Expanded Roles for Chk1 in Genome Maintenance*

Published, JBC Papers in Press, April 18, 2008, DOI 10.1074/jbc.R800021200

Greg H. Enders

From the Department of Medicine, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

Chk1 is a conserved kinase that imposes cell cycle delays in response to impediments to DNA replication. Recent experiments have further defined effects of Chk1 on the activity of mammalian origins of DNA replication and progression of replication forks. Moreover, Chk1 now appears to help defend genomic integrity through effects on several other pathways, including Fanconi anemia proteins, the mitotic spindle, and transcription of cell cycle-related genes. These findings can account for the requirement for Chk1 in normal proliferating cells of the early embryo and suggest the potential for diverse effects of Chk1 inhibition in cancer therapy.

The most precious possession for any organism is its genetic dowry. Eukaryotic cells store their genomic inheritance in DNA, partitioned into discrete chromosomes. DNA is an inherently stable biomolecule. Moreover, each strand in a double helix can serve as a functional copy to regenerate the native sequence during DNA replication and repair, processes executed with remarkable fidelity. Despite these safeguards, DNA damage occurs routinely due to such lesions as spontaneous and induced nucleotide modifications and impediments to replication. Eukaryotic cells have evolved sophisticated means to recognize DNA damage and replication defects and launch “checkpoint” responses that delay DNA replication and mitosis and facilitate DNA repair. A central player in this response is the Chk12 protein kinase (1–3). Chk1 has been known to inhibit cyclin-dependent kinase (Cdk) activity through destabilization and facilitate DNA repair. A central player in this response is the Chk12 protein kinase (1–3). Chk1 has been known to inhibit cyclin-dependent kinase (Cdk) activity through destabilization of the Cdk activator Cdc25A. Recent studies have delineated additional mechanisms through which Chk1 preserves genomic integrity. This work is of fundamental interest in cell biology and informs efforts to integrate Chk1 inhibitors into cancer chemotherapy (4). Treatment with genotoxic drugs remains the backbone of such therapy.

The Canonical Chk1 Pathway

DNA double-strand breaks and other impediments to progression of replication forks are detected by two “sensor” kinases of the phosphoinositol 3-kinase group: ATM and ATR. ATM is a clinical syndrome characterized by sensitivity to radiation and tumorigenesis. In turn, ATM and ATR activate the “effector” kinases Chk1 and Chk2. Double-strand breaks primarily activate ATM and Chk2, whereas replication defects, acting in part through generation of single-strand DNA, activate ATRIP (ATR-interacting protein), ATR, and Chk1 (5–7) (Fig. 1). Although distinct in outline, these pathways exhibit substantial cross-talk. Activation of Chk1 in S phase cells delays DNA replication, stabilizes stalled replication forks, prevents cell death, and blocks mitotic entry. The block to mitotic entry is best understood and is mediated by inhibitory phosphorylation of Cdk1 in the ATP-binding site. This phosphorylation is antagonized by Cdc25A phosphatase, which is targeted for degradation by direct Chk1-mediated phosphorylation (8). Chk1 is essential for mammalian development, an atypical property for a checkpoint protein. Homozygous deletion of Chk1 in the mouse results in early embryonic lethality (9, 10). Chk1-null mouse embryonic cells exhibit enhanced mitotic entry and death following treatment with a DNA polymerase inhibitor, ionizing irradiation, or ultraviolet radiation (10). Chk1 heterozygous mammary epithelia display evidence of early mitotic entry, prior to completion of DNA synthesis, accompanied by increased DNA damage (11). These and other data have firmly established a role for Chk1 in restraining mitotic entry in response to DNA damage and replication blocks, in part by inhibiting Cdk activity.

Further Definition of Roles in Regulating Replication

DNA damage that occurs during DNA replication normally activates a checkpoint that delays cells within S phase, mediated in part by Chk1 and Chk2 (7, 12). Early experiments revealed that Chk1 stabilizes replication forks and inhibits firing of origins of DNA replication that are normally activated in late S phase (13, 14). Recent experiments have extended our understanding of effects on origins of replication and identified delays in replication fork progression (15). Single DNA fibers pulse-labeled with modified nucleotides that can be detected with fluorescent antibodies have been used to analyze inter-origin distance and fork movement. In both human cells treated with Chk1 inhibitors and chicken cells induced to delete the chk1 gene, inter-origin distance was decreased. Thus, neighboring origins that usually remain quiescent were activated. This response appears to compensate for inefficient fork progression. Whether Chk1 acts directly at origins to inhibit their firing remains unclear. Treatment of mammalian cells with the topoisomerase inhibitor camptothecin, a well established mediator of DNA double-strand breaks, curtails elongation from initiated replication forks (16). This effect was rescued by co-treatment with small molecule Chk1 inhibitors or Chk1-directed RNA interference (16). Thus, Chk1 appears to suppress firing of some origins and curtail elongation of initiated replication forks.
MINIREVIEW: Expanded Roles for Chk1

**Function at Centrosomes**

Chk1 has been detected previously at centrosomes (17), sites of nucleation of the mitotic spindle. This localization has been thought to facilitate repression of the first wave of Cdk1 activation at mitosis, which may begin at centrosomes (18). Recent work shows that checkpoint irradiation or treatment with the replication inhibitor hydroxyurea leads to concentration of Chk1 on centrosomes (19). Chemical or genetic disruption of ATM/ATR activity did not prevent this accumulation and, in fact, enhanced it. However, centrosomal Chk1 appeared to be phosphorylated by ATM and/or ATR, suggesting that the sensor kinases may still contribute to centrosomal Chk1 activity (19). Targeting a Chk1 mutant with defective kinase activity to centrosomes yielded increased mitotic entry in irradiated cells, consistent with the notion that centrosomal Chk1 limits mitotic entry in this setting. In another study, centrosomal targeting of Chk1 harboring mutations in ATR-dependent phosphorylation sites reduced apoptosis (20). These results support a role for centrosomal Chk1 in checkpoint-mediated inhibition of mitotic entry. A centrosome checkpoint at the G1/S transition has recently been described (21). Whether Chk1 contributes to this checkpoint remains unclear.

**Optimizing Spindle Checkpoint Function**

DT40 chicken lymphoma cells and their Chk1-null derivatives were analyzed by time-lapse video microscopy while undergoing mitosis. In about one-third of Chk1-null cells, anaphase was observed to occur despite misalignment of chromosomes. Such defective anaphases imply defective spindle checkpoints and were not seen in parental cells (22). Findings similar to those in Chk1-null chicken cells were obtained in human cells rendered transiently Chk1-deficient by small interfering RNA. Chk1-green fluorescent protein was detected at kineto-ATM and ATR directly phosphorylate and activate Chk1 in response to spindle toxins and what sites become phosphorylated remain to be resolved. It is also unclear how spindle poisons affect ATM/ATR activity. Thus, the regulation of Chk1 in response to spindle disruption remains to be defined. Nonetheless, these results suggest that Chk1 contributes to the strength of cell cycle delays mediated by the spindle checkpoint.

**Moonlighting in the Fanconi Anemia Genomic Integrity Pathway**

Fanconi anemia (FA) is a rare genetic syndrome characterized by progressive bone marrow failure and predisposition to cancer (23). FA cells are defective in repair of DNA cross-links. There are 11 known complementation groups. Eight FA proteins form a complex that mediates monoubiquitination of FANCD2. Interactions between FA proteins and other DNA damage response and repair pathways have begun to emerge. Phosphorylation of histone variant H2AX, a component of the canonical DNA damage response pathway, is required for recruitment of FANCD2 to sites of DNA cross-links (24). Chk1 phosphorylates FANCE on two conserved sites (25). A FANCE mutant with a non-phosphorylatable residue at one of these sites can mediate monoubiquitination of FANCD2 but does not restore function of the FA pathway in DNA repair (25). Thus, FANCE may be a functional target of Chk1, suggesting that Chk1 may integrate a network of DNA damage checkpoint and repair responses.

**Direct Transcriptional Repression**

The most novel new function for Chk1 is as a chromatin-modifying transcription factor. Chromatin modifications have been shown to be central to regulation of transcription of many loci (26). Chk1 has been recognized for some time to be a...
nuclear protein with activity in normal cycling cells. DNA damage fosters cytoplasmic, particularly centrosomal, re-localization of Chk1, as touched on above. Among a set of common histone modifications examined in mammalian cells, only histone H3 Thr11 phosphorylation and Lys9 acetylation were markedly diminished following irradiation or treatment with other DNA-damaging agents or replication inhibitors (27). These modifications correlated, in the first hours of exposure to the DNA-damaging agent, with reduced transcript levels for several cell cycle proteins, including cyclins B and A and Cdk1. Residues surrounding histone H3 Thr11 fit a minimum consensus for Chk1 phosphorylation, and acute deletion of Chk1 in mouse embryonic fibroblasts durably reduced Thr11 phosphorylation and transiently reduced Lys9 acetylation. Treatment with caffeine prevented both the release of Chk1 from chromatin and the loss of Thr11 phosphorylation, implying that both effects are dependent on ATM/ATR activity. Mutation of ATR phosphorylation sites was already known to prevent Chk1 dissociation from chromatin in response to DNA damage (20). Ablation of Chk1 expression was associated with S phase arrest in mouse embryonic fibroblasts and decreased recruitment of the GCN5 transcription factor to cyclin B and cdk1 promoters (27). These data suggest that ATM/ATR-directed release of Chk1 from chromatin mediates concerted reductions in histone H3 Thr11 phosphorylation and Lys9 acetylation, GCN5 binding, and transcription of several cell cycle genes. Evidence was found for effects of Chk1, directly or indirectly, on the transcription of some 200 genes and phosphorylation of other sites in histones (27).

**Implications and Future Directions**

The results of these recent studies imply a broader role for Chk1 in maintaining genomic integrity than previously appreciated. Chk1 appears to help maintain basal transcription of some cell cycle genes in unperturbed proliferating cells, thus acting as a positive factor in cell cycle progression. This function is consistent with prior evidence that Chk1 is preferentially expressed in cycling cells, may be activated by E2F proteins (28), and is induced by Cdk2 activity (29). Moreover, it can account for the severe defects in cell proliferation observed in Chk1-null early embryos. Independent evidence has emerged that Chk1 phosphorylation of the retinoblastoma protein may enhance binding to E2F and repression of cell cycle transcription (30). Although mechanistically distinct, this finding also suggests relatively direct regulation of transcription by Chk1. Following DNA damage, Chk1 appears to inhibit elongation of replication forks as well as firing of replication origins, clarifying the mechanisms of S phase delay. Cdk inhibition and/or the newly recognized transcriptional effects of Chk1 may contribute to these activities. Centrosomal re-localization of Chk1 following DNA damage can now be viewed as having dual functions, exerting specific effects at these sites as well as inhibiting Chk1 action on chromatin. The enhancement of spindle checkpoint function by Chk1 may be complex, reflecting direct effects on centrosomes or spindle checkpoint proteins such as aurora B and indirect effects through Cdns and transcription. Chk1 appears to optimize FA pathway function through at least some direct effects, such as FANCE phosphorylation, thereby coordinating a broad cellular response to DNA cross-links. Such a broad role for Chk1 makes sense in light of the fact that cross-links and other forms of DNA damage impose blocks to replication, potentially requiring an integrated cellular response.

Future studies will better define how Chk1 is targeted to chromatin and, perhaps, to promoters of cell cycle genes. Chk1-mediated phosphorylation of additional sites on chromatin and/or chromatin-associated proteins may prove to be functionally important. Chk1 mutations that can selectively abrogate localization to chromatin, centrosomes, or possibly kinetochores will be valuable in further dissecting the relative contributions of Chk1 at these sites to DNA and spindle checkpoint responses. Better definition is needed of the mechanisms through which spindle toxins regulate Chk1, possibly through ATM/ATR. The effects of Chk1 on centrosomes warrant further study. Interesting evidence suggests that Chk1 may drive centrosomal replication (19), but the relationship of this activity to Chk1 function in genome maintenance remains to be explored. Additional mechanisms through which Chk1 integrates cellular genome maintenance pathways will likely be identified. These studies will elucidate the role of Chk1 mutations in colorectal carcinomas with defective mismatch repair (31, 32) and inform efforts to inhibit Chk1 to therapeutic benefit in cancer chemotherapy.

**Acknowledgment**—Tim Yen provided valuable comments on the manuscript.

**REFERENCES**

1. Boddy, M. N., Furnari, B., Mondesert, O., and Russell, P. (1998) Science 280, 909–912
2. Furnari, B., Rhind, N., and Russell, P. (1997) Science 277, 1495–1497
3. Sanchez, Y., Wong, C., Thoma, R. S., Richman, R., Wu, Z., Piwnica-Worms, H., and Elledge, S. J. (1997) Science 277, 1497–1501
4. Zhou, B. B., and Bartek, J. (2004) Nat. Rev. Cancer 4, 216–225
5. Ball, H. L., Myers, J. S., and Cortez, D. (2005) Mol. Biol. Cell 16, 2372–2381
6. Crotez, D., Guntuku, S., Qin, J., and Elledge, S. J. (2001) Science 294, 1713–1716
7. MacDougall, C. A., Byun, T. S., Van C., Yee, M. C., and Cimprich, K. A. (2007) Genes Dev. 21, 898–903
8. Sorensen, C. S., Syljuasson, R. G., Falck, J., Schroeder, T., Ronnstrand, L., Khanna, K. K., Zhou, B. B., Bartek, J., and Lukas, J. (2005) Cancer Cell 3, 247–258
9. Liu, Q., Guntuku, S., Cui, X. S., Matsuoka, S., Cortez, D., Tamai, K., Luo, G., Carattini-Rivera, S., DeMayo, F., Bradley, A., Donehower, L. A., and Elledge, S. J. (2000) Genes Dev. 14, 1448–1459
10. Takai, H., Tominaga, K., Motoyama, N., Minamishima, Y., Nagahama, H., Tsukiyama, T., Ikeda, K., Nakayama, K., Nakaniishi, M., and Nakayama, K. (2000) Genes Dev. 14, 1439–1447
11. Lam, M. H., Liu, Q., Elledge, S. J., and Rosen, J. M. (2004) Cancer Cell 6, 45–59
12. Falck, J., Petrin, J. H., Williams, B. R., Lukas, J., and Bartek, J. (2002) Nat. Genet. 30, 290–294
13. Larner, J. M., Lee, H., Little, R. D., Dijkwel, P. A., Schildkraut, C. L., and Hamlin, J. L. (1999) Nucleic Acids Res. 27, 803–809
14. Nitani, N., Nakamura, K., Nakagawa, C., Masukata, H., and Nakagawa, T. (2006) Genetics 174, 155–165
15. Conti, C., Sacca, B., Herrick, J., Lalou, C., Pommier, Y., and Bensimon, A. (2007) Mol. Biol. Cell 18, 3059–3067
16. Seiler, J. A., Conti, C., Syed, A., Aladjem, M. I., and Pommier, Y. (2007) Mol. Biol. Cell 27, 5806–5818
MINIREVIEW: Expanded Roles for Chk1

17. Kramer, A., Mailand, N., Lukas, C., Syljuasen, R. G., Wilkinson, C. J., Nigg, E. A., Bartek, J., and Lukas, J. (2004) *Nat. Cell Biol.* 6, 884–891
18. Jackman, M., Lindon, C., Nigg, E. A., and Pines, J. (2003) *Nat. Cell Biol.* 5, 143–148
19. Loffler, H., Bochtler, T., Fritz, B., Tews, B., Ho, A. D., Lukas, J., Bartek, J., and Kramer, A. (2007) *Cell Cycle* 6, 2541–2548
20. Niida, H., Katsuno, Y., Banerjee, B., Hande, M. P., and Nakanishi, M. (2007) *Mol. Cell. Biol.* 27, 2572–2581
21. Mikule, K., Delaval, B., Kaldis, P., Jurczyk, A., Hergert, P., and Doxsey, S. (2007) *Nat. Cell Biol.* 9, 160–170
22. Zachos, G., Black, E. J., Walker, M., Scott, M. T., Vagnarelli, P., Earnshaw, W. C., and Gillespie, D. A. (2007) *Dev. Cell* 12, 247–260
23. Garcia-Higuera, I., Taniguchi, T., Ganesan, S., Meyn, M. S., Timmers, C., Hejna, J., Grompe, M., and D’Andrea, A. D. (2001) *Mol. Cell* 7, 249–262
24. Bogliolo, M., Lyakhovich, A., Callen, E., Castella, M., Cappelli, E., Ramirez, M. J., Creus, A., Marcos, R., Kalb, R., Neveling, K., Schindler, D., and Surralles, I. (2007) *EMBO J.* 26, 1340–1351
25. Wang, X., Kennedy, R. D., Ray, K., Stuckert, P., Ellenberger, T., and D’Andrea, A. D. (2007) *Mol. Cell. Biol.* 27, 3098–3108
26. Kouzarides, T. (2007) *Cell* 128, 693–705
27. Shimada, M., Niida, H., Zineldeen, D. H., Tagami, H., Tanaka, M., Saito, H., and Nakanishi, M. (2008) *Cell* 132, 221–232
28. Ren, B., Cam, H., Takahashi, Y., Volkert, T., Terragni, J., Young, R. A., and Dynlacht, B. D. (2002) *Genes Dev.* 16, 245–256
29. Maude, S., and Enders, G. (2005) *Cancer Res.* 65, 780–786
30. Inoue, Y., Kitagawa, M., and Taya, Y. (2007) *EMBO J.* 26, 2083–2093
31. Bertoni, F., Codegoni, A. M., Furlan, D., Tibiletti, M. G., Capella, C., and Broggi, M. (1999) *Genes Chromosomes Cancer* 26, 176–180
32. Kim, C. J., Lee, J. H., Song, J. W., Cho, Y. G., Kim, S. Y., Nam, S. W., Yoo, N. I., Park, W. S., and Lee, J. Y. (2007) *Eur. J. Surg. Oncol.* 33, 580–585