upon CDK11 depletion, recruitment of the ESCRT machinery to the stem body occurred normally (Figs. 4A and Fig. S2F). We then followed the translocation of the ESCRT-III, using the CHMP4B subunit as a marker, from the stem body to one arm of the ICB as a reporter for abscission site assembly in post-anillin ICBs (Fig. 4A) (2). In CDK11-depleted cells, CHMP4B targeting to the abscission site was impaired; there were significantly fewer post-anillin ICBs stained with CHMP4B at the abscission site compared with ICBs at the same stage in cells treated with control siRNA (Fig. 4B).

We next asked whether CDK11p58 kinase activity was required for abscission site assembly. Significantly, although p58WT could rescue CHMP4B localization at the abscission site in late-stage ICBs, expression of the kinase-dead p58DN did not (Fig. 4C). Our data implicate CDK11p58 kinase activity in promoting CHMP4B relocation to, and consequently the assembly of, a functional abscission site.

**CDK11p58 activity opposes Aurora B kinase to regulate abscission**

The timing of abscission in mammalian cells is regulated by Aurora B activity. To determine whether the delay in abscission and increase in ICBs upon CDK11 depletion depends on Aurora B activity, CDK11-depleted cells were incubated with hesperadin, an inhibitor of Aurora B (40). Following CDK11 depletion, the number of ICBs was scored after cells were treated with varying concentrations of hesperadin for 1 h (Fig. 5A). In both CDK11 siRNA and control cells, treatment with 100 nm hesperadin led to a decrease in the number of ICBs. However, partial inhibition of Aurora B with 10 nm hesperadin (40) did not significantly affect ICB numbers in control cells but, crucially, suppressed the increase of ICBs in CDK11-depleted cells.

Figure 3. CDK11p58 kinase activity is required for abscission. A, whole-field micrographs of HeLa cells treated with either control siRNA or CDK11 siRNA for 48 h and then fixed and stained to visualize DNA using 4',6-diamidino-2-phenylindole (blue) and acetylated tubulin (green). Scale bars = 5 μm in whole-cell images and 1 μm in magnifications. B, cell division defects in HeLa cells treated with control or CDK11 siRNA were scored by the presence of binucleate cells and the number of ICBs per 100 cells. n = 4 experimental repeats, more than 200 cells scored per experiment; p values were generated by an unpaired t test. C, 32 h post-siRNA transfection, HeLa cells were synchronized by incubation with monastral for 16 h. Mitotic progression was scored by the number of cells undergoing chromosome segregation (post-anaphase onset mitotic cells, top panel) and the number of cells attached by an ICB (bottom panel). n = 3 experimental repeats, more than 200 cells scored per experiment. Asterisk marks the point in time when measurements in panel D were made. D, quantification of the percentage of cells connected by an ICB 48 h post-siRNA transfection and 8 h after release from 16-h monastral treatment. n = 3 experimental repeats, more than 200 cells scored per experiment. E, quantification of live-cell imaging, n = 8 and 9 cells for control and CDK11 depletion, respectively. Whiskers, boxes, and bars indicate range, 25–75 percentile, and mean, respectively. The p value was generated by an unpaired t test. F, expression of HA-CDK11 constructs was induced for 16 h 32 h post-siRNA transfection, and cell lysate was generated and analyzed by Western blotting to assess the level of CDK11 isoform expression compared with endogenous tubulin expression. G, expression of HA-CDK11 constructs was induced for 16 h 32 h post-siRNA transfection, and cytokinetic defects were scored by the number of ICBs per 100 cells. n = 3 experimental repeats, more than 200 cells scored per experiment. The p value was generated by an unpaired t test.

Figure 4. CDK11p58 kinase activity regulates CHMP4B targeting to the abscission site. A, transfection with control or CDK11 siRNA for 48 h, fixed in methanol, and stained with anti-tubulin, anti-CHMP4B, and anti-anillin antibodies. Arrowheads indicate localization at the stem body. Scale bars = 5 μm in whole-cell images and 1 μm in magnifications. DAPI, 4',6-diamidino-2-phenylindole; MT, microtubule. B, CHMP4B localization at the abscission site was scored in post-anillin ICBs. n = 81 and 75 post-anillin ICBs (CDK11 and control siRNA, respectively), compiled from three independent experimental repeats; the p value was generated by Fisher’s exact test. C, expression of HA-p58 constructs was induced for 16 h 32 h post-siRNA transfection. Translocation of CHMP4B in post-anillin ICBs was scored as above. n = 72 and 86 post-anillin ICBs (HA-p58WT and HA-p58DK, respectively), compiled from three independent experimental repeats; the p value was generated by Fisher’s exact test.
**CDK11<sup>p58</sup> is required for abscission**

**A**

![Graph showing the number of ICBs per 100 cells over time after addition of hesperadin.](image)

**B**

![Graph showing the percentage of post-anaphase onset mitotic cells over time after monastrol wash-out.](image)

**C**

![Graphs showing the number of ICBs per 100 cells over time after addition of hesperadin for Control RNAi and CDK11 RNAi.](image)

**D**

![Bar graph showing the number of ICBs per 100 cells for Control RNAi and CDK11 RNAi.](image)

**E**

![Micrographs comparing control RNAi and CDK11 RNAi.](image)
pleted cells to nearly normal levels. These data suggest that Aurora B and CDK11 have opposing effects on the progression of cytokinesis.

To confirm that the reduction in ICB frequency following Aurora B inhibition was due to increased abscission and not a result of defects earlier in mitosis or reduced formation of ICBs, cells were synchronized with monastrol, and hesperadin was added after the cells had completed furrow ingression (4 h after release from monastrol arrest, Fig. 5B). Inhibition of Aurora B activity in late mitosis increased the progression of cells through cytokinesis (Fig. 5, C and D), consistent with previous studies (41), and significantly reduced the number of ICBs in CDK11-depleted cells (Fig. 5, C and D). Therefore, the Aurora B activity and CDK11p58 kinases function antagonistically to regulate the timing of abscission.

One mechanism by which CDK11p58 could oppose Aurora B is by down-regulating Aurora B activity. To test this and focus only on Aurora B activity within the ICB, we followed Aurora B activation using an antibody that specifically recognizes Aurora B phosphorylated at threonine 232, the active form of Aurora B (42). In untreated and control siRNA–treated cells, Aurora B remained active throughout ICB formation and abscission site assembly (Fig. 5E). Likewise, upon depletion of CDK11 by siRNA, Aurora B remained active within the ICB during cytokinesis, indicating that Aurora B activity is maintained throughout the lifetime of the ICB and is independent of CDK11 activity. Thus, decreased Aurora B activity per se is unlikely to underlie abscission site assembly and completion of cytokinesis.

CDK11p58 activity opposes Aurora B kinase to regulate ESCRT-III targeting to the abscission site

To further understand the regulatory switch that CDK11p58 and Aurora B antagonistically regulate, we examined the dependence of the ESCRT-III marker CHMP4B targeting to the abscission site. We previously demonstrated that CHMP4B targeting to the abscission site required CDK11p58 kinase activity (Fig. 4). Next we treated CDK11-depleted cells with the Aurora B inhibitor hesperadin; under these conditions, CHMP4B targeting to the abscission site was restored (Fig. 6, A and B). These data suggest that, although Aurora B activity blocks ESCRT-III targeting to the abscission site, CDK11p58 activity is required for ESCRT-III targeting to the abscission site and that CDK11p58 drives this process through a mechanism independent of Aurora B deactivation (Fig. 6C).

Discussion

CDKs regulate many processes during the cell division cycle. Here we show that abscission in mammalian cells is subject to CDK regulation by the mitosis-specific CDK11p58–cyclin L1β complex. CDK11p58–cyclin L1β kinase activity regulates assembly of the abscission site, as judged by targeting of the ESCRT-III component CHMP4B to the abscission site. Our findings are consistent with previous observations suggesting a role of CDK11 in cytokinesis (25, 30–32) and consistent with the subcellular localization we observed of endogenous and ectopically expressed CDK11p58 and CyclinL1β. Other studies have implicated CDK11p58 in chromosome condensation and centrosome function; however, we did not observe those phenotypes or CDK11p58 and cyclin L1β localization to chromosomes or centrosomes. The causes of these different observations are not readily apparent.

Abscission is mediated by the ESCRT-III complex and requires relocation of CHMP4B and CHMP4C from the stem body to the site of abscission (12, 13, 15). CDK11p58 kinase activity promotes the relocation of CHMP4B to the abscission site, overcoming the negative regulation by Aurora B. When CDK11p58 activity is lost, Aurora B activity delays abscission, which consequently leads to accumulation of cells attached by an ICB (Fig. 4). However, we observed that Aurora B activity, detected using an antibody that recognizes an activated phospho-Thr232 epitope, remains at the stem body throughout the abscission process and is unaffected by depletion of CDK11. This observation suggests that CDK11 does not directly “switch off” Aurora B, nor does it appear to stimulate Aurora B degradation. One interpretation of our observations is that CDK11 operates in a pathway that opposes Aurora B activity rather than directly altering Aurora B activity. Alternatively, CDK11 could operate in a pathway that modifies Aurora B substrate specificity. For example, PKCε-mediated phosphorylation of Aurora B (Ser127) altered Aurora B substrate specificity, a process required for abscission (43).

Aurora B phosphorylation of the ESCRT-III component CHMP4C delays abscission (12, 13, 15), and it has been postulated that phosphorylation of CHMP4C by Aurora B maintains CHMP4C in a “closed” state, which prevents formation of ESCRT-III filaments and therefore delays abscission (12). CHMP4C is phosphorylated on three residues by Aurora B (15). In the triple-phosphorylated state, CHMP4C localizes to the arms of the ICB, but partial dephosphorylation allows it to be targeted to the stem body (15). Interestingly, although ectopically HA-tagged CHMP4C was observed in helical filaments extending from the stem body toward the abscission site, neither phospho-specific CHMP4C antibodies nor generic CHMP4C antibodies stained these filaments (15). These data suggest that successive CHMP4C dephosphorylation events

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**Figure 5. CDK11p58 kinase activity and Aurora B activity have opposing roles in abscission.** A, HeLa cells were transfected with control or CDK11 RNAi for 48 h, and hesperadin was added (to a final concentration of 0, 10, or 100 nM in DMSO) for the final 1 h. Cells were fixed and scored for number of ICBs per 100 cells, n = 3 experimental repeats, more than 200 cells scored per experiment; the p value was generated by ANOVA, n.s., not significant, b, hesperadin addition after cells completed anaphase. HeLa cells were transfected with control or CDK11 siRNA and arrested at prometaphase with monastrol for 16 h. Cells were then released into fresh medium, and mitotic progression was scored by cells that had entered anaphase (i.e. undergoing chromosome segregation). Hesperadin was added 4 h after release from monastrol arrest; n = 3 experimental repeats, more than 200 cells scored per experiment; C, HeLa cells were transfected with control or CDK11 siRNA and synchronized with monastrol. The kinetics of cytokinesis progression were scored by emergence of cells attached by an intercellular bridge (number of ICBs per 100 cells). Hesperadin was added to a final concentration of 0, 10, or 100 nM 4 h after release from monastrol arrest. n = 3 experimental repeats, more than 200 cells scored per experiment. D, comparison of the percentage of cells connected by an ICB at the final time point of the experiment in C. E, localization of active Aurora B, using an anti phospho-Thr232 Aurora B antibody, during ICB maturation. Scale bars = 5 μm in whole-cell images and 1 μm in magnifications.
are required for CHMP4C dynamics within the ICB and its ultimate targeting to the abscission site.

The mechanism for deactivation of Aurora B and/or release of the CHMP4C-dependent abscission delay remains to be resolved. If, as our observations suggest, Aurora B activity persists, then the simplest model would be that the inhibitory action of Aurora B that prevents abscission is overcome by increased phosphatase activity. Consistent with this model, studies in *Schizosaccharomyces pombe* indicate that phosphatase activity increases as mitosis progresses (44). In such a model, CDK11 would then operate in a pathway that regulates phosphatase activity.

In both yeast and mammalian cells, it has been proposed that the Aurora B kinase senses chromatin in the cleavage plane and delays abscission until conditions permit cytokinesis to proceed (the NoCut Pathway) (41, 45, 46). In mammalian systems, Aurora B activity also regulates cytokinesis in response to defects in nuclear membrane formation (47), DNA replication (48), and tension across the ICB (49–51), indicating that Aurora B’s role in regulating abscission timing is not strictly restricted to coordinating cytokinesis with the completion of chromosome segregation (reviewed in Ref. 11). These cellular processes, when incomplete, inhibit abscission in an Aurora B–dependent manner. What is unknown is how completion of these processes either leads to deactivation of Aurora B or up-regulation of a counteracting pathway. Understanding the cellular events and molecular details of how the abscission checkpoint monitors those events to ensure correct timing of abscission in relation to the rest of the cell cycle are the next challenges for the field.

**Experimental procedures**

**Cell growth and synchronization**

HeLa cells were cultured in DMEM (Sigma) supplemented with 10% fetal bovine serum (Sigma) and 1% penicillin/streptomycin (Invitrogen) in a 5% CO2 atmosphere at 37 °C. To arrest cells in prometaphase, HeLa cells were incubated in 100 μM monastrol (Sigma) for 16 h. To enrich cells in late mitosis, cells were synchronized at G1/S by a double thymidine block. Cells were cultured in DMEM plus 2 mM thymidine for 18 h, washed, and grown in DMEM for 9 h. Cells were then grown in DMEM plus 2 mM thymidine for 18 h, washed, and cultured for a further 12 h in DMEM, at which point the cell culture was enriched in late mitotic stages.

**Generation of stable cell lines**

Generation of stable FRT/TO HeLa cells (Table S1) was done using the Flp-InTM system (Invitrogen). Constructs were amplified by PCR (see Table S2 for oligo sequences) and cloned into pcDNA5/FRT/TO-derived vectors. Cell lines were then generated according to the manufacturer’s instructions as described previously (2).
**siRNA treatment and rescue**

HeLa cells were grown to 40% confluency and transfected with 60 nm double-stranded siRNA using Lipofectamine 2000 (Invitrogen). For rescue experiments, siRNA-resistant CDK11 isoforms (HA-p110WT, HA-p58WT, HA-p110MA, and HA-p58DN) were expressed 32 h after siRNA transfection; live-cell imaging or cell fixation was then carried out 48 h after siRNA transfection. All siRNAs were obtained from Integrated DNA Technologies. The siRNA for CDK11 (5'-gcaugcuagagugaaagaaagagag-3') was targeted to a region within the CDK11 coding sequence; the negative control siRNA sequence (NCI, Integrated DNA Technologies) was used was 5'-gguuacggauuaagcguat-3'.

**Quantitative RT-PCR (qRT-PCR)**

HeLa cells in 6-well dishes were transfected with 100 pmol of Cdk11 siRNA or negative control siRNA (Integrated DNA Technologies) using 5 μl of Lipofectamine 2000 (Thermo Fisher Scientific). Cells were harvested 48 h later for qRT-PCR of Cdk11 and β-actin transcripts. For qRT-PCR, total RNA was extracted using Nucleozol (Macherey-Nagel) according to the manufacturer’s instructions. One microgram of total RNA was treated with 0.5 units of DNase I (New England Biolabs) for 15 min. qRT-PCR was performed with 1 μl of a 1:10 dilution of DNase I-digested RNA (oligos are listed in Table S5) using Luna Universal qPCR Master Mix (New England Biolabs) in a total reaction volume of 20 μl in a MyIQ2 real-time system (Bio-Rad). The relative mRNA expression level was derived from $2^{-ΔΔCT}$ by use of the comparative threshold cycle method. The amount of mRNA in each sample was normalized to the amount of actin mRNA.

**Ligation-independent cloning (LIC)**

Unless stated otherwise, all plasmids used were generated by LIC (2, 52, 53). pcDNAs5/FRT/TO-derived vectors were converted into LIC vectors by replacing the multiple cloning site with a specifically designed ligation-independent cloning site (Tables S3 and S4). Linearized LIC vectors (Tables S3 and S4) were treated with T4 DNA polymerase in the presence of dATP to generate overhangs. PCR-generated LIC inserts (Table S2) were treated with T4 DNA polymerase in the presence of dTTP to generate overhangs complementary to the relevant LIC vector. 0.01 pmol of prepared LIC vector was then annealed to 0.04 pmol of prepared LIC insert for 30 min at room temperature. EDTA was added to a final concentration of 40 mM, and the annealing mixture was heated to 75 °C and cooled slowly to room temperature. The annealed products were then used to transform Top10 Escherichia coli cells, and positive constructs were identified by colony PCR. Final constructs were then confirmed by restriction endonuclease digestion and DNA sequencing.

**Immunofluorescence**

Cells cultured on glass coverslips (thickness no. 1 ½, size 22 x 22 mm, Electron Microscopy Services) were stained by standard methods after fixation with either ice-cold 10% TCA buffered in cytoskeleton buffer (10 mM MES (pH 6.1), 138 mM KCl, 3 mM MgCl₂, 2 mM EGTA, and 0.32 mM sucrose) and 4% paraformaldehyde buffered in PBS or 100% methanol, as described previously (2). Primary antibodies used in this study were mouse anti-tubulin (DM1A, Sigma, used at 1:1000), rabbit anti-CDK11 (ab19393, Abcam, used at 1:1000), rabbit anti-MKLP1 (AKIN06, Cytoskeleton, used at 1:500), rabbit anti-anillin (sc-67327, Santa Cruz, used at 1:1000), goat anti-anillin (sc-54859, Santa Cruz, used at 1:500), rabbit anti-SEPT11 and rabbit anti-SEPT9 (54) (gifts from W. Trimble, University of Toronto, used at 1:500), mouse anti-RhoA (sc-418, Santa Cruz, used at 1:1000), mouse anti-INCENP (39-2800, Invitrogen, used at 1:1000), mouse anti-HA (12CA5, used at 1:1000), rabbit anti-tubulin (ab18251, Abcam, used at 1:1000), goat anti-Alix (sc-49267, Santa Cruz, used at 1:500), and goat anti-RACGAP1 (NB100-884, Novus Biologicals, used at 1:1000).

Imaging was done using a Nikon TE2000 inverted confocal spinning disc microscope with a ×60/1.4 numerical aperture immersion objective lens and 1.515 immersion oil (Nikon) at room temperature. Images were acquired using Metamorph software (Molecular Devices) driving an electron-multiplying charge-coupled device camera (ImagEM, Hamamatsu). We acquired Z sections (0.2 μm apart) to produce a stack that was then imported into Autoquant X2 (Media Cybernetics) and subjected to deconvolution (10 iterations). Maximum projections were done using Metamorph. Individual channels were overlaid, rotated, and cropped in GIMP v2.8.4.

**Western blotting**

Cells cultured on circular glass coverslips (thickness no. 1, diameter 25 mm, Fisher Scientific) were treated as indicated and mounted in a heated chamber containing an air–5% CO₂ atmosphere at 37 °C (Live Cell Instrument Systems) in dye-free DMEM with 10% fetal bovine serum (Invitrogen) mounted on a Nikon TE2000 inverted microscope equipped with a spinning disk confocal scanning head driven by Metamorph software as described above. Time-lapse video microscopy was used to follow cells with a stack of images (z-step, 0.6 μm) taken every 5 min using a ×40/1.0 numerical aperture PlanApo oil immersion objective lens and 1.515 immersion oil (Nikon). Acquired images were then processed as described above.

**GFP-Trap**

Cells enriched for late mitosis and grown to confluency on a 15-cm dish were harvested by incubating with PBS and 2 mM EDTA at 4 °C and centrifuged, and the cell pellet was lysed in 200 μl of ice-cold nondenaturing lysis buffer (1% (v/v) Triton X-100, 150 mM NaCl, 10 mM sodium phosphate (pH 7.2), 2 mM EDTA, and 50 mM NaF) with 1% (v/v) protease inhibitor mixture (Roche). Lysates were then adjusted to 1 ml with wash buffer (150 mM NaCl, 10 mM sodium phosphate (pH 7.2), and 0.5 mM EDTA) and incubated with 20 μl of GFP-Trap beads (Chromotek) for 16 h at 4 °C with rotation. GFP-Trap beads were washed four times with wash buffer (150 mM NaCl, 10 mM sodium phosphate (pH 7.2), and 0.5 mM EDTA) and overlaid, rotated, and cropped in GIMP v2.8.4.

**CDK11p58 is required for abscission**

Cells cultured on glass coverslips (thickness no. 1 ½, size 22 x 22 mm, Electron Microscopy Services) were stained by standard methods after fixation with either ice-cold 10% TCA buffered in cytoskeleton buffer (10 mM MES (pH 6.1), 138 mM KCl, 3 mM MgCl₂, 2 mM EGTA, and 0.32 mM sucrose) and 4% paraformaldehyde buffered in PBS or 100% methanol, as described previously (2). Primary antibodies used in this study were mouse anti-tubulin (DM1A, Sigma, used at 1:1000), rabbit anti-CDK11 (ab19393, Abcam, used at 1:1000), rabbit anti-MKLP1 (AKIN06, Cytoskeleton, used at 1:500), rabbit anti-anillin (sc-67327, Santa Cruz, used at 1:1000), goat anti-anillin (sc-54859, Santa Cruz, used at 1:500), rabbit anti-SEPT11 and rabbit anti-SEPT9 (54) (gifts from W. Trimble, University of Toronto, used at 1:500), mouse anti-RhoA (sc-418, Santa Cruz, used at 1:1000), mouse anti-INCENP (39-2800, Invitrogen, used at 1:1000), mouse anti-HA (12CA5, used at 1:1000), rabbit anti-tubulin (ab18251, Abcam, used at 1:1000), goat anti-Alix (sc-49267, Santa Cruz, used at 1:500), and goat anti-RACGAP1 (NB100-884, Novus Biologicals, used at 1:1000).

Imaging was done using a Nikon TE2000 inverted confocal spinning disc microscope with a ×60/1.4 numerical aperture oil immersion objective lens and 1.515 immersion oil (Nikon) at room temperature. Images were acquired using Metamorph software (Molecular Devices) driving an electron-multiplying charge-coupled device camera (ImagEM, Hamamatsu). We acquired Z sections (0.2 μm apart) to produce a stack that was then imported into Autoquant X2 (Media Cybernetics) and subjected to deconvolution (10 iterations). Maximum projections were done using Metamorph. Individual channels were overlaid, rotated, and cropped in GIMP v2.8.4.
CDK11\textsuperscript{p58} is required for abscission

were then washed four times with wash buffer, boiled in SDS sample buffer, and analyzed by Western blotting with anti-CDK11 (Abcam, used at 1:500) and anti-GFP (Roche, used at 1:500) antibodies.

Statistical analysis

All experiments were repeated at least three times, and statistical analyses were conducted using R v3.0.1 (R Foundation for Statistical Computing). To determine statistical significance, unpaired \( t \) tests were performed using the R function \( ttest \), and error bars depict the standard deviation. Fisher’s exact tests were done using fisher.test, and analyses of variance were done using aov followed by Tukey’s post-test with TukeyHSD. \( p < 0.05 \) was considered statistically significant.

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