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RNAPol-ChIP analysis of transcription from FSHD-linked tandem repeats and satellite DNA

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Abstract

RNA interference (RNAi) is implicated in maintaining tandem DNA arrays as constitutive heterochromatin. We used chromatin immunoprecipitation with antibodies to RNA polymerase II (RNAPol-ChIP) to test for transcription of the following repeat arrays in human cells: subtelomeric D4Z4, pericentromeric satellite 2, and centromeric satellite α. D4Z4 has a promoter-like sequence upstream of an ORF in its 3.3-kb repeat unit. A short D4Z4 array at 4q35 is linked to facioscapulohumeral muscular dystrophy (FSHD). By RNAPol-ChIP and RT-PCR, little or no transcription of D4Z4 was detected in FSHD and normal myoblasts; lymphoblasts from an FSHD patient, a control, and a patient with D4Z4 hypomethylation due to mutation of DNMT3B (ICF syndrome); and normal or cancer tissues. However, RNAPol-ChIP assays indicated transcription of D4Z4 in a chromosome 4-containing human–mouse somatic cell hybrid. ChIP and RT-PCR showed satellite DNA transcription in some cancers and lymphoblastoid cell lines, although only at a low level. Given the evidence for the involvement of RNAi in satellite DNA heterochromatinization, it is surprising that, at most, a very small fraction of satellite DNA was associated with RNA Pol II. In addition, our results do not support the previously hypothesized disease-linked differential transcription of D4Z4 sequences in short, FSHD-linked arrays.
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Keywords: FSHD; Transcription analysis; Gene expression; Chromatin immunoprecipitation; Satellite DNA; DNA repeat array

1. Introduction

Constitutive heterochromatin containing long tandem arrays of DNA repeats, such as pericentromeric chromatin, has generally been considered transcriptionally inert. However, there are some exceptions, such as certain *Drosophila* pericentromeric genes [1]. Moreover, transcription of arrays of tandem DNA repeats has recently been implicated in maintaining constitutive heterochromatin in fission yeast, nematodes, plants, and vertebrates through the production of small interfering RNAs (siRNAs) from larger precursor RNAs by the RNA interference (RNAi) pathway [2]. For example, in a human–chick somatic cell hybrid (SCH), the importance of the RNAi machinery for normal structure and function of centromeres was demonstrated by conditionally knocking down the function of Dicer, the RNAi nuclease [3]. In normal murine tissues and embryonic stem cell cultures, low levels of transcripts from major and minor satellite DNAs adjacent to or within centromeres were detected [4–6]. The pericentromeric satellite III DNA at 9qh undergoes induction of RNA polymerase II-associated transcription in heat-shocked HeLa cells [7,8]. Accumulation of centromeric satellite DNA-derived transcripts was seen in murine cells induced to differentiate in vitro or treated with the DNA methylation inhibitor 5-azacytidine or the apoptosis-inducer staurosporine [9].

In this study, we addressed the question of whether we could detect and quantitate transcription of two satellite DNAs and a subtelomeric array of tandem DNA repeats by chromatin immunoprecipitation using antibodies to RNA polymerase II

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(RNAPol-ChIP) ([10–12]; www.genpathway.com). For each DNA region investigated, we monitored the amount of immunoprecipitation by real-time quantitative PCR (qPCR). Recently, RNAPol-ChIP has been used to quantitate occupancy of promoters (e.g., [13–15]) and to quantify transcription itself [10,12]. We analyzed the following repeat arrays: human satellite II DNA (Sat2), most of which is in the very long pericentromeric heterochromatin of chromosome 1 (1qh); centromeric satellite α DNA (Satα); and the subtelomeric D4Z4, which is associated with facioscapulohumeral muscular dystrophy (FSHD) [16,17]. D4Z4 arrays were included in this study because they contain a putative gene within their 3.3-kb repeat unit [18,19]. Furthermore, they might be subject to RNAi because it was proposed that all large arrays of tandem DNA repeats are templates for production of siRNA [6]. RNAPol-ChIP was chosen for the transcription analysis because it can directly measure transcription and is independent of posttranscriptional processing, differential RNA stability in vivo, and the size of the RNA product.

The analysis of transcription from D4Z4 arrays was of particular interest because of their involvement, by an unknown mechanism [20], in FSHD. These arrays are present at 4q35 and 10q26 and are highly polymorphic in size [21,22]. FSHD, a dominant disorder, is tightly linked to the number of repeat units in the D4Z4 array. Patients almost always have <11 repeat units at one of their two allelic 4q35 arrays while unaffected individuals have 11–100 repeat units at both 4q35 alleles [23] (Fig. 1A). By RT-PCR, Northern blotting, or cDNA cloning, Ding et al. [24] found D4Z4-like transcripts related to DUX4, an open reading frame (ORF) in the D4Z4 repeat unit, in several normal human tissues and a rhabdomyosarcoma cell line. However, sequencing revealed that they did not arise from D4Z4, but rather from partially homologous sequences elsewhere. Because DUX4 could encode a double-homeodomain protein [18,19] and a decreased size of murine transgene arrays can be associated with increased expression [25], it was hypothesized that a decrease in the number of D4Z4 repeat units at one allelic 4q35 position causes FSHD by upregulation of DUX4. We compared transcription at D4Z4 in disease and control cell populations, including myoblasts from FSHD patients, lymphoblasts from patients with the ICF syndrome (immunodeficiency, centromeric region instability, facial anomalies) that results in strong hypomethylation of D4Z4 [26] due to mutations in *DNMT3B* [27], and cancers with D4Z4 hypomethylation. The effect of hypomethylation of D4Z4 on its transcription is of special interest because FSHD cells exhibit moderate hypomethylation of their abnormally short, disease-linked D4Z4 array at 4q35 [28]. We also report the first analyses of transcription of Sat2 and Satα in control or transformed human cell populations displaying either the high levels of methylation that are normal for these sequences or hypomethylation [29,30].

2. Materials and methods

2.1. Cell culture and tissue samples

Myoblast cell strains (GM17899 from Coriell Institute and F1010 from our lab) were from biopsies of moderately affected deltoid skeletal muscle of FSHD patients with a confirmed short D4Z4 array at 4q35. A non-FSHD myoblast cell strain (GM17901, Coriell Institute) was established from a deltoid muscle biopsy of a patient with generalized muscle weakness and a diagnosis of dematomyositis. At the passage used for analysis, most of the cells in the myoblast cultures stained desmin-positive, indicating that they were myoblasts, and not contaminating fibroblast-like cells. Lymphoblastoid cell lines (LCLs) were B-cell lines from FSHD patients, GM17939 and GM17868 (Coriell Institute); normal controls, AG14836 and AG15022 (Coriell Institute) and three previously described controls [31]; and ICF patients, ICF C (GM08714, Coriell

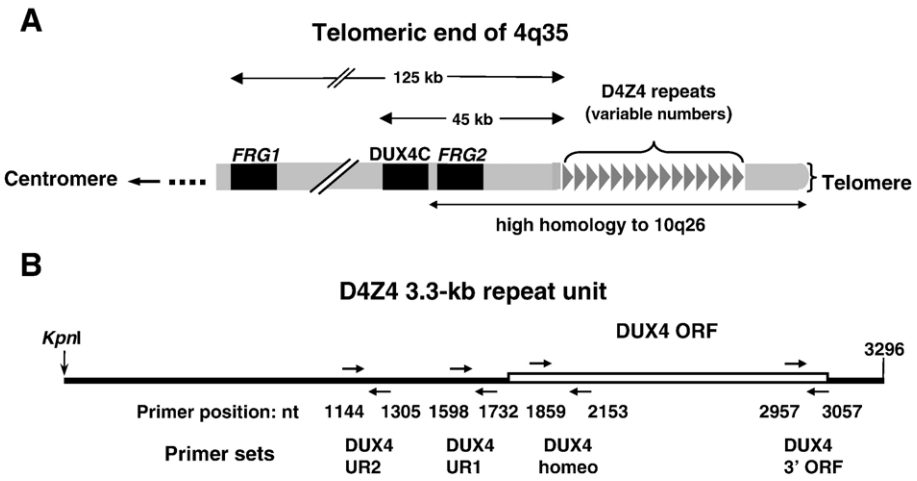

Fig. 1. Schematic illustration of the distal region of 4q35 and the repeat unit of D4Z4. (A) Positions of the size-polymorphic D4Z4 array; *FRG1* and *FRG2*, the only known genes in the distal portion of 4q35; and DUX4C, a putative gene, are illustrated (not to scale). From one to about 100 repeat units can be present in the D4Z4 array. Small numbers of repeat units at 4q35 are linked to FSHD. The region of 4q35 that is highly homologous to the subtelomeric region of 10q, including the D4Z4 array, is indicated. DUX4C is highly homologous to, but in the opposite orientation from, DUX4 in the D4Z4 array; *FRG2* is in the same direction as DUX4C. (B) The DUX4 ORF within the 3.3-kb D4Z4 repeat unit and the primers used to test for transcription from D4Z4 are shown with positions given relative to the first base of the *Kpn*I site in the 3.3-kb D4Z4 repeat unit (from GenBank AF11753). The DUX4 UR1 primers are partially homologous to a subrepeat called hhspm3 (GenBank X06587).

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