Mutat Res Fund Mol Mech Mutagen 800–802 (2017) 1–7

Contents lists available at ScienceDirect

Mutat Res Fund Mol Mech Mutagen

journal homepage: www.elsevier.com/locate/mut

Torsional stress promotes trinucleotidic expansion in spermatids

Olivier Simard, Seyedeh Raheleh Niavarani, Virginie Gaudreault, Guylain Boissonneault*

Department of Biochemistry, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Canada

ARTICLE INFO

Keywords:
Trinucleotide repeats
Chromatin remodeling
Spermiogenesis
Spermatids
Torsional stress
Protamines

ABSTRACT

Trinucleotide repeats are involved in various neurodegenerative diseases and are highly unstable both in dividing or non-dividing cells. In Huntington disease (HD), the age of onset of symptoms is inversely correlated to the number of CAG repeats within exon 1 of the *HTT* gene. HD shows paternal anticipation as CAG repeats are increased during spermatogenesis. CAG expansion were indeed found to be generated during the chromatin remodeling in spermatids where most histones are evicted and replaced by protamines. This process involves striking change in DNA topology since free supercoils must be eliminated. Using an *in vitro* CAG repeat reporter assay and a highly active nuclear extracts from spermatids, we demonstrate that free negative supercoils result in CAG TNR expansion at a stabilized hairpin. We also suggest a possible role for protamines in promoting localized torsional stress and consequently TNR expansion. The transient increase in torsional stress during spermiogenesis may therefore provide an ideal context for the generation of such secondary DNA structures leading to the paternal anticipation of trinucleotidic diseases.

1. Introduction

Variations in trinucleotidic repeats (TNRs) length are involved in many neurodegenerative diseases such as Huntington disease (HD) [1], various types of spinocerebellar ataxias, myotonic dystrophies and fragile X syndrome [2]. In HD, CAG repeats are located within exon 1 of the *HTT* gene and the pathology is generally observed once a threshold number of 40 TNRs is reached [1,3]. As for many other poly-glutamine diseases, the age of onset of symptoms are inversely correlated with repeats number so longer TNRs are observed when symptoms appears at a younger age [4]. TNRs are dynamic microsatellite sequences and their length is impacted by various DNA transactions such as replication [5,6], transcription [7] and repair [6,8]. Most studies agree that non-B DNA structure are generated during the process, mostly in the form of stabilized hairpin within repeats [9,10]. This, in turn, recruits the mismatch repair machinery leading to TNR instability [11–16].

TNRs instability has been widely observed in various somatic cells tissues [17] but also during gametogenesis, allowing either paternal or maternal anticipation [2,13,18,19]. HD displays paternal anticipation since expansion are detected during spermatogenesis in both mice and human [13,20]. Expansion in the germline may lead to precocious onset of the symptoms in the offspring. CAG expansion within *HTT* have been observed during meiosis [21], but also during the post-meiotic differentiation of haploid spermatids (spermiogenesis) [13]. Using a HD mouse model, harboring the human exon 1 of *HTT*, we previously showed that TNR expansion occurs during spermiogenesis at steps 13–14 where spermatids undergo a major chromatin remodeling process [22].

During chromatin remodeling in spermatids, most histones are replaced by protamines allowing greater compaction and stability of the nucleus [23]. This involves major changes in DNA topology where most of the freed nucleosomal DNA supercoils are lost likely through transient DNA strand breakage [23–25] and the deposition of protamines [26]. The timely expression of topoisomerase II β at these steps makes it a likely candidate for the efficient removal of the accumulating torsional stress [23,24]. We hypothesized that the transient increase in free supercoils resulting from the loss of nucleosomes would provide an ideal context for secondary structures formation such as hairpins at CAG repeats.

In the present study, we used a CAG repeat reporter assay to monitor TNR expansion generated by active nuclear extracts from elongating spermatids [27] and demonstrate that negative supercoils drive TNR expansion within a CAG hairpin. Our results shed some light on the origin of CAG expansion observed during chromatin remodeling in spermatids.

* Corresponding author at: Department of Biochemistry, Faculty of Medicine and Health Sciences, Université de Sherbrooke, 3201 Rue Jean Mignault, Sherbrooke (Québec), J1E4K8, Canada.

E-mail address: guylain.boissonneault@usherbrooke.ca (G. Boissonneault).

http://dx.doi.org/10.1016/j.mrfmmm.2017.04.001
Received 9 January 2017; Received in revised form 7 April 2017; Accepted 7 April 2017
Available online 09 April 2017
0027-5107/ © 2017 Elsevier B.V. All rights reserved.

2. Methods

2.1. Animals

All spermatids nuclear extracts were prepared from sexually mature (9 weeks old and above) male CD-1 mice obtained from Charles River and were maintained under standard housing conditions. Mice were anesthetized by isoflurane and euthanized by CO_2 asphyxiation. Animal care was in accordance with the Université de Sherbrooke animal care and use committee.

2.2. Preparation of plasmid

Supercoiled pBL302 was prepared using Fast Plasmid Maxi prep (cat# B22213, Biotool). Relaxed pBL302 was prepared by digesting supercoiled pBL302 with Nt.BsmAI (cat# R0121S NEB) for 1 h at 37 °C, then inactivated at 65 °C for 20 min. ATP was added to a final concentration of 1 mM, then 400 U of T4 DNA ligase (cat# M0202S NEB) was added to each reaction and incubated at RT for 1 h. Repaired plasmids were purified by ethanol precipitation.

2.3. S1 nuclease assays

S1 nuclease digestions were performed using 1 μg of plasmid, 5 μL of S1 Nuclease buffer (5x) and 10 U of S1 nuclease (ThermoFisher, cat# EN0321) and incubated at RT for 30 min. Reactions were stopped by adding 2 μL of 0.5 M EDTA followed by incubation at 70 °C for 10 min. Reactions were precipitated by ethanol prior to electrophoresis. Determination of S1 nuclease digestion at CAG repeats was determined by quantitative PCR using the following primers F2: GCAAAAACTCGGTTTGACGCCTCC and R2: TGCTTCTCCTCTATGTC GGCGTCT with the following reagents: 1 x Advanced™ qPCR master mix (Wisent, cat# 800-431-UL,), 0.2 μM of each primer, 1 pg of DNA sample and the following PCR program cycles: 95 °C for 1 min, 40 cycles of 95 °C for 15 s, 55 °C for 20 s and 72 °C for 20 s.

2.4. Preparation of spermatids nuclear extracts

All the following steps were performed on ice, with ice-cold solutions, while centrifugations were carried out at 4 °C. Testes from 6 mice were decapsulated and homogenized in cold PBS. Homogenized cells were then filtered using a BD Falcon™ 40 μm Cell Strainer (BD Biosciences, USA). Filtered cells were washed 3 times with PBS and centrifuged at 1000*g* for 1 min between washes. Pellets were resuspended in 1,2 mL of lysis solution (320 mM Sucrose, 10 mM Tris–HCl pH 7.5, 5 mM $MgCl_2$, 1% Triton X-100) and incubated on ice for 5 min for a complete lysis of the cytoplasm. Nuclei were washed once with 800 μL of lysis solution, then twice with 750 μL of PBS-PIC (PBS supplemented with 1X Protease Inhibitor Cocktail) (cat# B14001, Biotool). Nuclei were centrifuged at 1000*g* for 3 min between washes. Pellets containing nuclei were resuspended in sonication buffer (10 mM Tris pH 7.5, 10 mM NaCl, 1.5 mM $MgCl_2$, 10% Glycerol, 1X Protease inhibitor cocktail) and sonicated using a Misonix sonicator, with the following sequence: 10 pulses of 30 s at an amplitude of 25, with 15 s breaks between pulses. Sonication resulted in the lysis of nuclei from spermatogonias, spermatocytes and steps 1–9 spermatids. Supernatants, which correspond to nuclear extract from these cells were kept at −80 °C for immunoblots. Remaining sonication-resistant nuclei (steps 10–16 spermatids confirmed by microscopy) were washed twice with PBS-PIC, resuspended in 180 μL of extraction buffer (10 mM Tris pH 7.5, 10 mM NaCl, 1.5 mM $MgCl_2$, 10% Glycerol, 1X PIC, 350 mM Na_2SO_4) and incubated on ice for 30 min. Nuclei were centrifuged at 13,000*g* for 15 min. Supernatants, which contain nuclear proteins from steps 10–16 spermatids, were aliquoted and kept at −80 °C.

2.5. Immunoblots

Immunoblots targeting Top2β and Prm1 were performed using 20 μg of either spermatids or NIH 3T3 nuclear extracts and the following antibodies: Rb anti Ms-Top2β, (Santa Cruz BioTechnology, cat# Sc-13059), Ms anti-protamine P1 (cat. # Mab-001 Hup 1N, Briar Patch Biosciences LLC), Dk anti-Rb IgG Dylight 800 Conjugate (Jackson ImmunoResearch, cat# DkxRb-003-F800NHSX), Gt anti-mouse IgG DyLight 680 (ThermoScientific, cat#35518,). Fluorescence was visualized using the Odyssey Infrared Imaging System (Li-Cor Biosciences, model9120).

2.6. In vitro NHEJ assays

The DNA template for *in vitro* repair was generated by digestion of pET28a using NheI and SacI restriction enzyme. Digestions were performed sequentially and pET28a was purified after each digestion using a gel extraction kit (Omega, #D2500-01). *In vitro* DNA repair reactions were performed in a final volume of 15 μL for 16 h at 34 °C under the following conditions: 50 mM Tris HCl, 10 mM $MgCl_2$, 1 mM ATP, 10 mM DTT, 100 ng of digested pET28a, 0.5 mM each dNTPs, 1.5 μL of nuclear extract from spermatids and 0.5 mM SCR7 (Selleckchem, cat.# S7742,) when indicated. Reactions were inactivated at 80 °C for 10 min followed by Proteinase K digestion (40 μg/reaction) at 55 °C for 3 h and a final inactivation step at 80 °C for 20 min. The repair efficiency of each reaction was measured by qPCR using the following primers F1: CAGCAGCCAACTCAGCTT and R1: AGCCATCATCATCATCATCACA with the following reagents: 1 x Advanced™ qPCR master mix (Wisent, cat# 800-431-UL), 0.2 μM each primer, 1 pg of DNA sample and the following PCR program cycles: 95 °C for 1 min, 40 cycles of 95 °C for 15 s, 62 °C for 20 s and 72 °C for 20 s.

2.7. In vitro TNR expansion assays

In vitro TNR expansion assays were performed as described by Stevens et al. [27]. Briefly, 5 μg of nuclear extract was incubated with 3μg of pBL302 plasmid (supercoiled or relaxed), 10 μL of 5X TNR buffer (500 μM dNTPs, 1 mM CTP, 1 mM GTP, 1 mM UTP, 20 mM ATP, 200 mM creatine phosphate, 35 mM magnesium acetate, 150 mM Hepes pH 7.8, 2.5 mM DTT), 48 μg of creatine kinase, in a final reaction volume of 50 μL. Reactions were performed for 4 h at 34 °C, then inactivated by adding 50 μL of 2X stop buffer (2% SDS, 50 mM EDTA, 2 mg/mL proteinase K) and incubated at 34 °C for 30 min, then 80 °C for 10 min pBL302 was purified from reactions by using a PCR purification kit (Omega D6492-02).

2.8. TNR expansion analysis and validation

In vitro TNR expansion were analyzed as described by Stevens et al. [27]. Briefly, purified pBL302 from reactions was transformed in *S. Cerevisiae* strain BL1435, and plated on either SC-His counting plates or SC-His analysis plate. After 36 h, SC-His analysis plates were replicated with a sterile velvet, then replicated onto three SC-His waste plate, and finally onto a SC-His-Arg plates supplemented with canavanine 500 mg/L. Canavanine plates were grown for 3–4 days and growing colonies were counted. For validation, at least 20 canavanine-positive colonies per plate were picked and lysed in zymolyase for 30 min at 37 °C, then 80 °C for 10 min. 5 μL of each lysed colony was used for PCR, using the primer F3: 5′/TYE705/GCAAAAACTCGGTTTGACGCC-TCC and R2: TGCTTCTCCTCTATGTCGGCGTCT with the following reagents: 1x OneTaq® standard buffer, 0.2 mM dNTPs, 0.2 μM each primer, 1 U of OneTaq® polymerase and the following PCR program cycles: 94 °C for 1 min, 40 cycles of 94 °C for 1 min, 58 °C for 1 min, 68 °C for 1 min, with a final elongation at 68 °C for 10 min. PCR products were resolved by electrophoresis using a 6% polyacrylamide-

Download English Version:

https://daneshyari.com/en/article/5528704

Download Persian Version:

https://daneshyari.com/article/5528704

Daneshyari.com