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Editorial

Special section: Replication stress, a threat to the nuclear and mitochondrial genome

Dysfunction of chromosomal DNA repair was recognized as disease mechanism in the sixties, when nucleotide excision repair defects were identified as the cause of *Xeroderma pigmentosum* [1]. In the seventies the key mechanisms to ensure discontinued DNA replication past a lesion were discovered: (i) switching between the parental strands involving the Bloom syndrome and Fanconi anemia (Fanc) complementation group proteins to bypass an interstrand DNA crosslink [2] and (ii) direct DNA synthesis across DNA lesions via specialized translesion synthesis (TLS) polymerases. In the nineties polymerase η defects were connected with *Xeroderma pigmentosum variant* [3]. Since then the list of Fanc proteins, TLS polymerases, RecQ helicases and other disease-associated factors involved in replication fork recovery has been growing. Even for breast cancer susceptibility genes like *BRCA2* pathogenic mutations causing replication fork protection without affecting classical disease-related functions in homologous recombination were described [4].

As outlined in this Special Section by Jean-Sebastien Hoffmanńs team, replication stress can be dissected into distinct stages starting with slowing, stalling all the way to collapsing of replication forks [5]. It arises as a consequence of either exogenous or endogenous challenges ultimately creating an obstacle for replication progression or compromising the replicative machinery by an imbalance of the enzymes or nucleotide pools. Each stage of replication stress can introduce genomic alterations, ranging from point mutations generated by TLS, to sister chromatid exchanges at stalled forks and non-allelic homologous recombination between repeats upon fork collapse. For many years the impact of replication-associated DNA damage signaling on the development of diseases was underestimated, because mechanistic knowledge was lagging behind. As an example, chromosome aberrations involving short homologous sequences at break junctions were exclusively attributed to DNA double-strand break (DSB) repair by non-homologous end joining before break-induced replication (MMBIR) emerged as a pathway reactivating collapsed forks via template switching [6]. The most severe outcome of replication stress is catastrophic chromosome shattering, so-called chromothripsis, which results in highly complex genome rearrangements followed by reiterative fork stalling and template switching (FoSTeS) between non-allelic sequences.

Replication-based genomic changes have been associated with human diseases, neurological disorders, immunodeficiencies and cancer. In fact, replication stress triggers genomic instability in the very early stages during tumorigenesis [7]. In this Special Section Sinai and Kerem [8] focus on the most vulnerable loci in the human genome, the so-called Common Fragile Sites (CFSs). These elements can microscopically be detected as gaps and breaks in metaphase chromosomes in response to even mild replication stress. They combine several destabilizing features: AT-richness with proneness to secondary structure formation; delayed origin firing with replication into G2/M-phase; large genes, so that transcription cannot be finished before S-phase, which inevitably causes collisions between transcription and replication machineries. R-loops, i.e. the RNA-DNA hybrid transcription intermediates which impede replication, were only recently understood as a major endogenous source of replication stress in cancer [9]. CFSs are themselves sites of carcinogenic chromosome rearrangements in early preneoplastic lesions; the mutated gene products exacerbate replication stress and thus drive the neoplastic transformation process in a vicious cycle.

The cellular toolbox to restart replication at an obstacle contains dormant origin activation, downstream repriming and several bypass mechanisms orchestrated by PCNA, namely homologous recombination, template switch and TLS. Bournique and colleagues [5] provide a detailed overview of TLS, an emergency program to prevent transmission of replication damage to daughter cells. After slow-down of replicative polymerases, TLS polymerases synthesize across non B-DNA structures such as formed by DNA damage or by natural impediments in genomic regions like repetitive sequences, telomeres or CFSs. However, TLS polymerases can perform only short-range DNA synthesis with low fidelity. Consequently, TLS must be under tight control. Specific ubiquitin and SUMO modifications of PCNA creating the so-called PCNA switchboard weigh fast, low-fidelity TLS against slow, high-fidelity template switch. The different TLS polymerases show specialization on particular structural challenges during DNA synthesis. More recent works revealed additional links with other DNA damage response pathways. Thus, TLS polymerase η also mediates DNA synthesis at strand invasion intermediates during homologous recombination, TLS polymerase ι template switch by fork regression and polymerase θ functions in replication timing and alternative end-joining at collapsed forks for replication restart.

Flach and Milyavsky [10] comprehensively overview the existing knowledge of replication stress in hematopoietic stem cells (HSCs) and progenitors and its consequences. HSCs are normally in a dormant state, which minimizes DNA replication and metabolic activity associated with reactive oxygen species (ROS) production. Quiescence prevents formation of fork stalling lesions and ensures an intact genome. Enforced cell cycle entry by inflammatory stimuli, the need for excessive blood cell renewal or transplantation provokes replication stress. Repeated or chronic exposure causes premature exhaustion of HSCs via senescence or apoptosis driving the aging process. Manifestations of aging are reduced bone marrow reconstitution capacity and skewing towards the myeloid lineage. Consequences are a declining competence of the innate and adaptive immune

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system and increased incidence of myeloproliferative diseases including hematopoietic malignancies. Direct evidence of a causal relationship between replication stress and premature aging in HSCs was recently provided by a mouse model inactivating the PCNA switchboard [11]. However, even though extremely valuable, simple extrapolation of mouse data to human systems is dangerous due to the significant differences that have been observed in HSC biology and DNA damage responses between the two species. While data sets on natural aging in human individuals are still rare, valuable information was retrieved from patients with premature aging syndromes, i.e. progerias.

Several progerias are caused by hereditary mutations in genes with functions in DNA replication fork protection such as Bloom syndrome, Rothmund-Thompson syndrome, Werner syndrome and Fanconi anemia [12]. All of them affect stem cell compartments. Fanconi anemia patients show features of the normal aging process of HSCs with bone marrow failure, genomic instability and leukemia proneness. Failure to resolve replication damage has been exploited for diagnostic purposes via *ex vivo* exposure of patient cells to interstrand DNA crosslinking agents like Mitomycin C. These treatments mimic endogenous crosslinking processes e.g. at abasic sites generated by spontaneous hydrolysis or the action of a DNA glycosylase [13]. Frederico et al. [14] provide insight into the activities of FancD2 in replication fork protection and replication-coupled DNA repair activities. FancD2 is particular in that it represents a central node of the Fanconi anemia repair pathway and exerts critical functions independently of the core Fanc protein complex. In the early phase of the replication damage response FancD2 coordinates engagement of TLS and homologous recombination in concert with PCNA. At later stages it controls DSB repair pathway usage at collapsed forks ensuring high fidelity repair by homologous recombination rather than non-homologous end joining. Together with the Bloom syndrome product BLM, FancD2 protects hard-to-replicate regions like CFSs from persistent replication stress. At these sites it promotes not only DNA replication progression but also termination. Failure leads to the formation of ultrafine chromosome bridges labeling unresolved connections between chromatids in anaphase. FancD2 therefore also ensures proper chromosome segregation and prevents transmission of replication damage to daughter cells.

Mitochondria make use of nuclear-encoded DNA replication proteins, and even FancD2 was found to play a role in mitochondria, namely in ATP production [15]. Notably, perturbations of DNA replication both in the nucleus and in mitochondria have been associated with organismal aging [16]. However, many details of DNA replication in mitochondria have remained enigmatic. In this Special Section Ricchetti [17] summarizes our current knowledge of replication stress in mitochondria. Despite the shared use of enzymes and links with aging and diseases, there are significant differences between DNA replication in mitochondria as compared with the nucleus, due to an orders of magnitude (i) smaller size and (ii) higher copy number of the genome, (iii) its circular organization and dense packing with coding sequences, (iv) a distinct mechanism with asymmetry between leading and lagging strand synthesis generating intermediates with a long stretch of single-stranded DNA. Despite all these differences replication stress arises in both organelles after perturbation of the nucleotide pool, the replication machinery or the DNA template such as by oxidative DNA damage. In recent years powerful methods to study mitochondrial DNA replication have been developed such as mTRIP, which relies on fluorescence in situ hybridization of the active replication origin [18] and single molecule fiber analysis detecting nascent mitochondrial DNA synthesis [19]. Mitochondrial replication stalling may cause slippage between direct repeats and result in a DNA deletion. Mutations causing DNA replication failure such as in the nuclear gene encoding polymerase γ are associated with diseases characterized by premature aging or neurological decline and promote aerobic glycolysis in cancer cells [20]. Much remains to be learnt about the coordination of mitochondrial and nuclear replication processes and signaling in response to replication stress. Another challenge will be to understand the communication between heterogeneous mitochondria within the same cell, which can neutralize detrimental effects of mutations by sharing and exchanging genetic material by fusion and fission processes or mitophagy.

With the availability of new and powerful methods we have begun to grasp the critical role of nuclear and mitochondrial DNA replication stress in natural aging and disease mechanisms. Stunningly, after decades of effective use of replication stress-inducing cytostatics, only now we realize the great potential of drugs manipulating these processes.

References

[1] J.E. Cleaver, Defective repair replication of DNA in xeroderma pigmentosum, Nature 218 (1968) 652–656.
[2] D.A. Shafer, Replication bypass model of sister chromatid exchanges and implications for Bloom's syndrome and Fanconi's anemia, Hum. Genet. 39 (1977) 177–190.
[3] C. Masutani, R. Kusumoto, A. Yamada, N. Dohmae, M. Yokoi, M. Yuasa, M. Araki, S. Iwai, K. Takio, F. Hanaka, The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta, Nature 399 (1999) 700–704.
[4] K. Schlacher, H. Wu, M. Jasin, A distinct replication fork protection pathway connects fanconi anemia tumor suppressors to RAD51-BRCA1/2, Cancer Cell 22 (2012) 106–116.
[5] E. Bournique, M. DalÍOsto, J.-S. Hoffmann, V. Bergoglio, Role of specialized DNA polymerases in the limitation of replicative stress and DNA damage transmission, Mutat. Res. pii: S0027-5107 (August (17)) (2017) 2–30098, http://dx.doi.org/10.1016/j.mrfmmm.2017.08.002.
[6] M. Deniz, K. Holzmann, L. Wiesmüller, Functional analysis-make or break for cancer predictability, Mutat. Res. 743–744 (2013) 132–141.
[7] T.D. Halazonetis, V.G. Gorgolis, J. Bartek, An oncogene-induced DNA damage model for cancer development, Science 319 (2008) 1352–1355.
[8] M. Irony-Tur Sinai, B. Kerem, DNA replication stress drives fragile site instability, Mutat. Res. pii: S0027-5107 (october (17)) (2017), http://dx.doi.org/10.1016/j.mrfmmm.2017.10.002 (30110-0).
[9] P. Kotsantis, L.M. Silva, S. Irmscher, R.M. Jones, L. Folkes, N. Gromak, E. Petermann, Increased global transcription activity as a mechanism of replication stress in cancer, Nat. Commun. 7 (2016) 13087.
[10] J. Flach, M. Milyavsky, Replication stress in hematopoietic stem cells in mouse and man, Mutat. Res. pii: S0027-5107 (october (17)) (2017) 30033–30037, http://dx.doi.org/10.1016/j.mrfmmm.2017.10.001.
[11] B. Pilzecker, O.A. Buoninfante, P. van den Berk, C. Lancini, J.-Y. Somg, E. Citterio, H. Jacobs, DNA damage tolerance in hematopoietic stem and progenitor cells in mice, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E6875–E6883.
[12] A.A. Freitas, J.P. de Magalhaes, A review and appraisal of the DNA damage theory of ageing, Mutat. Res. 728 (2011) 12–22.
[13] D.R. Semlow, J. Zhang, M. Budzowska, A.C. Drohat, J.C. Walter, Replication-dependent unhooking of DNA interstrand cross-links by the NEIL3 glycosylase, Cell 167 (2016) 498–511.
[14] M.B. Federico, P. Campodonico, N.S. Paviolo, V. Gottifredi, Beyond interstrand crosslinks repair: contribution of FANCD2 and other Fanconi Anemia proteins to the replication of DNA, Mutat. Res. pii: S0027-510 (September (14)) (2017) 30114–30118, http://dx.doi.org/10.1016/j.mrfmmm.2017.09.004.

[15] P. Jayabal, C. Ma, M. Nepal, Y. Shen, R. Che, J. Turkson, P. Fei, Involvement of FANCD2 in energy metabolism via ATP5α, Sci. Rep. 7 (2017) 4921.
[16] I.N. Shokolenko, G.L. Wilson, M.F. Alexeyev, Aging: a mitochondrial DNA perspective: critical analysis and an update, World J. Exp. Med 4 (2014) 46–57.
[17] M. Ricchetti, Replication stress in mitochondria, Mutat. Res. (2018) in press.
[18] L. Chartre, M. Ricchetti, Prevalent coordination of mitochondrial DNA transcription and initiation of replication with the cell cycle, Nucleic Acids Res. 41 (2013) 3068–3078.
[19] A.F. Phillipps, A.R. Millet, M. Tigano, S.M. Dubois, H. Crimmins, L. Babin, M. Charpentier, M. Piganeau, E. Brunet, A. Sfeir, Single-molecule analysis of mtDNA replication uncovers the basis of the common deletion, Mol. Cell 65 (2017) 527–538.
[20] M. Scheibye-Knudsen, E.F. Fang, D.L. Croteau, D.M.3rd Wilson, V.A. Bohr, Protecting the mitochondrial powerhouse, Trends Cell. Biol. 25 (2015) 158–170.

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