February 28, 2020

Dear Editors:

Please find enclosed our manuscript titled "Transcription-dependent DNA supercoils determine the 3D organization of dinoflagellate genomes", which we would like to submit for publication in *Science*.

The past decade has seen an explosion of interest in understanding the three dimensional organization of genomes and its functional relationship to gene regulation. The vast majority of studies in this area have focused on the genomes of mammalian species as well as, to a lesser extent, those of other classical model organisms such as *Drosophila*, *C. elegans* and yeast. As a result, we have begun to understand some of the topological forces driving 3D folding of metazoan genomes. In mammals, constraints on loop extrusion imposed by the insulator protein CTCF result in the formation of topologically associated domains (TADs), and are the dominant feature of three dimensional genome organization. Loop extrusion TAD domains have so far appeared to be exclusive to metazoans; they are superimposed over so called "compartment" interactions, driven by associations between similar chromatin states. Compartments are generally thought to be the main topological force in organisms without loop extrusion domains.

However, the eukaryotic diversity as a whole has been largely unexplored even though it includes lineages whose chromatin biology is known to represent radical departures from the conventional eukaryotic norms, and which represent natural experiments that can reveal deeper principles of chromatin organization that are largely masked in most other eukaryotes.

Dinoflagellates are the most striking such case, as, even though nucleosomal chromatin is an ancestral and extremely strongly conserved feature of all other eukaryotic lineages, it has been largely dispensed with in dinoflagellates, whose genomes are generally not packaged by histones (other proteins, of viral and bacterial origin, are instead the main packaging components). In addition, dinoflagellates have also largely eliminated gene regulation at the transcriptional level, and their genes exist in long polycistronic arrays, from which mature mRNAs are generated through *trans*-splicing.

Dinoflagellate genomes are also mostly extremely large and repetitive, which has until very recently prevented the generation of chromosome-scale genome assemblies.

In this study, we map the three-dimensional genome organization of the coral symbiont *Breviolum minutum* using deep Hi-C profiling, which we also use to obtain a chromosome-level scaffolding of its genome. We then analyze the Hi-C data with respect to the functional organization of the *B. minutum* genome, and make the remarkable discovery that dinoflagellate chromosomes are organized into very strong topological domains with properties not previously observed in any other organism (which we term "dinoTADs"). DinoTADs are, unlike mammalian TADs, not associated with chromatin loops. They instead coincide with pairs of divergent polycistronic gene arrays with their boundaries corresponding to the points of convergence between gene arrays. This suggested that plectoneme formation due to transcription-induced supercoiling is the main driver behind the formation of dinoTADs, a model that is supported by the observation (made through multiple orthogonal transcription inhibition experiments) that dinoTADs are decompacted in the absence of active transcription. In addition, phylogenomic analysis reveals a massive expansion of topoisomerase genes that is specific to dinoflagellate genomes.

Out results begin to unravel the mystery of dinoflagellate chromatin, which has fascinated researchers since the mid-20th century, while also highlighting previously underappreciated fundamental principles of physical genome organization in all domains of life. As transcription-induced torsional stress is present in all organisms, our observations point to it being a primary topological force in all genomes, one whose effects are obscured by the presence of conventional chromatin and the interactions between nucleosomes in most eukaryotes. They also identify the question of how exactly torsional stress is dealt with in the context of mammalian chromatin as a key one to pursue in the future.

We respectfully request that the manuscript not be sent for review to XXX WHO TO EXCLUDE XXX due to conflicts of interest. We would also like to suggest the following list of potential reviewers for it:

- suggested reviewer 1

- suggested reviewer 2
- suggested reviewer 3

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal.

Sincerely,

Georgi K. Marinov
William J. Greenleaf