Chromatin structure of yeast minichromosomes containing triplet repeat sequences associated with human hereditary neurological diseases

Mitsuhiro Shimizu, Ryo Fujita, Nobuyuki Tomita, Heisaburo Shindo¹ and Robert D. Wells²
Department of Chemistry, Meisei University, Hino, Tokyo 191-8506, Japan, ¹School of Pharmacy, Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo 192-0392, Japan and ²Institute of Biosciences and Technology, Texas A & M University, Houston, Texas 77030-3303, USA

ABSTRACT

Expansion of triplet repeat sequences such as \((\text{CTG})_n\), \((\text{CGG})_n\), and \((\text{GAA})_n\) causes human genetic diseases. Since DNA is packaged into arrays of nucleosomes in eukaryotic cells, chromatin may be involved in the mechanism of triplet repeat diseases. To elucidate this issue, we have examined effects of triplet repeat sequences on the chromatin organization in vivo using well defined yeast minichromosomes. We show here that \((\text{CGG})_{12}\) disrupts an array of positioned nucleosomes, whereas \((\text{CTG})_{12}\) promotes the nucleosome formation. Thus, triplet repeat sequences can affect the chromatin organization in vivo, which may contribute to the triplet repeat expansion or alterations in the expression of genes associated with triplet repeat diseases.

INTRODUCTION

Since 1991, more than 10 human hereditary diseases, including Fragile X syndrome, myotonic dystrophy, Huntington's disease and Friedreich's ataxia have been identified to be associated with the expansion of \((\text{CTG})_n\), \((\text{CGG})_n\), and \((\text{GAA})_n\) (1). The expansions occur in both the coding segments of the exons and the non-coding regions such as 5'- and 3'-untranslated regions or introns. Triplet repeat sequences associated with the diseases can form a variety of alternative DNA conformations including hairpins, slipped strand structures, quadruplex DNA and intramolecular triplexes. Thus, it has been proposed that DNA secondary structure is a causative factor for the expansion. However, mechanisms of the expansion and the diseases remain to be elucidated.

In eukaryotic cells, DNA is packaged into nucleosomes; the primary structural units of chromatin, and chromatin alteration is an essential step for regulation of gene expression. Recently, we showed that an unusual DNA conformation adopted by poly dA-poly dT disrupts an array of positioned nucleosomes (2), indicating that alternative DNA structures act as a determinant of nucleosome organization. Thus, triplet repeat sequences may affect chromatin organization through their unique conformations, which may cause genetic instability.

Herein, we have examined the effect of triplet repeat sequences on the organization of nucleosomes in vivo using well-defined yeast minichromosomes.

MATERIALS AND METHODS

A set of oligonucleotides of triplet repeat sequences synthesized chemically was annealed and ligated with the vectors, TALS-pBR322ΔRI or TRP1/ARS1-pBR322ΔRI (2). After the construction of plasmids, pBR322 backbone was eliminated, and TALS or TRP1/ARS1 derivatives were then isolated, ligated and introduced into the \(S.\) cerevisiae strain FY24 (\(MAT\alpha ura3-53\) trp1Δ63 leu2Δ1). Chromatin structure of yeast minichromosomes was analyzed by micrococcal nuclease digestion as described previously (2-4).

RESULTS AND DISCUSSION

Recently, we developed an assay system with which to examine the effects of DNA sequences on nucleosome formation in yeast minichromosomes in vivo (2). Fig. 1 shows chromatin structures of TRP1/ARS1 and TALS minichromosomes used as vectors in this study. When a repeat is inserted into the \(SacI\) site in the nucleosome IV in TALS, it lay around the dyad of the positioned nucleosome. On the other hand, when a repeat inserted into the \(EcoRI\) site in the nuclease hypersensitive site (HSR B) in TRP1/ARS1, it lay in the nucleosome free region. Thus, this assay allows the careful examination of the effect of inserts at specific locations on nucleosome formation in chromatin assembled physiologically in living yeast cells.

We focus on \((\text{CGG})_n\), \((\text{CTG})_n\) and \((\text{GAA})_n\), which are located in non-coding regions of genes responsible for...
Fragile X syndrome, myotonic dystrophy and Friedrich's ataxia, respectively. When a (CGG)$_{12}$ was inserted into the positioned nucleosome IV in TALS minichromosomes, the region became hypersensitive to micrococcal nuclease, indicating that CGG repeats disrupt an array of positioned nucleosomes. When the (CGG)$_{12}$ was inserted into the nucleosome free region in TRP1/ARS1, the nuclease hypersensitivity remains unchanged. Such destabilization of nucleosome can be accounted for in terms of formation of stable higher-order DNA structures (hairpins, quadruplex or slipped structures) or high energy cost of nucleosome formation with the sequences.

In contrast, when a (CTG)$_{12}$ was inserted into the nucleosome free region in TRP1/ARS1 minichromosomes, the region was protected from nuclease digestion, indicating that CTG repeats promote nucleosome formation. Consistent with this result, (CTG)$_{12}$ was incorporated into the positioned nucleosome IV in TALS. Since nucleosomes acts as a general repressor for gene expression, the CTG repeats may modulate expression of disease-causing genes via chromatin structural changes.

In summary, triplet repeat sequences can affect the chromatin organization in vivo, which may contribute to the expansion phenomenon and the disease phenotypes.

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REFERENCES


Figure 1. Chromatin structures of TALS and TRP1/ARS1 minichromosomes (2-4). TRP1/ARS1 minichromosomes consist of three stable nucleosomes (nucleosomes I – III) and of four nucleosomes of limited stability. The TALS plasmid was constructed by inserting 356 bp of STE6 promoter, including the α2 operator (indicated by the hatched box) into the EcoRI site in the HSR B in the TRP1/ARS1 plasmid. TALS minichromosomes consist of ten nucleosomes, in which nucleosomes I to V are precisely positioned. Triplet repeat sequences, (CTG)$_n$, (CGG)$_n$ and (GAA)$_n$ were inserted into either at the SacI site in the center of nucleosome IV in TALS or at the EcoRI site in the nuclease hyper sensitive region B (HSR B) in TRP1/ARS1.