

SUMMARY OF FINAL REPORT

Is localization of DNA replication origins linked to triplet repeat instability? DNA replication origin mapping in the spinocerebellar ataxia type VII (SCA7) locus

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Dates of project: February 2008 – February 2010

Aims:

The overall purpose of this project was to identify and characterize the origins of DNA replication of the spinocerebellar ataxia type 7 (SCA7) locus in various cell types from normal individuals and established cell lines from people with spinocerebellar ataxia, and to assess whether a correlation exists between origin activity and genomic instability at this locus.

Background:

Abnormal expansion of trinucleotide repeats (TNR) is the hallmark of increasing numbers of human pathological conditions, among which are several types of hereditary ataxias. TNR increase most likely arises from the tendency of these repetitive sequences to promote the formation of unusual secondary structures during various DNA transactions, and, in particular, during DNA replication, probably through the formation of a slipped strand in lagging-strand synthesis. In this respect, the localization of origins of DNA replication might in principle affect replication fork progression. No studies to date have directly demonstrated alterations of DNA replication origins location in human disease, most likely as a consequence of the technical difficulty of origin identification in mammalian cells. The purpose of this project is to identify and characterize the origins of DNA replication in the SCA7 locus in various cell types from normal individuals and cell lines from patients with spinocerebellar ataxia, and to establish whether a correlation exists between origin activity and genomic instability at this locus.

The preliminary results obtained using a newly developed DNA library enriched in origins of replication (Todorovic et al, Mol Cell, 2005) have indicated that at least 4 different DNA replication origins are localized in a ~200 kb region encompassing the SCA7 locus, one of which is located very close to the tract containing the TNR expansion. In the context of this project, these origins will now be mapped at high resolution and their activity characterized in replicating human cells of different histological derivation, in both normal conditions or after cell treatment with agents affecting DNA replication fork dynamics, and in cells lines from patients with spinocerebellar ataxia.

Results:

We have identified four putative DNA replication origins within the SCA7 locus, active in wild-type human cells. Two of them have been fine mapped and validated through well-established nascent DNA strand characterization. We also investigated the possibility that trinucleotide repeat expansion could induce a re-arrangement in replication origins distribution, possibly activating normally silent origin sites. The results obtained are in favour of this possibility, showing cryptic origin activation in the proximity of the TNR locus in two patients' cell lines.

Lay summary:

SCA7 is caused by the abnormal expansion of a specific DNA region within the ataxin7 gene. This region corresponds to a DNA tract consisting of tandem repetitions of a trinucleotide sequence. The genomes of normal individuals carry from 6 to 25 repetitions, those of people with SCA7 carry up to 300 repetitions. The repeated tract length is associated with disease severity and age of onset. Expansion of this triplet repeat represents a dynamic mutation of the human genome, as its length is not stably transmitted to the offspring (as in most inherited diseases), but often increases at each generation. The molecular reasons for the expansion of the number of repeats in the affected individuals are still largely unknown.

In this project we explored the possibility that an alteration in the process of DNA replication might be responsible for abnormal triplet expansion. In all cells, DNA replications starts at specific sites in the genome, named DNA replication origins. The distribution of these origins is usually tightly regulated and their activity well orchestrated in order to guarantee complete and accurate genome duplication in an adequate lapse of time. We hypothesized that misregulation of DNA replication origins usage at the SCA7 locus might account for the genomic instability, eventually giving rise to pathological repeat expansion.

To address this possibility, we first determined the distribution of DNA replication origins in the SCA7 locus. We found that at least four, evenly distributed replication origins are present at this locus. The trinucleotide repeated tract is located between the second and the third newly identified origins, in a roughly central position. This organization is supposed to ensure the proper replication of the SCA7 locus, allowing the faithful duplication of repeated tract without the generation of expansions. We also found that when healthy cells were treated with drugs known to impair distribution of DNA replication origins, the stability of the CAG repeated tract was severely affected, consistent with the possibility that aberrant origins might be activated upon treatment with these drugs. Of great interest, next we found that, in two cell lines from people with SCA7, origin distribution in the SCA7 locus was altered, with the appearance of a novel DNA replication origin in close proximity to the region carrying the repeated tract. This origin, which is cryptic in normal individuals, co-localizes with a DNA segment previously reported to affect the stability of the SCA7 locus in transgenic animals.

Taken together, our results indicate a crucial role of DNA replication origin localization in determining genetic stability of the trinucleotide repeated tract in the SCA7 genetic locus.

This work was undertaken as a PhD project and the student working on the project has successfully obtained his PhD.

Benefits to people with ataxia arisen/likely to arise from this research:

This is essentially a basic research project with no apparent immediate relevance for patient care. However, in this respect it appears important to emphasize that, after two decades of intense research on disease due to triplet expansion and the characterization of the molecular mechanisms leading to expansion itself, as yet no clue exists concerning the genetic elements that are the actual cause of the disease itself. Our research now clearly indicates that DNA replication and, in particular, origin activation is a critical determinant of triplet repeat instability at the SCA7 locus.

Publications arisen from this project:

Carrer A, Cleary J, Pearson CE, Giacca M, DNA replication origins positioning at the SCA7 locus is crucial for trinucleotide stability (in preparation)

Conferences/ meetings where this research has been presented:

None yet

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