Dicer in action at replication-transcription collisions

Jie Ren¹, Stephane E Castel¹,², and Robert A Martienssen¹,*

¹Howard Hughes Medical Institute-Gordon and Betty Moore Foundation; Watson School of Biological Sciences; Cold Spring Harbor Laboratory; Cold Spring Harbor, NY USA;
²Present Address: New York Genome Center; New York, NY USA

Keywords: Dicer, genome stability, replication stress, transcription termination, tumor suppressor

Abbreviations: CFS, common fragile sites; DSB, double strand break; γ-H2A.X, phosphorylation of the histone variant H2A.X; HR, homologous recombination; Hs, Homo sapiens; Pol II, RNA polymerase II; RNAi, RNA interference; Sp, Schizosaccharomyces pombe; sRNA, small RNA.

Maintaining genome stability at sites of transcription and replication collision is a major challenge to cells. Recently, we have shown that in Schizosaccharomyces pombe Dicer promotes transcription termination at these sites, facilitating DNA replication and preventing replication fork restart that would otherwise occur via homologous recombination at the expense of genome stability. This novel role of Dicer could further explain its previously described role as a tumor suppressor.

Transcription-replication collisions have been studied in a broad spectrum of organisms, from bacteria to human. These studies deemed the transcription complex “as a natural impediment of replication” and revealed that forks stalled by collisions must be protected from collapse before restart. ¹ On the opposing side, the trapped RNA polymerase may not be released through canonical termination pathways. Thus, unresolved conflicts will eventually lead to fork collapse, DNA damage, consequently genome instability as precancerous conditions. Such conflict seems inevitable at highly transcribed genes and very long transcripts because of the temporal and spatial overlap, which represent natural “hard-to-replicate sites” and common fragile sites (CFS). ¹ In tumor cells, proliferation driven by oncogene activation leads to a hyper-replicative state. ¹ DNA double strand breaks (DSB), as marked by the phosphorylation of the histone variant H2A.X (γ-H2A.X), are highly elevated in dividing cancer cells, and are especially enriched at sites of transcription-replication collision, presumably as a result of fork collapse. ² Such genome instability further drives metastasis. ³ Notably, many components of pathways that prevent and resolve transcription-replication conflicts are tumor suppressors, e.g. the RAD52 epistasis group (for homologous recombination (HR) and DSB repair), tumor protein p53-binding protein 1 (TP53BP1), and PIF1 (an essential DNA helicase for replication fork progression).

Recently, we proposed a novel role for Dcr1 (Hs DICER1 homolog) in resolving transcription-replication conflicts in Schizosaccharomyces pombe,⁴ an excellent model organism to study such conflicts because its genome organization is very similar to that of higher eukaryotes.⁵ Dicer is an RNase III family nuclease, which cleaves double stranded RNA substrate into small RNA (sRNA) to load into the RNA interference (RNAi) pathway to mediate silencing at both the post-transcriptional and transcriptional levels.⁶ Previously we showed that S. pombe pericentromeric heterochromatin has an alternating arrangement of replication origins and transcription units, which are transcribed at S phase when DNA is replicating. Such competition between transcription and replication requires RNAi machinery to release stalled RNA polymerase II (Pol II), allowing the completion of replication. Without RNAi, Pol II fails to release and stalled replication forks need to be repaired by HR at the expense of genome integrity and heterochromatin inheritance.⁷

Now using Pol II accumulation as a hallmark for polymerase collision, we identified a genome-wide role for Dcr1 in promoting transcription termination and maintaining genome stability (Fig. 1). Outside the pericentromeric regions, Dcr1 releases Pol II from the 3’ end of highly transcribed protein coding genes, and surprisingly from antisense transcription of tDNA and rDNA, which are normally transcribed by Pol III and Pol I. Dicer-dependent sRNA were detected at these Dicer-regulated genes, providing evidence for direct activity. Unlike at the pericentromeric regions, this novel function of Dcr1 does not rely on the other

(C) Jie Ren, Stephane E Castel, and Robert A Martienssen
*Correspondence to: Robert A Martienssen; Email: martiens@cshl.edu
Submitted: 11/14/2014; Submitted: 11/19/2014; Accepted: 11/20/2014
http://dx.doi.org/10.4161/23723556.2014.991224
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.
RNAi pathway components. Particularly, these novel Dcr1-regulated genes are strongly correlated with sites of replication stress and DNA damage, indicated by their co-localization with Rad52 and Ctb2 (Hs 53BP1 homolog), which denote transcription-replication conflicts. We also found that loss of Dcr1 results in the reduction of repetitive rDNA copy number, likely as a result of increased recombination at collision sites within the repeats. Remarkably, rDNA restructuring is one of the most common chromosomal alterations in adult tumors. Finally, we tested our model by increasing replication stress via repression of Pfh1 (Hs PIF1 homolog). Similar to cancer cells, the consequent replication stress results in DNA damage as marked by γ-H2A. Supporting our model, we found evidence for both increased Dcr1 activity (in the form of sRNA), and an enhancement of copy number loss in the absence of Dcr1 when replication stress was increased. Dicer has been identified as a hallmark of tumor suppressor gene, and mutations in the Dicer gene have been found in cancer cells from diverse tissues. Repression of Dicer often associates with poor patient outcome and can even stimulate metastasis in many mouse tumor models. However, homozygous deletion of Dicer has not been reported in cancer. This unique dosage effect is in line with Dicer’s role in suppressing genome instability that triggers tumorigenesis, and its potential indispensability during elevated replication stress in cancer cells. Thus it might act as a tumor suppressor in healthy cells, and an oncogene in cancerous cells. Yet most of the studies to-date focus on Dicer’s role in gene silencing, specifically the deregulation of miRNA biogenesis, to explain tumorigenesis associated with DICER1 mutations. We hope that this novel role of Dicer in replication stress can provide an additional angle for further study.

A more comprehensive understanding of Dicer’s function in the face of replication stress may inform cancer treatment, since it suppresses genome instability, the driving force behind tumorigenesis and metastasis. DICER1 levels are regulated by replication stress both directly and indirectly, for example, up-regulation by transcription factors MITF and tumor protein p63 (summarized in ref. 9), both of which respond to replication stress; and down-regulation by hypoxia, a common feature of tumor and induces replication stress, which confers accumulation of the repressive H3K27me3 mark over the DICER1 promoter via inhibition of its oxygen-dependent demethylases (erasers). Therefore, modulating DICER1 levels may provide a valuable, and as of yet unexplored treatment option for some tumors. Along these lines, it has been shown that metformin, which inhibits oxygen consumption by intoxication of mitochondrial respiratory chain, elicits anti-cancer effect through upregulation of DICER1.10

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed

Funding
This work was supported by grants from the National Institutes of Health (GM076396), the Howard Hughes Medical Institute-Gordon and Betty Moore Foundation (GBMF3033).

References
1. Magdalou I, Lopez BS, Pasero P, Lambert SAE. The causes of replication stress and their consequences on genome stability and cell fate. Semin Cell Dev Biol 2014; 30:154-64; PMID:24818779; http://dx.doi.org/10.1016/j.semcdb.2014.04.035
2. Seo J, Kim SC, Lee HS, Kim JK, Shon HJ, Salleh NML, Desai KV, Lee JH, Kang ES, Kim JS, et al. Genome-wide profiles of H2AX and γ-H2AX differentiate endogenous and exogenous DNA damage hotspots in human cells. Nucleic Acids Res 2012; 40:5965-74; PMID:22467212; http://dx.doi.org/10.1093/nar/gks287
3. Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, Stebbings LA, Monks BA, Martin C, McLaren S, Lin M-L, et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature 2010; 467:1109-13; PMID:20981101; http://dx.doi.org/10.1038/nature09460
4. Castel SE, Ren J, Bhattacharjee S, Chang A-Y, Sanchez M, Valbuena A, Antequera F, Martienssen RA. Dicer promotes transcription termination at sites of replication stress to maintain genome stability. Cell 2014; 159:572-83; PMID:25417108; http://dx.doi.org/10.1016/j.cell.2014.09.031
5. Sabouri N, McDonald KR, Webb CJ, Cristea IM, Zaksian VA. DNA replication through hard-to-replicate sites, including both highly transcribed RNA Pol II and Pol III genes, requires the S. pombe Pfh1 helicase. Gen Dev 2012; 26:581-93; PMID:22426534; http://dx.doi.org/10.1101/gad.184097.111
6. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 2001; 409:363-6; PMID:11201747; http://dx.doi.org/10.1038/35053110
7. Zarategui M, Castel SE, Irvine DV, Kloe A, Ren J, Li F, de Castro E, Marin L, Chang A-Y, Goto D, et al. RNAi promotes heterochromatin silencing through transcription-coupled release of RNA Pol II. Nature 2012; 479:135-8; http://dx.doi.org/10.1038/nature10501

Figure 1. Dicer termination of Pol II transcription at stalled replication forks maintains genome stability. Transcription by RNA polymerase II (Pol II) (blue) and replication by replisome (green) collide, stalling fork progression and holding Pol II to the template. Dicer (orange) releases Pol II at sites of collision, allowing the completion of replication, leaving small RNA (sRNA) as by-product (yellow). Without Dicer, the collision needs to be resolved by homologous recombination and results in genomic instability and copy number change, which contribute to tumorigenesis.
8. Stults DM, Killen MW, Williamson EP, Hourigan JS, Vargas HD, Arnold SM, Moscow JA, Pierce AJ. Human rRNA Gene Clusters Are Recombinational Hotspots in Cancer. Cancer Res 2009; 69:9096-104; PMID:19920195;  http://dx.doi.org/10.1158/0008-5472.CAN-09-2680

9. van den Beucken T, Koch E, Chu K, Rupaimoole R, Prickaerts P, Adriaens M, Voncken JW, Harris AL, Buifa FM, Haider S, et al. Hypoxia promotes stem cell phenotypes and poor prognosis through epigenetic regulation of DICER. Nat Commun 2014; 5:1-13; PMID:25351418

10. Blandino G, Valerio M, Cioce M, Mori F, Casadei L, Pulito C, Sacconi A, Biagioni F, Cortese G, Galanti S, et al. Metformin elicits anticancer effects through sequential modulation of DICER and c-myc. Nat Commun 2012; 3:865-11; PMID:22643892;  http://dx.doi.org/10.1038/ncomms1859