

11. Goodell, M. A. *et al.* Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nature Med.* **3**, 1337–1345 (1997).
12. Rando, T. A. & Blau, H. M. Primary mouse myoblast purification, characterization, and transplantation for cell-mediated gene therapy. *J. Cell Biol.* **125**, 1275–1287 (1994).
13. Mauro, A. Satellite cells of skeletal muscle. *J. Biophys. Biochem. Cytol.* **9**, 493–495 (1961).
14. Partridge, T. A., Morgan, J. E., Coulton, G. R., Hoffman, E. P. & Kunkel, L. M. Conversion of mdx myofibers from dystrophin negative to positive by injection of normal myoblasts. *Nature* **337**, 176–179 (1989).
15. Partridge, T. A. Invited review: myoblast transfer: a possible therapy for inherited myopathies? *Muscle Nerve* **14**, 197–212 (1991).
16. Acsadi, G. *et al.* Human dystrophin expression in mdx mice after intramuscular injections of DNA constructs. *Nature* **352**, 815–818 (1991).
17. Acsadi, G. *et al.* Human dystrophin expression in mdx mice after intramuscular injections of DNA constructs. *Nature* **352**, 815–818 (1991).
18. Ragot, T. *et al.* Efficient adenovirus-mediated transfer of a human minidystrophin gene to skeletal muscle of mdx mice. *Nature* **361**, 647–650 (1993).
19. Kochanek, S. *et al.* A new adenoviral vector: replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and b-galactosidase. *Proc. Natl Acad. Sci. USA* **93**, 5731–5736 (1996).
20. Rafael, J. A. *et al.* Prevention of dystrophic pathology in mdx mice by a truncated dystrophin isoform. *Hum. Mol. Genet.* **3**, 1725–1733 (1994).
21. Phelps, S. F. *et al.* Expression of full-length and truncated dystrophin mini-genes in transgenic mdx mice. *Hum. Mol. Genet.* **4**, 1251–1258 (1995).
22. Pereira, R. F. *et al.* Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. *Proc. Natl Acad. Sci. USA* **92**, 4857–4861 (1995).
23. Saito, T. *et al.* Myogenic expression of mesenchymal stem cells within myotubes of mdx mice *in vitro* and *in vivo*. *Tissue Eng.* **1**, 327–343 (1995).
24. Prockop, D. J. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* **276**, 71–74 (1997).
25. Asahara, T. *et al.* Isolation of putative progenitor endothelial cells for angiogenesis. *Science* **275**, 964–967 (1997).
26. Shi, Q. *et al.* Evidence for circulating bone marrow-derived endothelial cells. *Blood* **92**, 362–367 (1998).
27. Bjornson, C. R., Rietze, R. L., Reynolds, B. A., Magli, M. C. & Vescovi, A. L. Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells *in vivo*. *Science* **283**, 534–537 (1999).
28. Baroffio, A., Bochaton-Piallat, M.-L., Gabbiani, G. & Bader, C. R. Heterogeneity in the progeny of single human muscle satellite cells. *Differentiation* **59**, 259–268 (1995).
29. Lichter, P. *et al.* Rapid detection of human chromosome 21 aberrations by *in situ* hybridization. *Proc. Natl Acad. Sci. USA* **85**, 9664–9668 (1988).
30. Gussoni, E. *et al.* A method to codetect introduced genes and their products in gene therapy protocols. *Nature Biotech.* **14**, 1012–1016 (1996).

Acknowledgements

We thank E. Snyder for the mouse Y-chromosome probe. This work was supported by the Muscular Dystrophy Association (L.M.K.), the Bernard and Alva Gimbel Foundation and Family (L.M.K.), the Howard Hughes Medical Institute (L.M.K., R.C.M.) and the NIH (R.C.M.). L.M.K. and R.C.M. are investigators of the Howard Hughes Medical Institute.

Correspondence and requests for materials should be addressed to R.C.M. (e-mail: mulligan@rascal.med.harvard.edu) or L.M.K. (e-mail: kunkel@rascal.med.harvard.edu).

Evidence that a free-running oscillator drives G1 events in the budding yeast cell cycle

Steven B. Haase & Steven I. Reed

Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 94303, USA

In yeast and somatic cells, mechanisms ensure cell-cycle events are initiated only when preceding events have been completed¹. In contrast, interruption of specific cell-cycle processes in early embryonic cells of many organisms does not affect the timing of subsequent events², indicating that cell-cycle events are triggered by a free-running cell-cycle oscillator. Here we present evidence for an independent cell-cycle oscillator in the budding yeast *Saccharomyces cerevisiae*. We observed periodic activation of events normally restricted to the G1 phase of the cell cycle, in cells lacking mitotic cyclin-dependent kinase activities that are essential for cell-cycle progression. As in embryonic cells, G1

events cycled on schedule, in the absence of S phase or mitosis, with a period similar to the cell-cycle time of wild-type cells. Oscillations of similar periodicity were observed in cells responding to mating pheromone in the absence of G1 cyclin (Cln)- and mitotic cyclin (Clb)-associated kinase activity, indicating that the oscillator may function independently of cyclin-dependent kinase dynamics. We also show that Clb-associated kinase activity is essential for ensuring dependencies by preventing the initiation of new G1 events when cell-cycle progression is delayed.

In wild-type budding yeast, completion of cell-cycle events in one cell-cycle phase is essential for the transition to the next phase¹, although mutants have been identified that can initiate synchronous, periodic rounds of budding in the absence of DNA replication and mitosis³. Strains bearing temperature-sensitive mutations in genes involved in protein degradation, *cdc4*, *cdc34* or *cdc53*, cannot enter S phase or mitosis but do initiate synchronous rounds of budding at the restrictive temperature⁴. The molecular basis for the inability of these mutants to replicate DNA or complete mitosis is related to the stabilization of the Clb/Cdc28-specific inhibitor, Sic1p, and the consequent loss of Clb-kinase activity⁵. That *cdc4*, *cdc34* and *cdc53* cells produce multiple buds indicates that the initiation of G1 events may be uncoupled from the completion of S phase or mitosis by the loss of Clb-associated kinase activity.

To investigate this idea, we analysed events normally restricted to G1 in synchronous populations of cells bearing the temperature-sensitive mutation *cdc4-3*, or cells disrupted for all six B-type cyclin genes. Synchronous populations of G1 cells were collected by centrifugal elutriation, and then shifted to the restrictive temperature (36°C). Budding and transcript levels were analysed at 10-min intervals. Synchronous oscillations of *CLN2* transcript levels were reproducibly observed with periods coinciding with the budding cycles (Fig. 1d–f, g–i). The cycling of G1 events indicates the existence of an oscillator that cycles independently of B-type cyclin activity and the completion of S phase or mitosis. The period of budding and transcription cycles in cells lacking B-type cyclin activity is very similar to the period observed in normally dividing wild-type cells (Fig. 1a–c), indicating that G1 events in normally dividing cells may be entrained to the same independent oscillator.

Activation of Cln/Cdc28 kinase is a critical G1 event that is required for the initiation of budding and the transition from G1 to S phase⁶. Therefore, we investigated whether the oscillations in *CLN2* transcript levels in cells lacking Clb activity led to functional oscillations in Cln2-associated kinase. Indeed, Cln2-associated kinase did oscillate synchronously with the appearance of new buds in *cdc4-3* cells (Fig. 1j, k). However, it is likely that the oscillation of Cln2-associated kinase activity is not essential for driving budding cycles, as constitutive expression of Cln2 from the *GAL1* promoter did not prevent rebudding in *cdc4-3* cells (data not shown). Other events essential for rebudding cycles may be entrained to the oscillator independently of Cln2-associated kinase activity (see below).

As Clns can auto-activate their own transcription^{7,8} and also appear to stimulate their own proteolysis^{9,10}, they could conceivably define an oscillator that drives G1 events in the absence of cell-cycle progression. Our finding that *cdc4-3 cdc28-4* double mutant cells do not exhibit budding or G1-specific transcription cycles indicates that Cln/Cdc28 kinase activity may be essential for these events (data not shown). However, our experiments do not distinguish whether Clns are components of an oscillator or are simply entrained to a Cln-independent oscillator, as the outputs we measured (budding and transcription) are themselves dependent on Cln/Cdc28-kinase activity.

Previous observations indicate that measurable oscillations may occur independent of Cln/Clb-associated kinase activity. In response to mating pheromone, budding yeast adopt a unique morphology, termed 'shmoo', by polarizing growth to form a

mating projection. Cells that do not conjugate with a partner of the opposite mating type can initiate multiple projections over time¹¹. The formation of mating projections is similar to budding, requiring many of the same genes¹² and involving the polarization of cortical F-actin to the projection site¹³. However, projection formation does not require Cdc28-kinase activity. To determine whether mating-projection formation occurs with a periodicity characteristic of budding cycles, we examined the formation of projections in a synchronous population of G1 cells deleted for all three *cln* genes.

We treated an elutriated G1 population of *cln1,2,3* mutant cells with the yeast mating pheromone, α -factor, and found that cells initiated multiple synchronous rounds of projection formation (Fig. 2a, c). The appearance of the second projection depends on the continued presence of α -factor (data not shown), indicating that the second projection may be a normal response to mating pheromone. As Cln activity is required for Clb expression¹⁴, *cln1,2,3* cells lack both Cln- and Clb-associated kinase activity. Thus the synchronous rounds of mating projection formation are triggered independently of Cln- and Clb-associated Cdc28-kinase activity. The kinetics of mating projection formation mirrored the kinetics of multiple bud formation in synchronized *clb1,2,3,4,5,6* mutant cells (Fig. 2a–c). Additionally, analysis of actin polarization by rhodamine–phalloidin staining showed that polarized actin is re-localized in cells immediately preceding the appearance of both new buds and new mating projections (Fig. 2b, c). Collectively, these data indicate that the relocalization of polarized growth machinery during mating projection cycles or budding cycles is entrained to the activity of a Cln/Clb-independent oscillator.

It is likely that the extended period of oscillations in these

experiments (~100 min) relative to those presented in Fig. 1 (70–80 min) reflects the lower temperature (30 °C) at which these experiments were performed. The period of budding cycles and mating-projecting cycles is similar to the cell-cycle time of wild-type cells growing at 30 °C (ref. 19), indicating that the same Cln/Clb-independent oscillator may also direct the polarized growth machinery as well as the initiation of other G1 events in normally dividing cells.

That G1 events may be triggered by the activity of an independent oscillator in normal cycling cells is not irreconcilable with previously established dependencies observed in the yeast cell cycle. When cell-cycle progression is perturbed, checkpoint signals delay progression into the next phase and may also directly or indirectly attenuate the activity of the oscillator. As we have shown that the loss of Clb-associated kinase activity uncouples the initiation of G1 functions from the completion of S phase or mitosis, we considered that Clb-associated kinase activity may be involved in preventing the activation of G1 events during a checkpoint arrest.

In budding yeast, cells arrest in mitosis with elevated Clb/Cdc28 kinase activity in response to incomplete DNA replication or spindle dysfunction^{16,17}. We investigated whether Clb/Cdc28-kinase activity is required to prevent G1-specific transcription and budding during spindle and DNA-replication checkpoint arrests. To eliminate Clb activity under checkpoint arrest conditions, we constructed strains that expressed a hyperstabilized allele of the Clb/Cdc28 inhibitor, *SIC1- Δ 3P*¹⁸, from the inducible *GAL1* promoter in wild-type cells and in a strain bearing a temperature-sensitive mutation in the thymidylate kinase gene¹⁹, *cdc8*.

In separate experiments, spindle integrity and DNA-replication checkpoint arrests were induced in the absence of *Sic1- Δ 3P* expression by the addition of the microtubule-destabilizing drug nocodazole (Fig. 3a–c), or by shifting *cdc8-1* cells to the restrictive temperature (Fig. 3d–f), respectively. When ~90% of the cells arrested with large buds, *Sic1- Δ 3P* expression was induced at 0 min

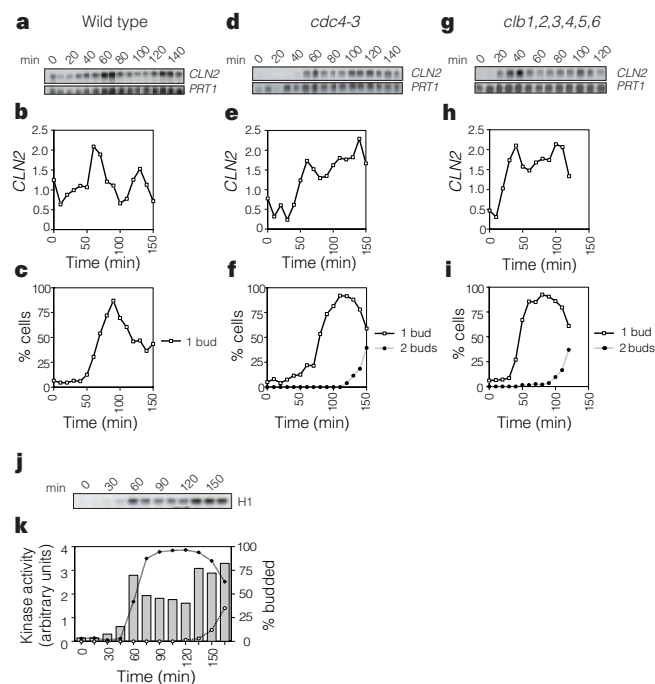


Figure 1 G1-specific transcription, budding cycles and Cln2-associated kinase activity in wild-type cells and cells lacking Clb activity. **a–i**, Time course following elutriation of G1 cells for wild-type cells (**a, c**), *cdc4-3* (**d–f**) and *clb1,2,3,4,5,6* cells (**g–i**). Analyses of *CLN2* transcripts (**a, b, d, e, g, h**) and budding indices (**c, f, i**) are shown. As the normalization transcript *PRT1* exhibits weak oscillations with the same periodicity as *CLN2* (ref. 30), the oscillation of *CLN2* is likely to be underestimated. Wild-type cells divided, so only single buds were scored. **j, k**, Synchronous oscillations of Cln2-associated kinase activity in *cdc4-3 CLN2-HA₃* cells. Time course following elutriation is shown. **j**, H1-kinase activity of anti-HA precipitates. **k**, Quantification of H1-kinase activity (solid bars) and budding index (filled symbols, 1 bud; open symbols, 2 buds). All results shown are representative of multiple experiments.

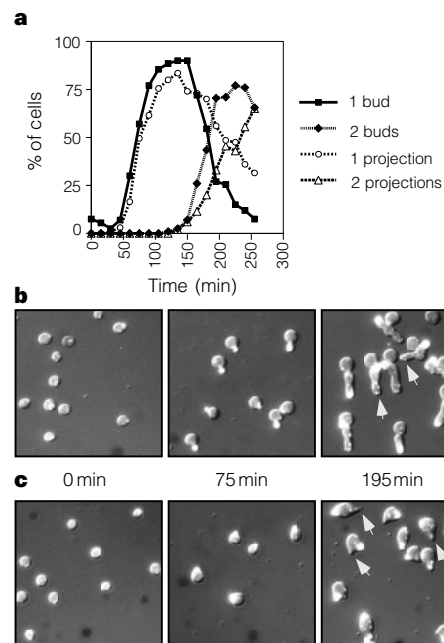


Figure 2 Synchronous mating projection cycles in a *cln1,2,3*-null mutant cells have similar kinetics to budding cycles in *clb1,2,3,4,5,6* mutant cells. Time course of synchronous G1 cells collected by elutriation. **a–c**, Mating projections were scored for *cln1,2,3* cells (**a**, open symbols; **c**) and buds were scored for *clb1,2,3,4,5,6* cells (**a**, filled symbols; **b**). Arrows indicate some sites of initial buds or mating projections (**b** and **c**, respectively). Phalloidin staining indicates presence of cortical F-actin.

while maintaining the checkpoint-arrest conditions. Upon induction of Sic1- Δ 3P, new buds emerged from the large budded cells in both the spindle and DNA-replication checkpoint-arrested cells expressing Sic1- Δ 3P, but not in control cells lacking Sic1- Δ 3P (Fig. 3c, f). Also, *CLN2* transcription was activated coincidentally with the appearance of new buds in cells expressing Sic1- Δ 3P, but not in control cells (Fig. 3a, b, d, e). DNA staining confirmed that rebudded cells had a single nucleus, indicating that mitosis had not occurred in response to the shift to galactose medium (data not shown). Similar results have been observed for cells in which Clb-associated kinase activity was eliminated in cells arrested during a spindle-checkpoint arrest^{20,21}.

These results indicate a role for B-type cyclin-associated kinase in preventing the initiation of new G1 events during checkpoint arrests, possibly by downregulating *CLN2* transcription²² or by directly attenuating the activity of the independent oscillator.

Our data support a model for the budding yeast cell cycle in which G1 events are triggered by a Cln/Clb-independent cell-cycle oscillator in normal cycling cells. That an independent oscillator may regulate some aspects of the yeast cell cycle indicates that cell-cycle regulation in embryonic cells and somatic cells may be fundamentally similar except that checkpoint mechanisms, absent in embryonic cells, exert an additional level of control in somatic cells. Our data also indicate that, in yeast, the activity of B-type cyclin-associated kinase attenuates the activity or outputs of the independent oscillator when cell-cycle progression is blocked and checkpoints are activated.

Previous findings have indicated that a *cdc2*-independent biochemical oscillator may control CO₂ production in the yeast *Schizosaccharomyces pombe*²³. The mechanism for generating periodic oscillations in yeast remains to be determined. Other known *Saccharomyces cerevisiae* cyclin/cdk systems could theoretically define an oscillator. Three members of the *PCL* family of cyclin-like genes can activate the cyclin-dependent kinase Pho85 and exhibit cell-cycle-regulated gene expression. However, as the expression of these genes is downregulated in response to α -factor²⁴, it is unlikely that they define the oscillator described in Fig. 2. Simple single-enzyme biochemical systems can generate sustained oscillations²⁵. The free-running oscillator described here, therefore, could reflect the self-generated fluctuations of an enzyme. Alter-

natively, as Ca²⁺ oscillations have been associated with cell-cycle functions in many systems²⁶, self-limiting ion-channel-regulated Ca²⁺ fluctuations might provide the basis for a cell-cycle regulatory oscillator. □

Methods

Strains, cell synchronization and checkpoint arrests

All strains are derivatives of BF264-15Dau, and were constructed by standard yeast methods. Yeast strains were grown in rich YEP medium (1% yeast extract, 2% peptone, 0.012% adenine, 0.012% uracil) containing 2% dextrose for all strains except for the galactose-dependent strains, *clb1,2,3,4,5,6 GAL1-CLB1* and *cln1,2,3 GAL1-CLN3*, which were grown in YEP medium with 2% galactose. For synchrony experiments, temperature-sensitive strains were grown at 25 °C before elutriation. All other strains were grown at 30 °C. For *clb1,2,3,4,5,6 GAL-CLB1* and *cln1,2,3 GAL1-CLN3* cells, dextrose was added to YEP + 2% galactose medium 45 min before elutriation to terminate *CLB1* and *CLN3* expression from the *GAL1* promoter. After elutriation, *clb1,2,3,4,5,6 GAL1-CLB1* cells (Fig. 1) were maintained in G1 for 80 min by treatment with 60 ng ml⁻¹ α -factor to ensure complete destruction of Clb1 and then released at 0 min. After elutriation all strains in Fig. 1 were grown in rich YEP + 2% dextrose + 1M sorbitol at 36 °C at a density of ~10⁷ per ml. Sorbitol was added to stabilize cells with elongated buds. Elutriated cells in Fig. 2 were grown in YEP dextrose medium without sorbitol at 30 °C. After elutriation, *cln1,2,3 GAL1-CLN3* were treated with 60 ng ml⁻¹ α -factor.

For arrest with nocodazole, cells were pre-synchronized in S phase by being grown for 2 h in YEP + 4% raffinose medium containing 5 mg ml⁻¹ sulphamylamide and 200 μ g ml⁻¹ amethopterin (Sigma). At this time most cells were arrested in S phase with large buds. Cells were collected by centrifugation and released into YEP + 2% raffinose medium containing 15 μ g ml⁻¹ nocodazole for 2 h. *cdc8* strains were arrested in YEP + 2% sucrose medium for 2 h.

RNA analysis, kinase assays and quantification

We used standard yeast RNA methods²⁷. Kinase assays were performed on immunoprecipitates at 25 °C, as described¹⁵. Hybridizations and H1 kinase assays were quantified using a Phosphorimager (Molecular Dynamics). Transcript signals were normalized to the levels of the constitutive *PRT1* transcript²⁸ as indicated.

Microscopy

Cells were stained for actin using rhodamine-conjugated phalloidin as described²⁹. Differential interference (DIC) and fluorescence images were collected using a Nikon Edipse E800 microscope with a 60 \times objective, and a Quantix CCD camera (Photometrics).

Received 26 May; accepted 15 July 1999.

- Hartwell, L. H. & Weinert, T. A. Checkpoints: Controls that ensure the order of cell cycle events. *Science* **246**, 629–634 (1989).
- Murray, A. W. & Kirschner, M. W. Dominoes and clocks: the union of two views of the cell cycle. *Science* **246**, 614–621 (1989).
- Hartwell, L. H. Genetic control of the cell division cycle in yeast. *Exp. Cell Res.* **69**, 265–276 (1971).
- Mathias, N. et al. Cdc53p acts in concert with Cdc4p and Cdc34p to control the G1-to-S-phase transition and identifies a conserved family of proteins. *Mol. Cell. Biol.* **16**, 6634–6643 (1996).
- Schwob, E., Bohm, T., Mendenhall, M. D. & Nasmyth, K. The B-type cyclin kinase inhibitor p40^{SIC1} controls the G1 to S transition in *S. cerevisiae*. *Cell* **79**, 233–244 (1994).
- Reed, S., Wittenberg, C., Lew, D., Dulic, V. & Henze, M. G1 control in yeast and animal cells. *Cold Spring Harb. Symp. quant. Biol.* **56**, 61–67 (1992).
- Dirick, L. & Nasmyth, K. Positive feedback in the activation of G1 cyclins in yeast. *Nature* **351**, 754–757 (1991).
- Cross, F. R. & Tinkelenberg, A. H. A potential positive feedback loop controlling *CLN1* and *CLN2* gene expression at the start of the yeast cell cycle. *Cell* **65**, 875–883 (1991).
- Tyers, M., Tokiwa, G., Nash, R. & Futcher, B. The Cln3-Cdc28 kinase complex of *S. cerevisiae* is regulated by proteolysis and phosphorylation. *EMBO J.* **11**, 1773–1784 (1992).
- Lanker, S., Valdivieso, M. H. & Wittenberg, C. Rapid degradation of the G1 cyclin Cln2 induced by CDK-dependent phosphorylation. *Science* **271**, 1597–1601 (1996).
- Bucking-Throm, E., Duntze, W., Hartwell, L. H. & Manney, T. R. Reversible arrest of haploid yeast cells in the initiation of DNA synthesis by a diffusible sex factor. *Exp. Cell Res.* **76**, 99–100 (1973).
- Chenevert, J., Valtz, N. & Herskowitz, I. Identification of genes required for normal pheromone-induced cell polarization in *Saccharomyces cerevisiae*. *Genetics* **136**, 1287–1296 (1994).
- Gehring, S. & Snyder, M. The *SPA2* gene of *Saccharomyces cerevisiae* is important for pheromone-induced morphogenesis and efficient mating. *J. Cell Biol.* **111**, 1451–1464 (1990).
- Surana, U. et al. The role of *CDC28* and cyclins during mitosis in the budding yeast *S. cerevisiae*. *Cell* **65**, 145–161 (1991).
- Basco, R. D., Segal, M. D. & Reed, S. I. Negative regulation of G1 and G2 by S-phase cyclins of *Saccharomyces cerevisiae*. *Mol. Cell. Bio.* **15**, 5030–5042 (1995).
- Stueland, C. S., Lew, D. J. & Reed, S. I. Full activation of p34^{CDC28} histone H1 kinase activity is unable to promote entry into mitosis in checkpoint-arrested cells of the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13**, 3744–3755 (1993).
- Sorger, P. K. & Murray, A. W. S-phase feedback control in budding yeast independent of tyrosine phosphorylation of p34^{CDC28}. *Nature* **355**, 365–368 (1992).
- Verma, R. et al. Phosphorylation of Sic1p by G1 Cdk required for its degradation and entry into S phase. *Science* **278**, 455–460 (1997).
- Scalfani, R. A. & Fangman, W. L. Yeast gene *CDC8* encodes thymidylate kinase and is complemented by the herpes thymidine kinase gene *TK*. *Proc. Natl Acad. Sci. USA* **81**, 5821–5825 (1984).
- Schwab, M., Lutum, A. S. & Seufert, W. Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. *Cell* **90**,

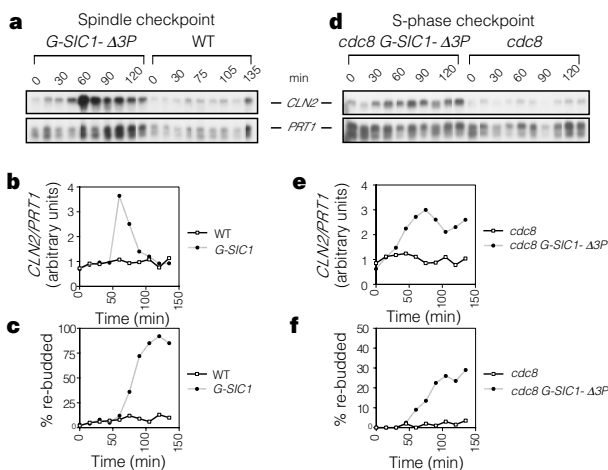


Figure 3 Clb activity is required to prevent the activation of G1 transcription. **a–c**, Time course of *GAL-SIC1- Δ 3P* and wild-type (WT) cells arrested at the spindle checkpoint by nocodazole treatment. Cells were shifted to galactose medium containing nocodazole at *t* = 0 min to induce Sic1- Δ 3P expression. **d–f**, Time course of *cdc8 GAL-SIC1- Δ 3P* and *cdc8* control cells arrested at the DNA-replication checkpoint by shifting to restrictive temperature (36 °C). Cells were shifted to galactose medium at *t* = 0 min to induce Sic1- Δ 3P expression and maintained at 36 °C. RNA analyses (**a, b, d, e**) and budding indices (**c, f**) are shown. Large budded cells with new buds emerging were scored as rebudded.

683–693 (1997).

21. Dahmann, C., Diffley, J. F. & Nasmyth, K. A. S-phase-promoting cyclin-dependent kinases prevent re-replication by inhibiting the transition of replication origins to a pre-replicative state. *Curr. Biol.* **5**, 1257–1269 (1995).
22. Amon, A., Tyers, M., Futcher, B. & Nasmyth, K. Mechanisms that help the yeast cell cycle clock tick: G2 cyclins transcriptionally activate G2 cyclins and repress G1 cyclins. *Cell* **74**, 993–1007 (1993).
23. Novak, B. & Mitchison, J. M. Change in the rate of CO2 production in synchronous cultures of the fission yeast *Schizosaccharomyces pombe*: a periodic cell cycle event that persists after the DNA-division cycle has been blocked. *J. Cell. Sci.* **86**, 191–206 (1986).
24. Measday, V. et al. A family of cyclin-like proteins that interact with the Pho85 cyclin-dependent kinase. *Mol. Cell. Biol.* **17**, 1212–1223 (1997).
25. Ngo, L. G. & Roussel, M. R. A new class of biochemical oscillator models based on competitive binding. *Eur. J. Biochem.* **245**, 182–190 (1997).
26. Whitaker, M. & Patel, R. Calcium and cell cycle control. *Development* **108**, 525–542 (1990).
27. Collart, M. A. & Oliviero, S. in *Current Protocols in Molecular Biology* Vol. 2.13.12 (Current Protocols, John Wiley and Sons, New York, 1993).
28. Xu, H., Kim, U. J., Schuster, T. & Grunstein, M. Identification of a new set of cell cycle-regulatory genes that regulate S-phase transcription of histone genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**, 5249–5259 (1992).
29. Adams, A. E. M. & Pringle, J. R. Staining of actin with fluorochrome-conjugated phalloidin. *Methods Enzymol.* **194**, 729–731 (1991).
30. Spellman, P. T. et al. Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol. Biol. Cell* **9**, 3273–3297 (1998).

Acknowledgements

We thank R. Deshaies for the stabilized Sic1-Δ3P construct; D. Stuart for the triple *cln* null mutant strain; M. Grunstein for the *HTA1/PRT1* probe; C. Wittenberg, N. Rhind and K. Sato for critical review of the manuscript; and members of the Reed laboratory for helpful discussions. This work was supported in part by the Leukemia Society of America and the NIH.

Correspondence and requests for materials should be addressed to S.I.R. (e-mail: sreed@scripps.edu).

Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination

Roger D. Johnson*, Nan Liu† & Maria Jasin*

* Cell Biology Program, Memorial Sloan-Kettering Cancer Center, and Cornell University Graduate School of Medical Sciences, 1275 York Avenue, New York, New York 10021, USA

† Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California 94551, USA

The repair of DNA double-strand breaks is essential for cells to maintain their genomic integrity. Two major mechanisms are responsible for repairing these breaks in mammalian cells, non-homologous end-joining (NHEJ) and homologous recombination (HR)^{1,2}: the importance of the former in mammalian cells is well established³, whereas the role of the latter is just emerging. Homologous recombination is presumably promoted by an evolutionarily conserved group of genes termed the *Rad52* epistasis group^{4–11}. An essential component of the HR pathway is the strand-exchange protein, known as RecA in bacteria⁸ or Rad51 in yeast⁶. Several mammalian genes have been implicated in repair by homologous recombination on the basis of their sequence homology to yeast Rad51 (ref. 11): one of these is human *XRCC2* (refs 12, 13). Here we show that *XRCC2* is essential for the efficient repair of DNA double-strand breaks by homologous recombination between sister chromatids. We find that hamster cells deficient in *XRCC2* show more than a 100-fold decrease in HR induced by double-strand breaks compared with the parental cell line. This defect is corrected to almost wild-type levels by transient transfection with a plasmid expressing *XRCC2*. The repair defect in *XRCC2* mutant cells appears to be restricted to

recombinational repair because NHEJ is normal. We conclude that *XRCC2* is involved in the repair of DNA double-strand breaks by homologous recombination.

Similar to yeast mutants that affect DNA double-strand break (DSB) repair by HR⁶, hamster cells that lack *XRCC2* are hypersensitive to ionizing radiation (about 2-fold) and crosslinking agents (60- to 100-fold), and show an increase in chromosome instability^{13,14}. In contrast to yeast, all characterized mammalian DSB-repair mutants have been found to be defective in NHEJ. Thus, the role of *XRCC2* in DNA repair is unclear. To determine whether the hamster cell line *irs1*, which is deficient in *XRCC2* (refs 12, 13), can repair DSBs by HR, we used a novel recombination reporter substrate SCneo (Fig. 1a). SCneo contains two nonfunctional copies of the neomycin phosphotransferase (*neo*) gene. One copy, designated 3' *neo*, is a 5' truncation of the *neo* gene¹⁵. The second copy, designated S2*neo*, is mutated at an *NcoI* site by deletion of 4 base pairs (bp) of *neo* gene coding sequence and insertion of the 18-bp site for the rare-cutting *I-SceI* endonuclease¹⁶. The two *neo* genes are in direct orientation and are separated by a functional hygromycin

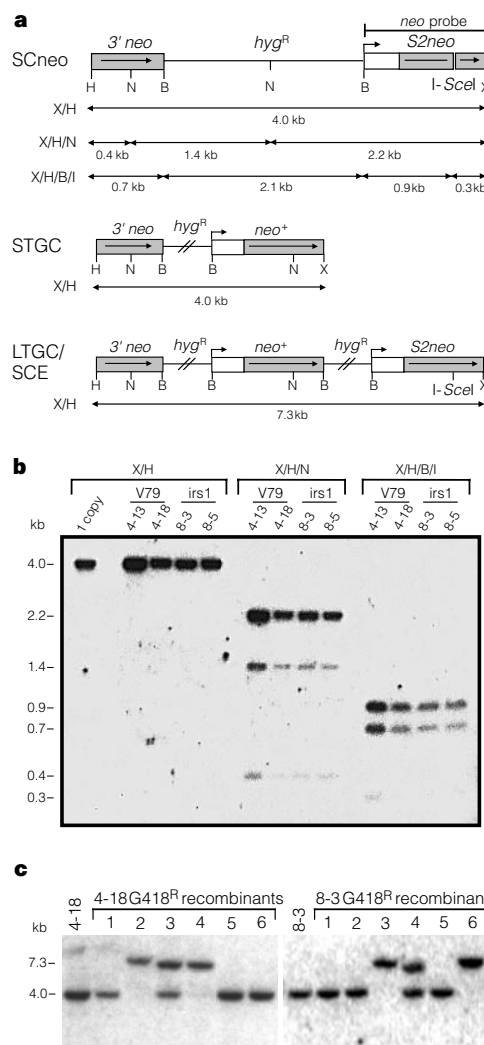


Figure 1 Recombination reporter substrate SCneo. **a**, Structure of SCneo and predicted HR products. The *neo* probe is indicated. X/H, *XhoI/HindIII*; X/H/N, *XhoI/HindIII/NcoI*; X/H/B/I, *XhoI/HindIII/BamHI/I-SceI*. **b**, Southern blot analysis of SCneo cell lines. Each cell line contains a single copy of SCneo, except the parental cell line 4-13 which contains two copies. **c**, Southern blot analysis of cell lines 4-18 (V79) and 8-3 (*irs1*) and G418^R recombinants derived from them. Genomic DNA was digested with *XhoI/HindIII* to distinguish STGC (4.0 kb) and LTGC/SCE (7.3 kb). Recombinants with both fragments probably underwent two recombination events.