Telomere project: outline of main results

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Contents

Abstract

Presented at the top level of the outline are the main assertions and conclusions concerning the telomere project and what will be discussed in the manuscript. The main pieces of evidence that support these conclusions are discussed, as well as the weak points.

1 Telomere sizes can be assessed using STORM microscopy

1.1 Reasoning: The radius of gyration is a good measure of size

The radius of gyration of a point cloud that represents one telomere reflects the radius of gyration (R_g) of the underlying telomere. This is largely an assertion based on what we know of how STORM microscopy works and not necessarily a conclusion that can be strictly proven.

1.2 Support: Our numbers are similar to the Zhuang lab paper

The Zhuang lab measured diameters of the localization distribution. This number is roughly the same as our mean radius of gyration. While not direct evidence of this point, we can claim consistency with prior results. Also, the genomic lengths of their mouse embryonic fibroblasts are similar to ours.

1.3 Support: Size correlates to the genomic distance

Hela S telomeres have shorter genomic lengths than Hela L. They also have smaller measured sizes.

1.4 Weak point: the telomeres are not fully labeled

The average number of localizations found in a telomere is between 100 and 200. However, given a label size of 18 base pairs and an average genomic size of 10 kb for Hela S and 25 kb for Hela L, we know that this corresponds to a labeling efficiency of $\frac{100}{10000/18} = 0.18$, or about 20%. This means undersampling might bias the results.

1.4.1 TODO variation in R_g is small when subsampling

We can remove points from telomere clusters and show the dependence of R_g on the number of points removed. This would tell us how strongly the value depends on any given set of points.

1.4.2 TODO simulations of real R_g vs. subsampled R_g

We can simulate a large number of polymers and simulate what happens if we label only a fraction of their points.

1.5 Weak point: the telomere size depends on number of frames

We see a slight increase (~5 nm, or about 5% to 6% of the mean) in average size when the we image with 30,000 frames. This could be due to stage drift. It's supposedly corrected for using the autocorrelation method, but how precise is this method?

1.6 Weak point: cluster sizes depend on staining

Immunofluorescence staining of TRF1 showed a smaller telomere size than DNA FISH staining. **But**, these measurements label different parts and may have different linker sizes between the label and the fluorescent marker. Is this a fair comparison?

1.7 Weak point: small telomeres will appear disproportionately large

Smaller telomere sizes will *appear* disproportionately larger than their real size than larger ones due to the localization uncertainty.

1.7.1 Counter argument : this can be taken into account

If we know the localization precision, we can correct for this. In fact, this effect is already taken into account in our estimation of the model parameters for compaction and persistence length.

1.8 Weak point: DNA FISH ruins the local DNA structure

I'm not sure if we can really debate this point or whether its known to what extent higher order structure is destroyed when performing FISH.

1.8.1 Counter argument: IF TRF1 measurements show similar sizes

The immunofluorescence labeling can actually be a strong point in this case. Because we only measure a small change in size from IF, we can claim that the compaction of is roughly preserved when FISH is performed.

The actual difference in Hela L TRF1 IF and Hela L WT FISH is about 20 nm in the mean radius of gyration.

What constitutes a small change in size here?

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2 Hela L telomeres are more compact than Hela S telomeres

2.1 Support: The density increases with volume when comparing Hela types

We can easily make this statement by taking the wild type mean radius of gyration for both cell types as a characteristic radius of a telomere. The mean gyration radii for Hela L and Hela S are $R_g \approx 100\,nm$ and $R_g \approx 77\,nm$ respectively. The average genomic lengths are $N = 25\,kb$ and $N = 10\,kb$, respectively.

The ratio of the average telomere volume is therefore

$$\frac{R_g^3\,(L)}{R_g^3\,(S)} \approx 2.2$$

However, the ratio of the genomic lengths is

$$\frac{N(L)}{N(S)} = 2.5$$

Because the ratio of genomic lengths is larger than the volume, this suggests that there is more Hela L telomeric DNA per volume than Hela S DNA.

/textbf{Note that some of the biochemical tests have placed the average of the Hela L genomic length at an even larger value, which would mean there's even more compaction.}

Also, the ratio of the volumes using the median radius of gyration is 2.1148, similar to the ratio of means.

2.2 Support: There are more heterochromatic marks on Hela L

There are more H3K9me3 marks on Hela L cells, a mark that correlates to heterochromatin.

2.3 Support: Polymer modeling places larger compaction limit on Hela L

The results of the polymer modeling places the upper limit on the packing ratio for Hela L at about 50 bp/nm, where as the upper limit is about 40 bp/nm in Hela S.

This is not proof that Hela L is more compact, but supports the other conclusions listed here.

2.4 Weak point: Small changes to compaction

I've made estimates that we need a precision in the mean values for the radius of gyration that's between plus or minus 5 and 15 nm to make accurate statements about compaction of one cell type vs. another. The precision is probably closer to 15 nm since it is derived from the scaling law for a Gaussian chain ($N \sim R_g^2$) and the 5 nm value is derived from the scaling law for constant density ($N \sim R_g^3$). Other work suggests the scaling at this length scale is closer to the Gaussian chain (Bancaud, 2012).

We're probably right at the limits of the achievable precision to assess compaction differences.

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3 There is no 30 nm-fiber like structure in Hela telomeres

3.1 Reasoning: Best-fit packing ratios are well below 30 nm fiber ratio

The highest upper limit on the packing ratios that describe our data is about 50 bp/nm, but the 30 nm fiber is twice this at 100 bp/nm.

3.2 Support: parameter estimations undoubtedly say this

The parameter plots place upper bounds on the polymer parameters that best describe the telomeres, and these upper bounds lie nowhere near the 30 nm packing density of 100 bp/nm. I believe this is both a strong and unambiguous conclusion.

The only weak points concern the application of the worm-like chain model to the data.

3.3 Weak point: t-loops

T-loops will make part of the polymer appear as circular, not linear. One of the best arguments we have for not including them is that t-loops contribute to the higher order structure in a way that influences the packing density. Since we assess the packing density in our model, we indirectly measure the effect of t-loops.

3.3.1 Counter argument: t-loops are highly heterogeneous

Not all in vitro telomeres in the Zhuang paper have t-loops. Furthermore, if they did, the size of the loop relative to the entire size of the telomere varied by a very large amount (it was almost uniform, see Fig. 2G in the paper).

Therefore, not accounting for the t-loop probably has less of an effect on our results than if every telomere had a t-loop. If every telomere had a t-loop, the linear polymer model would be much more biased.

3.3.2 Counter argument: t-loops are included in the packing density

We measure the packing density of the linear polymer, in which higher order structure abstracted way into the details of the polymer fiber itself. t-loops may be one such higher order structure.

3.3.3 Counter argument: they've only been observed in vitro

Is this true? Is it really a good argument to suggest they don't exist because we've never seen them in vivo?

3.4 Weak point: G-quadruplexes

We don't specifically allow for G-quadruplexies, at least directly...

3.4.1 Counter argument: These actually cause greater compaction

Packaging by G-quadruplexes should effectively appear as a larger telomeric compaction in our model. Since we account for/measure compaction, we are indirectly including them. This also follows the same line of reasoning as the t-loops.

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4 TRF1 and TRF2 knockdowns affect telomere size

4.1 Evidence: Visual comparison of distributions

We see a consistent difference in R_g distributions for Shelterin knockdowns.

4.2 Evidence: Significance tests

The significance tests that I performed showed differences and a typical uncertainty in the mean of about 5 nm.

4.3 Evidence: ChIP shows a correlation

ChIP measurements show that TRF2 knockdowns should have a large amount of heterochromatic marks.

4.4 Weak point: the controls differed from the wild type

The control means differ from the wild type means, but not by as much as the Shelterin knockdowns. We can claim that this puts a limit of about 5 nm on what we can tell based on distributions.

4.5 Weak point: day-to-day variation is large

The day-to-day variation in the means (and medians) is roughly the same as the minimum precision I estimated above that was necessary to unequivocally assess compaction changes between two cell types. Is this enough precision?
