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Dicer in action at replication-transcription collisions

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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

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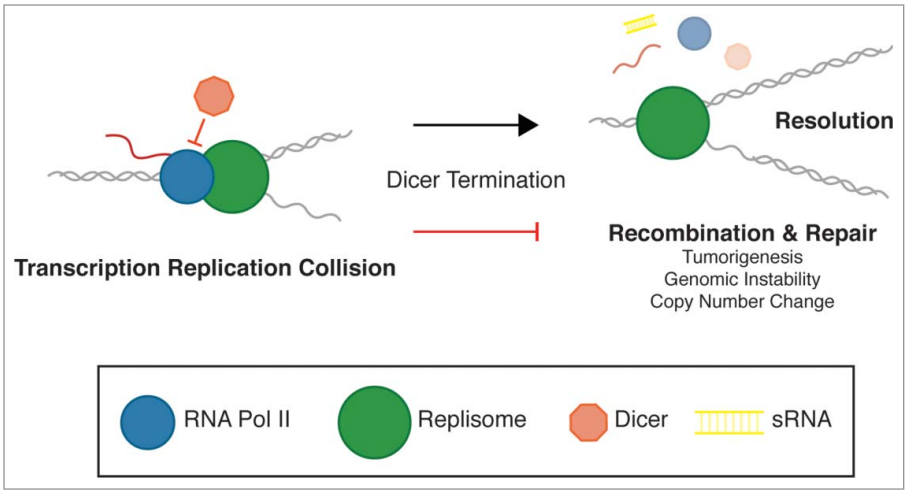

Figure 1. Dicer termination of Pol II transcription at stalled replication forks maintains genome stability. Transcription by RNA polymerase II (Pol II) (blue) and replication by replisome (green) collide, stalling fork progression and holding Pol II to the template. Dicer (orange) releases Pol II at sites of conflict, 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.

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 Crb2 (*Hs* 53BP1 homolog), which denote transcription-replication conflicts.[5] 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 alteration in adult tumors.[8] 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.[5] 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.

DICER1 has been identified as a haploinsufficient tumor suppressor gene, and mutations in the *DICER1* gene have been found in cancer cells from diverse tissues.[9] Repression of Dicer often associates with poor patient outcome and can even stimulate metastasis in many mouse tumor models.[9] However, homozygous deletion of *DICER1* 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.[3] 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).[9] 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

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