Altogether, the results of Xia et al. offer new perspectives on MVA-inhibitor mechanisms of action and potential therapeutic uses. Optimizing immune adjuvant by acting on dendritic cells' endosomal maturation kinetics is indeed a highly innovative concept. Therapeutic use of MVA-pathway inhibitors for anti-infectious vaccines or in the aim of boosting antitumor responses in combination with anti-PD1 or PDL-1 antibodies is an attractive objective.

Although neither statins nor bisphosphonates were initially supposed to have any effect on tumor biology, several epidemiological studies suggest that these two drug families might have an antitumor effect (Altwairgi, 2015; Gnant and Clézardin, 2012; Iannelli et al., 2018). Some hypotheses supported by preclinical studies put forward a direct antitumor effect of the MVA pathway blockade via its interaction with essential oncogenic actors or pathways such as p53, MYC, PI3-kinase, and MAP-kinase pathways (Iannelli et al., 2018). The present work, demonstrating that these drugs might also act via an indirect immunotherapeutic effect, will probably result in a strong resurgence of interest in exploring the antitumor effect of MVA-pathway inhibitors in cancer therapy.

However, the dosing, as well as the mode of administration of such inhibitors, will probably constitute critical issues. As suggested by the authors, oral administration of statins might not be the optimized way to induce a strong adjuvant effect as compared with intradermal, subcutaneous, or intratumor approaches. Importantly, immunosuppressive effects have been observed with higher doses of MVA inhibitor (Ghittoni et al., 2006). One can suppose that at high concentrations or in different environmental contexts, a strong endosomal maturation slowdown could result in a totally arrested maturation that could lead to a subsequent decrease of antigen presentation.

Finally, this work constitutes a solid preclinical basis to raise hopes toward new and exciting avenues that might be capable of enhancing both our anti-infection and antitumor immune defenses with a potential huge impact on the collectivity.

REFERENCES

Altwairgi, A.K. (2015). Statins are potential anticancerous agents (review). Oncol. Rep. *33*, 1019–1039.

Drenth, J.P., Waterham, H.R., Kuis, W., Houten, S.M., Frenkel, J., Wanders, R.J., Poll-The, B.T., and van der Meer, J.W. (2000). [Identification of the gene for hyper-IgD syndrome: a model of modern genetics]. Ned. Tijdschr. Geneeskd. *144*, 782–785.

Ghittoni, R., Napolitani, G., Benati, D., Ulivieri, C., Patrussi, L., Laghi Pasini, F., Lanzavecchia, A., and Baldari, C.T. (2006). Simvastatin inhibits the MHC class II pathway of antigen presentation by impairing Ras superfamily GTPases. Eur. J. Immunol. *36*, 2885–2893.

Gnant, M., and Clézardin, P. (2012). Direct and indirect anticancer activity of bisphosphonates: a brief review of published literature. Cancer Treat. Rev. *38*, 407–415.

Goldstein, J.L., and Brown, M.S. (1990). Regulation of the mevalonate pathway. Nature *343*, 425–430.

Iannelli, F., Lombardi, R., Milone, M.R., Pucci, B., De Rienzo, S., Budillon, A., and Bruzzese, F. (2018). Targeting Mevalonate Pathway in Cancer Treatment: Repurposing of Statins. Recent Patents Anticancer. Drug Discov. *13*, 184–200.

Machesky, L.M., and Hall, A. (1997). Role of actin polymerization and adhesion to extracellular matrix in Rac- and Rho-induced cytoskeletal reorganization. J. Cell Biol. *138*, 913–926.

Manthiram, K., Zhou, Q., Aksentijevich, I., and Kastner, D.L. (2017). The monogenic autoinflammatory diseases define new pathways in human innate immunity and inflammation. Nat. Immunol. *18*, 832–842.

Wang, M., and Casey, P.J. (2016). Protein prenylation: unique fats make their mark on biology. Nat. Rev. Mol. Cell Biol. *17*, 110–122.

Xia, Y., Xie, Y., Yu, Z., Xiao, H., Jiang, G., Zhou, X., Yang, Y., Li, X., Zhao, M., Li, L., et al. (2018). The Mevalonate Pathway Is a Druggable Target for Vaccine Adjuvant Discovery. Cell *175*, this issue, 1059–1073.

The Helix Twist: Damage and Repair Follows the DNA Minor Groove

Eran Kotler[1,2,*] and Eran Segal[1,2,*]

[1]Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot 7610001, Israel
[2]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 7610001, Israel
*Correspondence: eran.kotler@weizmann.ac.il (E.K.), eran.segal@weizmann.ac.il (E.S.)

https://doi.org/10.1016/j.cell.2018.10.034

Mutation frequencies vary along the genome, but the factors determining this variability are only partially understood. Pich et al. unravel a ~10 bp periodicity in mutation rates at nucleosome-proximal regions that follows minor groove orientation. Opposing differential DNA damage and repair processes could shape genetic divergence irrespective of selection.

Eukaryotic DNA is tightly packed into chromatin by wrapping around nucleosomes consisting of histone octamers. To facilitate this compaction, the DNA double helix sharply bends every helical repeat in a ~10 bp periodicity, when the DNA major groove faces toward the nucleosome core (Richmond and Davey, 2003). Consistently, A/T di-nucleotides (WW) are favored

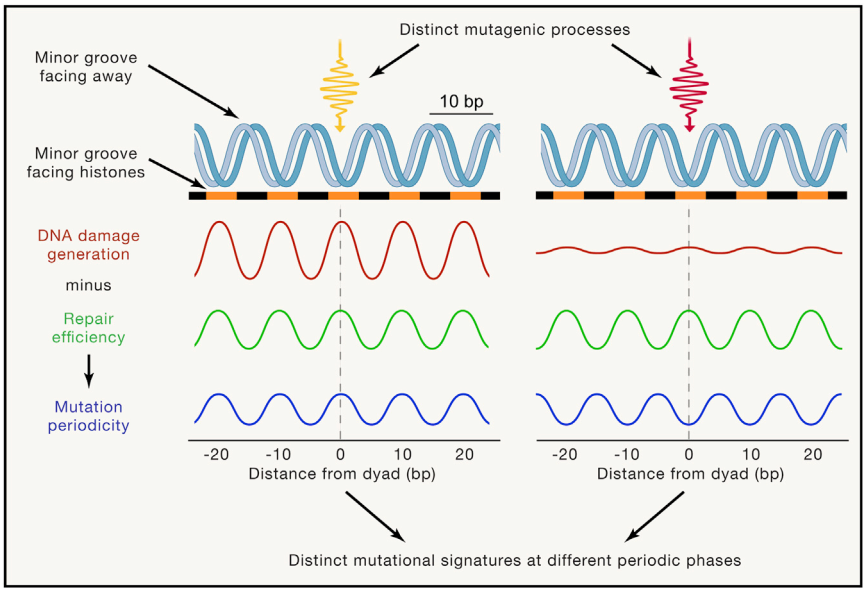

Figure 1. A Combination of DNA Damage and Repair Processes Determines Periodic Mutation Signatures

Distinct mutational processes differentially affect the generation of DNA damage in stretches of DNA in which the minor groove faces toward or away from histones (or irrespective of nucleosome rotational positioning). Similarly, DNA repair efficiency varies with respect to minor groove orientation in nucleosome-proximal regions. These opposing forces combinatorically shape the mutational landscape, forming a ~10 bp periodicity in mutation rates in which distinct mutational signatures exhibit different periodic phases.

at sequence positions in which the DNA minor groove faces the histones, forming a similar ~10 bp periodicity thought to play a role in rotational positioning of DNA relative to nucleosomes (Segal et al., 2006). In addition to rotational positioning, the translational positioning of nucleosomes along the DNA sequence is affected by sequence determinants (Struhl and Segal, 2013) and plays a role in fundamental biological processes such as transcriptional regulation and replication timing (Azmi et al., 2017; Choi and Kim, 2009). Separately, it has long been appreciated that mutations are non-randomly distributed along the genome (Wolfe et al., 1989). In this issue of *Cell*, Pich et al. examine the relationship between nucleosome positions and mutations by computationally interrogating the effects of nucleosomal positioning on mutation rate variability prior to the action of evolutionary selection forces (Pich et al., 2018).

Hypothesizing that nucleosome translational positioning could affect mutation rates, Pich et al. examine the frequency distributions of somatic mutations across ~3,500 tumors of different types. This analysis reveals a periodic mutation enrichment signal at ~191 bp intervals, corresponding to the approximate length of nucleosome plus linker DNA stretches. Intriguingly, the phase of this periodic signal differs between tumor cohorts. Thus, in most tumor types, high mutation rates are periodically found within nucleosomes, whereas in other types of cancer, mutations are enriched in linker regions (e.g., in lung adenocarcinomas and squamous-cell carcinomas) or do not exhibit a clear periodic mutation pattern (e.g., in ovarian cancer).

To examine the effects of structural differences in DNA on mutation rates, the authors perform a similar analysis with higher resolution, focusing on DNA stretches adjacent to nucleosome cores. Remarkably, this exposes a strong ~10 bp periodicity in mutation rates that follows the orientation of the DNA minor groove at distinct phases (i.e., facing toward or away from histones) depending on tumor type. The authors further hypothesize that differences in mutational processes playing a dominant role in different tumor types may lead to this discrepancy in periodic mutation distributions. To this end, they deconstruct the contribution of distinct mutational signatures (as defined by Alexandrov et al. [2013]) to each tumor and find that dominant signatures (associated with defined mutational processes) are major determinants of the observed periodicity phase in nucleosome-covered regions. Pooling together mutations corresponding to each mutation signature reveals a strong correspondence between mutation signatures and the orientation of mutation-rate periodicity. For instance, mutations from signatures 4 and 7 (associated with tobacco carcinogens and with UV light, respectively) are periodically enriched in stretches where the minor groove faces away from histones. In contrast, mutations from signatures 10 and 17 (associated with POLE mutations and with guanine oxidation, respectively) accumulate in DNA regions where the minor groove faces the histones.

The observed periodicities in mutation rates represents an integration over DNA damage-generating processes (forming mutagenic damaged bases) and DNA repair (correcting damaged bases). To decouple these processes, the authors examine the positional distribution of DNA lesions induced by UV irradiation and by the alkylating agent methyl methanesulfonate (MMS) and compare them to the repair-rates of these lesions, which are mediated by the nucleotide-excision repair (NER) and the base-excision repair (BER) pathways, respectively. While both repair mechanisms are more efficient in DNA stretches in which the minor groove faces away from histones (due to increased accessibility), only UV-induced mutations but not MMS-induced mutations exhibit a ~10 bp periodic signal. This analysis demonstrates the interplay between DNA damage occurrence and repair efficiency within nucleosome-proximal DNA regions, explaining the outcome of such processes (Figure 1).

The authors then ask whether a similar periodicity in mutation rates could be observed in germline mutations and in patterns of evolutionary genetic divergence. Consistently, rare variants in both human and *Arabidopsis* as well as the positional distributions of C>T genetic divergence (in close species) exhibit similar periodicity with higher variability in DNA stretches in which the minor groove faces the histones.

These findings suggest that similar principles underlie germline and somatic mutation rate variability.

The pervasive WW periodicity is commonly thought to be related to the structural constraints associated with DNA bending around nucleosomes. However, since this periodicity follows the same minor groove orientation signal that correlates with differential mutation rates, the authors further speculate that it could have resulted from the combined effects of a C>T mutation bias (product of spontaneous methylcytosine deamination) and an increased mutation frequency at DNA stretches where the minor groove faces the histones. Using a computational simulation framework, they demonstrate that in the absence of purifying selection, such differential DNA damage and repair processes that depend on the helical orientation of nucleosome-bound DNA could generate the observed WW periodicity found across eukaryotic genomes. It would be interesting to expand current evolutionary models to account for the effects of nucleosomal positions and minor groove orientation on mutation rates for distinct mutational signatures. Such models may provide insights into mechanisms of neutral divergence along evolution.

Mutation rate variability occurs at different scales and has been proposed to be affected by sequence-context determinants, position within chromosomes, transcriptional activity, and other factors. The work by Pich et al. contributes to our understanding of the forces shaping the mutational landscape and genetic divergence in presumably neutral regions. These findings raise a variety of interesting questions regarding the interplay between nucleosomes, mutational processes, and repair mechanisms. Histones are subjected to a variety of post-translational modifications that modulate their biochemical properties and play a role in fundamental biological processes such as DNA replication, transcriptional regulation, and DNA damage repair. Many of these processes involve chromatin remodeling and nucleosome shifting. Plausibly, histone modifications and changes in chromatin organization could further affect mutation frequencies in nucleosome-proximal regions. Furthermore, distinct patterns of nucleosome shifting could be required to facilitate efficient repair via distinct mechanisms, properly orienting the damaged region with respect to the histones.

This study lays the groundwork for further investigations into the underlying mechanisms of distinct DNA repair pathways. Which pathways require direct interaction with the minor groove? Does nucleosome rotational positioning play a role in the occurrence of double-strand breaks and their repair? While these questions are complex, the analytical tools for addressing them have been portrayed.

ACKNOWLEDGMENTS

E.S. is supported by the Crown Human Genome Center, the Else Kroener Fresenius Foundation, D.L. Schwarz, J.N. Halpern, L. Steinberg, J. Benattar, Aliza Moussaieff, Adelis Foundation, and grants funded by the European Research Council and the Israel Science Foundation.

REFERENCES

Alexandrov, L.B., Nik-Zainal, S., Wedge, D.C., Aparicio, S.A.J.R., Behjati, S., Biankin, A.V., Bignell, G.R., Bolli, N., Borg, A., Børresen-Dale, A.-L., et al.; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MMML-Seq Consortium; ICGC PedBrain (2013). Signatures of mutational processes in human cancer. Nature *500*, 415–421.

Azmi, I.F., Watanabe, S., Maloney, M.F., Kang, S., Belsky, J.A., MacAlpine, D.M., Peterson, C.L., and Bell, S.P. (2017). Nucleosomes influence multiple steps during replication initiation. eLife *6*, e22512.

Choi, J.K., and Kim, Y.-J. (2009). Intrinsic variability of gene expression encoded in nucleosome positioning sequences. Nat. Genet. *41*, 498–503.

Pich, O., Muiños, F., Sabarinathan, R., Reyes-Salazar, I., Gonzalez-Perez, A., and Lopez-Bigas, N. (2018). Somatic and germline mutation periodicity follow the orientation of the DNA minor groove around nucleosomes. Cell *175*, this issue, 1074–1087.

Richmond, T.J., and Davey, C.A. (2003). The structure of DNA in the nucleosome core. Nature *423*, 145–150.

Segal, E., Fondufe-Mittendorf, Y., Chen, L., Thåström, A., Field, Y., Moore, I.K., Wang, J.-P.Z., and Widom, J. (2006). A genomic code for nucleosome positioning. Nature *442*, 772–778.

Struhl, K., and Segal, E. (2013). Determinants of nucleosome positioning. Nat. Struct. Mol. Biol. *20*, 267–273.

Wolfe, K.H., Sharp, P.M., and Li, W.H. (1989). Mutation rates differ among regions of the mammalian genome. Nature *337*, 283–285.