

Telomere Master Notes

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1 Introduction

Telomeres consist of DNA tandem repeat sequences, their associated binding proteins, and a non-coding RNA transcript. They are located at the end of chromosomes and address two important problems in eukaryotes: the end-replication problem and the end-protection problem. A nice summary is provided in [1].

1.1 Telomere composition and structure

In humans, the telomere tandem repeat sequence is TTAGGG. A telomere's size lies between 5 and 15 kb in humans. A key feature of the telomere end in all organisms is the 3' single-stranded G-rich overhang. In mammals, these overhangs are 30-500 nucleotides long.

Associated with the telomeric DNA are proteins of the shelterin complex. Shelterin is responsible for solving the end-protection problem, though it acts in a complicated manner.

The six known shelterin proteins are TRF1, TRF2, RAP1, TIN2, TPP1, and POT1. TRF1 and TRF2 bind to the duplex region of the DNA; POT1 coats the overhang with oligonucleotide/oligosaccharide binding folds. TIN2 has a bridging function since it interacts simultaneously with TRF1, TRF2, and TPP1.

In addition to the shelterin proteins, there are telomere-associated proteins that are recruited by shelterin to act as “accessory factors.”

The long non-coding RNA might be important for telomere maintenance and function, though little is known about the underlying molecular mechanisms.

1.2 Telomeres and the end-replication problem

The telomerase enzyme solves the end-replication problem and helps counteract telomere erosion. The mechanisms underlying telomere length regulation in mammalian cells are not fully understood, though some key factors are known.

For example, overexpressing TRF1 in cancer-derived human cells leads to telomere shortening, whereas depleting telomeres of TRF1 results in elongation. Reducing levels of TRF2 and TIN2 leads to telomere extension. There are also repressive histone marks on telomeric chromatin. Deletion of these marks correlates with large elongations of telomeres.

1.3 Mammalian telomeres have nucleosomes

Mammalian telomeres are enriched with repressive histone marks, including H3K9me3 and H4K20me3 and the heterochromatin-specific factors chromobox homolog (CBX) 1, 3, and 5. Depleting these marks correlates with telomere elongation.

2 Shelterin and its role in telomere functioning

- Do the shelterin proteins always come in a complete unit, or do the ratios of the amounts of the various shelterin proteins vary from telomere to telomere?
- Do the increased sizes in TRF1 correspond to telomeres having longer genomic lengths?

2.1 TRF1 controls human telomere lengths

TRF1 was found to control the length of human telomeres [2]. The authors both overexpressed TRF1 and reducing the binding of TRF1 to telomeres and observed a gradual shortening and lengthening of telomeres. The expression of telomerase was not changed, which suggested that TRF1 somehow inhibits the action of telomerase.

2.2 TRF2 is linked to the DNA repair machinery

Deleting TRF2 results in the activation of the kinase ATM and triggers the accumulation of DNA damage factors, including H2AX and 53BP1 [1]. Furthermore, in the absence of TRF2, ligase 4- and Ku-mediated NHEJ repair is activated at telomeres, which results in chromosome end-to-end fusions [1].

3 Polymer simulation and chromatin modeling

Polymer simulations are now becoming useful tools for modeling experimental data on chromatin and predicting other structural features of DNA [3, 4].

Within the context of this telomere project, we wanted to simulate a particular chromatin model, the wormlike chain, and compare it to the measured telomere sizes. This would allow us to estimate what combination of values for the model parameters, the chain’s contour length and its persistence length, was most likely to have produced our data.

3.1 Introduction to the polymer models

Polymers are molecular and macromolecular chains that are found throughout both the natural and the man-made world. Because of their ubiquity, tools for studying their properties find great utility in the physical and life sciences.

The study of polymer physics involves coping with complexity. Many polymers are comprised of a very large number of subunits. For example, DNA is a polymer that consists of a linear chain of tens of millions of nucleic acids. Because of the large number of molecular degrees of freedom, statistical mechanics and computer simulations are the basic tools for understanding polymer structure.

This work focuses on the computer simulation of one important polymer model, the wormlike chain (WLC). It was originally developed by Kratky and Porod to incorporate the flexibility of a polymer into the models of the time [5]. This flexibility emerges from the underlying details of the electronic bonds that hold the atoms and molecules in the chain together. Despite the fact that it is now over 65 years old, the WLC still finds many uses in describing physical phenomena. Furthermore, its details are still being uncovered by scientists.

This work first describes the basic theory. After this, the algorithm used to generate the 3D chains is explained. It is one of the simpler algorithms and produces the essential results of the model. Finally, I describe how the algorithm is implemented on the computer, using Python as a programming language.

3.2 Theory of the wormlike chain

In the simplest WLC model, we treat the polymer as a semiflexible and homogeneous rod with a negligible thickness and a length L_c , known as the contour length. The flexibility of the rod is described by its persistence length ℓ_p . Intuitively, the persistence length is the average length over which the polymer remains approximately straight. Polymers with a longer persistence length will be more rigid than shorter ones.

Mathematically, the persistence length is the characteristic length describing the exponential decay of the tangent-tangent correlation function [6],

$$\langle \mathbf{t}(s) \cdot \mathbf{t}(0) \rangle \sim \exp(s/\ell_p) \quad (1)$$

where $\mathbf{t}(s)$ is the unit vector tangent to the polymer at the one-dimensional coordinate s along the polymer. For distances s much greater than ℓ_p , (1) states that there will be no correlation in the direction that the tangent vectors point.

The subunits that make up most polymers are very small molecules and are thus subject to agitation by the random collisions with other molecules in their environment. This is especially pertinent to polymers in aqueous environments, where collisions with the solvent molecules cause the polymer to change shape and conformation many times a second. According to Boltzmann’s statistics, the probability that a semiflexible polymer in thermodynamic equilibrium will be found in one of any of its possible conformations at an instant in time is proportional to the Boltzmann factor

$$P(U) \sim \exp\left(-\frac{U}{k_B T}\right) \quad (2)$$

where $P(U)$ represents of the probability of observing a polymer conformation with associated internal energy U , k_B is Boltzmann’s constant and T is the temperature of the system. The fact that it takes energy to bend the polymer into a particular conformation reflects the “semiflexible” qualities of the polymer.

The energy U required from the environment to achieve a given conformation can be determined by dividing the polymer into many short sections such that it can be represented as the summation of the energies of many small circular arcs. The energy required to bend a rod with Young’s modulus E , moment of

3.3 Simulation Testing

3.3.1 Accuracy as a function of segment length and number of chains

The purpose of the simulations is to compute the probability distribution function for randomly selecting a telomere with a certain radius of gyration from a population of telomeres. This requires that a large number of conformations to be generated so that the distribution function is accurate. First, we must know how many conformations and are required and the size of the individual segments to accurately reflect the mean radius of gyration of the corresponding theoretical polymer.

In the simulation, the polymer is constructed from a number of equal-sized segments. (The last segment can be shorter than all the others if a non-integer value is supplied as a parameter for the polymer contour length.) In addition, the Path object and its children in the simulation code treat all distances in segment units, i.e. a distance of 1 represents 1 segment.

The agreement of the mean radius of gyration from the simulation and the theoretical expression for the radius of gyration is a function of this segment length. Intuitively, the segment length must be short enough to accurately model the conformations of the polymer; if it’s too large, the accuracy of the mean R_g values can be so large that we cannot expect that the probability distribution of R_g values to reflect the model.

Fig. 1 displays the errors between the simulated mean radius of gyration and the theoretical one using version 0.1 of the code. The polymer chains were 25,000 bp long. Values for the linear packing density and the persistence length were taken from the range [10, 30, 50, 70, 90] and [10, 30, 50, ..., 170, 190], respectively. For each pair of these values, 1000 polymer confirmations were simulated and their mean R_g calculated from the discretized probability distribution generated by the simulation. Importantly, the segment length in user-defined units was 0.4 nm, or 25 times smaller than the smallest persistence length. The highest error is 0.05%. Note that errors are defined by the absolute difference between simulated and theoretical R_g , divided by the theoretical value for R_g .

In 2, the errors are displayed for the same simulation as above, but with a segment length equal 1 nm, or 10 times smaller than the smallest persistence length tested. The errors appear roughly the same, which suggests that a segment length on the order of 1 nm is a good length for accurate simulation of chromatin polymers in these range of parameters.

Finally, in 3 the errors are displayed for a simulation with the same range of values for packing density and persistence length, a segment length of 0.4 nm (25 times less than the smallest persistence length tested, and only 100 polymers simulated for each pair of values. Note that range of

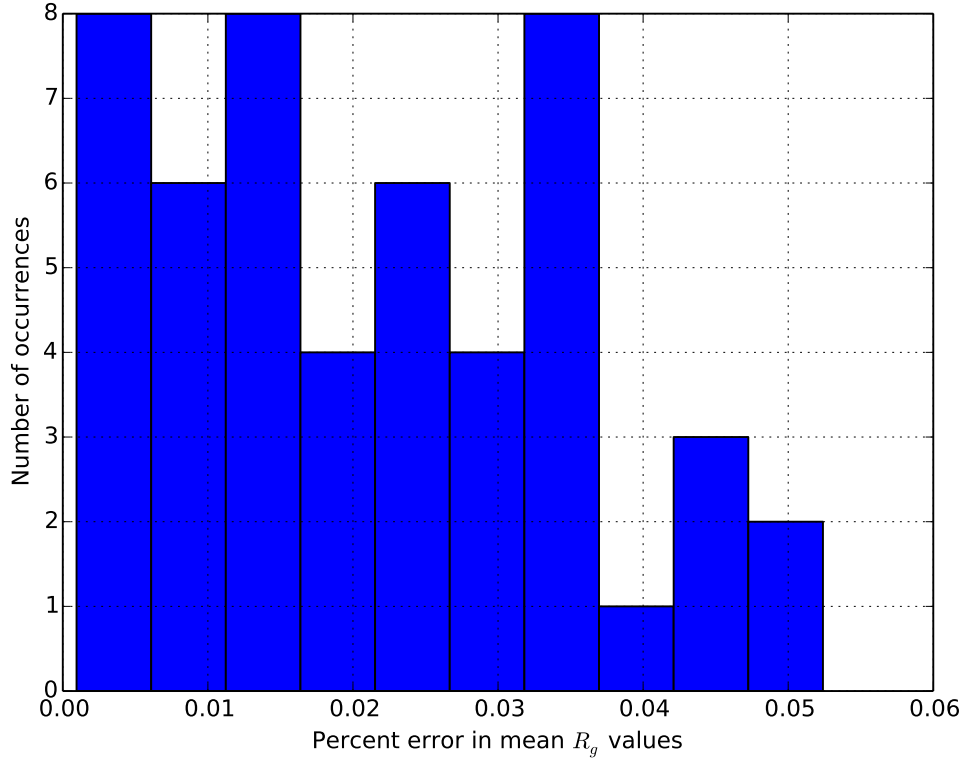


Figure 1: The accuracy of the mean radii of gyration for a number of polymer experiments with different packing densities and persistence lengths. 1000 polymer conformations were simulated for each parameter pair. Each segment was 0.4 nm long.

errors is about three times as high as when 1000 polymers were tested.

These results suggest that a segment length of 0.1 nm and a minimum of 1000 bp be used to create the probability distribution function for a chromatin polymer of a single contour length (25000 bp in these examples). When the population also involves sampling multiple contour lengths, more polymers will likely be required.

3.3.2 Tests to be performed

These are the crucial tests to be performed on the polymer simulation code before simulating polymer conformations.

1. ☒ The dependence of the simulated mean RG on segment length. This test was performed with code version 0.1 on <2014-11-28 Fri>.
2. ☒ The dependence of the simulated mean RG on the number of paths. Test performed with code version 0.1 on <2014-11-28 Fri>

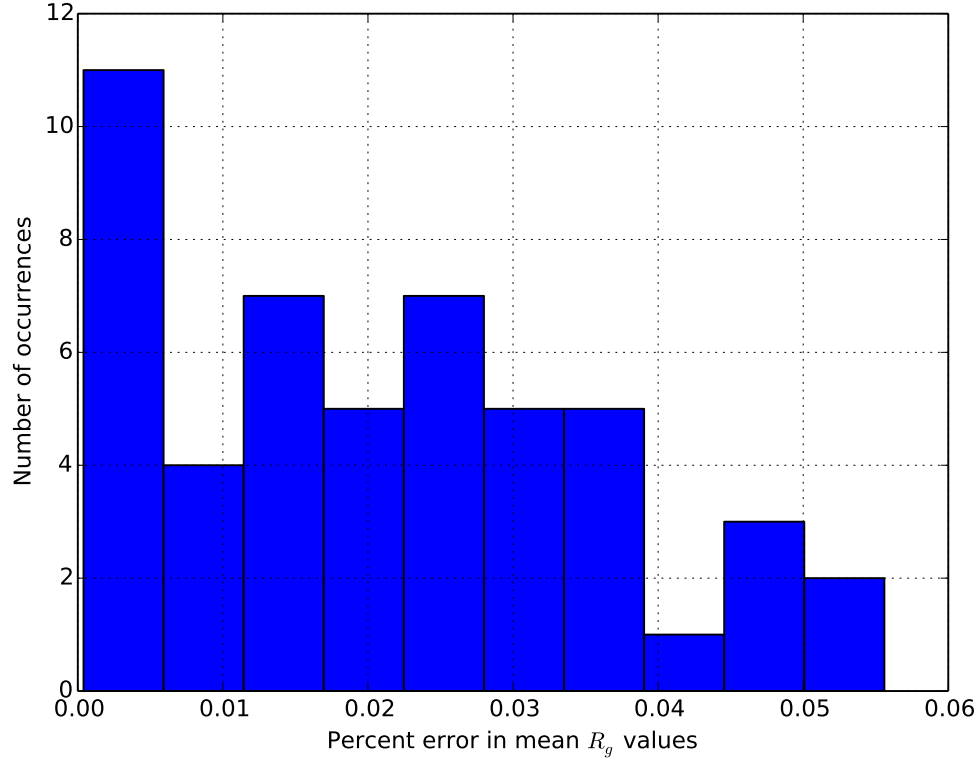


Figure 2: Errors between simulation and theory for the same simulation as parameters as in the previous graph, but with a segment length of 1 nm.

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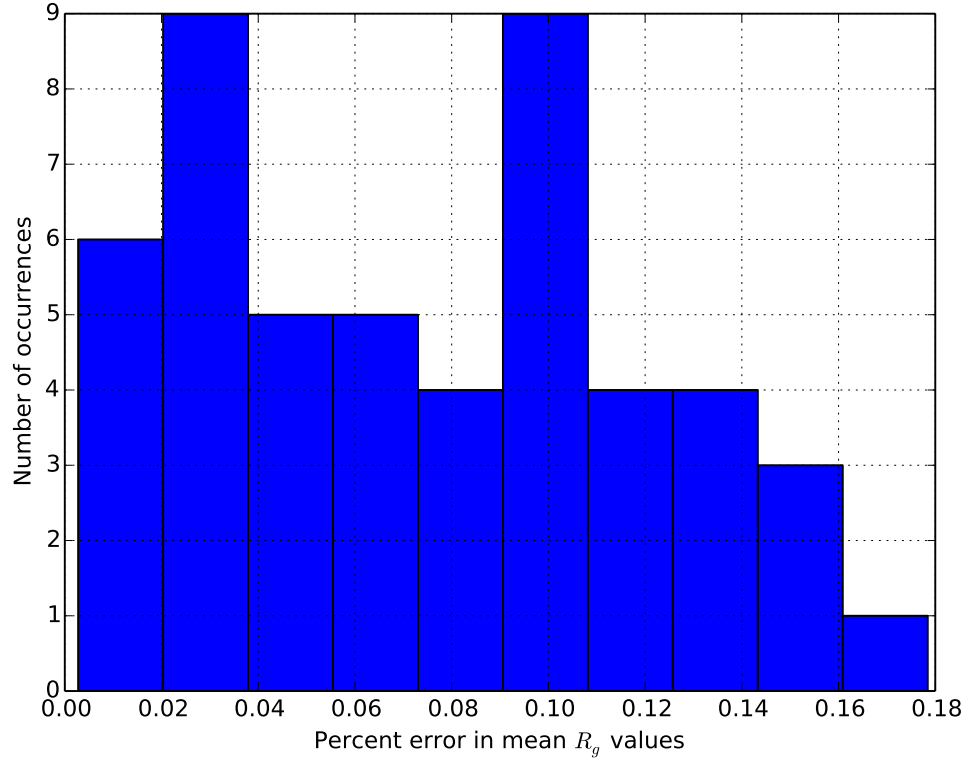


Figure 3: Errors for a simulation with 100 polymer conformations and 0.4 nm length segments.

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