

A Role for Dormant Origins in Tumor Suppression

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In this issue of *Molecular Cell*, Kawabata et al. (2011) show that mice hypomorphic for the replication licensing protein Mcm4 show spontaneous DNA replication defects due to a lack of dormant origins, potentially explaining why these mice are cancer prone.

In late mitosis and G1, origin DNA is licensed for replication by loading Mcm2-7 complexes. To prevent reduplication of sections of the genome, origins must fire no more than once per cell cycle. This is achieved by preventing further loading of Mcm2-7 onto origins during S phase and G2. Therefore, even if replication forks have stalled, new origins cannot be licensed during S phase to ensure complete genome duplication. Cells overcome this limitation by loading excess Mcm2-7 onto chromatin prior to S phase, licensing dormant origins that do not fire during unperturbed S phases but that can be activated to promote complete genome replication (Woodward et al., 2006; Ge et al., 2007; Gilbert, 2007; Ibarra et al., 2008) (normal cells in Figure 1). Mice homozygous for the *MCM4*^{Chaos3} mutation are cancer prone (Shima et al., 2007). A new paper by Shima and colleagues in this issue (Kawabata et al., 2011) shows that *MCM4*^{Chaos3/Chaos3} mice exhibit reduced levels of chromatin-bound Mcm2-7 protein and a corresponding inability to activate dormant origins when challenged with aphidicolin, an inhibitor of replicative DNA polymerases (MCM hypomorph in Figure 1). Strikingly, even in the absence of externally supplied replication stresses, *MCM4*^{Chaos3/Chaos3} cells have an increased number of stalled replication forks, DNA damage foci, and abnormal mitoses. This genetic instability very likely explains why the mice are tumor prone. These findings demonstrate the critical importance of dormant replication origins for cells in a physiological setting and have implications for the genetic instability commonly seen in cancer cells.

The *MCM4*^{Chaos3} mutant studied by Kawabata et al. (2011) could potentially affect Mcm2-7 function in a number of

different ways. Two related functions in the *MCM4*^{Chaos3} mutant, the replication fork speed and the helicase (DNA-unwinding) activity of Mcm2-7 complexes, are essentially normal. In contrast, *MCM4*^{Chaos3} mutant Mcm2-7 complexes are relatively unstable, reducing total Mcm2-7 levels by ~60%. Similar levels of RNAi-induced Mcm2-7 knockdown in tissue culture cells cause a major defect in the activation of dormant origins (Ge et al., 2007). Despite having fewer dormant origins, *MCM4*^{Chaos3} mutant cells have essentially normal rates of DNA replication, with only marginal or undetectable activation of DNA damage response kinases. The major problem for *MCM4*^{Chaos3/Chaos3} cells therefore appears to be a reduction in the number of dormant origins available for use when replication forks stall, consistent with another report studying mice expressing an MCM2 mutant (Kunnev et al., 2010).

MCM4^{Chaos3/Chaos3} cells in an unchallenged S phase showed a small increase in RAD51, RPA32, and RAD17 foci, but no increase in homologous recombination, indicative of a slight increase in DNA damage. More dramatically, immunofluorescence analysis of mitotic *MCM4*^{Chaos3/Chaos3} cells revealed a 50% increase in FANCD2 foci (a Fanconi anemia protein involved in resolving stalled replication intermediates), accompanied by >2-fold increase in aberrant anaphases and formation of micronuclei. This suggests that cells without sufficient dormant origins have problems properly resolving DNA damage normally processed by the Fanconi anemia pathway. Why these structures do not robustly activate intra-S phase or G2/M checkpoints is currently unclear. The absence of cell-cycle checkpoint activation allows

a significant percentage of cells to progress into mitosis with unresolved replication intermediates, most likely contributing to the aneuploidy in these cells.

Primary cells progressing through late G1 can activate a “licensing checkpoint” that blocks entry into S phase if they have licensed an insufficient number of replication origins (Blow and Gillespie, 2008). The licensing checkpoint depends on a number of genes that are commonly mutated in cancer. Previous evidence shows that the licensing checkpoint is engaged when levels of chromatin-bound Mcm2-7 are strongly reduced, but it remains unclear how sensitive the licensing checkpoint is to smaller reductions, such as those seen in *MCM4*^{Chaos3/Chaos3} cells. Neither the *MCM4* nor the *MCM2* hypomorphic cells display gross proliferation defects. One possibility is that dormant origin defects are seen in *MCM4*^{Chaos3/Chaos3} and *MCM2* mutant mice because the reduction is too modest to reproducibly engage the licensing checkpoint. The levels of *MCM2-7* could just be enough to enter S phase, causing replication defects that are too minor to strongly activate checkpoints but, in the long term, substantial enough to cause genomic instability and cancer. Following this interpretation, mice with more severe reductions in origin licensing might additionally display defects in developing tissues due to checkpoint-induced G1 delay. This sort of developmental defect might explain the stem cell deficiency reported for *MCM2* hypomorphic mice (Pruitt et al., 2007).

One unexpected feature of the *MCM* mutant mice is the different tumors that they experience: the original *MCM4*^{Chaos3/Chaos3} mice suffered mainly mammary adenocarcinomas (Shima

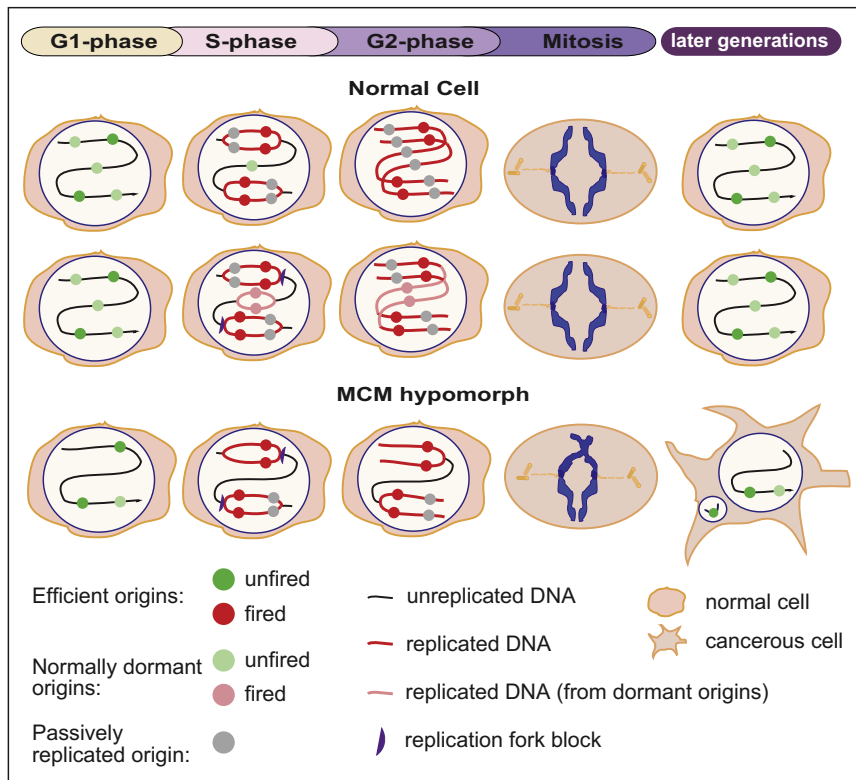


Figure 1. A Role for Dormant Origins in Maintaining Genomic Stability

A small segment of the genome is shown during the cell cycle of normal (top) and MCM hypomorph (bottom) cells. In G1, efficient and normally dormant (inefficient) origins are licensed by loading MCM2-7 onto chromatin (green and light green dots). In S phase, some origins fire (red dots) and replication forks move bidirectionally away from them, passively replicating other origins (gray dots) until forks stall or terminate. Fork stalling triggers the firing of some origins that usually remain dormant (light red dots) to ensure complete genome duplication. Lack of dormant origins can result in progression of cells into mitosis with unreplicated segments of the genome, leading to chromosome breakage and formation of micronuclei.

et al., 2007), while the original MCM2 mutant mice suffered mainly thymomas (Pruitt et al., 2007). Rather than this reflecting a relationship between MCM mutation and tumor type, it is now clear that the genetic background of the mutant mice has a strong influence on the type of cancer arising. When the MCM mutants were crossed into different genetic backgrounds, a range of different tumors was seen, including liver and lung tumors

for the MCM2 mutant (Kunnev et al., 2010) and histiocytic carcinomas and lymphomas for the *MCM4^{Chaos3/Chaos3}* mice (Kawabata et al., 2011). This suggests that the genetic instability of MCM mutants promotes oncogenesis in a wide variety of different tissues, with the predominant tumor types depending on other factors.

The current paper by Kawabata et al., along with other recent papers (Ge et al.,

2007; Ibarra et al., 2008; Blow and Ge, 2009; Kunnev et al., 2010), shows the importance of dormant origins for maintaining genetic stability and preventing tumorigenesis. Misregulation of the licensing system appears to be a very early event in tumorigenesis, and Mcm2-7 proteins show promise as diagnostic markers for early-stage cancer (Blow and Gillespie, 2008). This typically determines whether cells have properly exited from the cell cycle and lost Mcm2-7 expression. The current work suggests that important information about tumor behavior may also be gained by determining the quantity of Mcm2-7 loaded onto DNA in proliferating cells as a possible predictor of genetic instability or sensitivity to chemotherapy (Blow and Gillespie, 2008).

REFERENCES

- Blow, J.J., and Ge, X.Q. (2009). *EMBO Rep.* 10, 406–412.
- Blow, J.J., and Gillespie, P.J. (2008). *Nat. Rev. Cancer* 8, 799–806.
- Ge, X.Q., Jackson, D.A., and Blow, J.J. (2007). *Genes Dev.* 21, 3331–3341.
- Gilbert, D.M. (2007). *Chromosoma* 116, 341–347.
- Ibarra, A., Schwob, E., and Méndez, J. (2008). *Proc. Natl. Acad. Sci. USA* 105, 8956–8961.
- Kawabata, T., Luebben, S., Yamaguchi, S., Ilves, I., Matisse, I., Buske, T., Botchan, M., and Shima, N. (2011). *Mol. Cell* 41, this issue, 543–553.
- Kunnev, D., Rusiniak, M.E., Kudla, A., Freeland, A., Cady, G.K., and Pruitt, S.C. (2010). *Oncogene* 29, 3630–3638.
- Pruitt, S.C., Bailey, K.J., and Freeland, A. (2007). *Stem Cells* 25, 3121–3132.
- Shima, N., Alcaraz, A., Liachko, I., Buske, T.R., Andrews, C.A., Munroe, R.J., Hartford, S.A., Tye, B.K., and Schimenti, J.C. (2007). *Nat. Genet.* 39, 93–98.
- Woodward, A.M., Göhler, T., Luciani, M.G., Oehlmann, M., Ge, X., Gartner, A., Jackson, D.A., and Blow, J.J. (2006). *J. Cell Biol.* 173, 673–683.