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Genomic deletions and point mutations induced in *Saccharomyces cerevisiae* by the trinucleotide repeats (GAA·TTC) associated with Friedreich's ataxia

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ABSTRACT

Expansion of certain trinucleotide repeats causes several types of human diseases, and such tracts are associated with the formation of deletions and other types of genetic rearrangements in *Escherichia coli*, yeast, and mammalian cells. Below, we show that long (230 repeats) tracts of the trinucleotide associated with Friedreich's ataxia (GAA·TTC) stimulate both large (>50 bp) deletions and point mutations in a reporter gene located more than 1 kb from the repetitive tract. Sequence analysis of deletion breakpoints indicates that the deletions reflect non-homologous end joining of double-stranded DNA breaks (DSBs) initiated in the tract. The tract-induced point mutations appear to reflect a different mechanism involving single-strand annealing of DNA molecules generated by DSBs within the tract, followed by filling-in of single-stranded gaps by the error-prone DNA polymerase zeta.

1. Introduction

In humans, certain genetic diseases are caused by expansions of tri-, tetra-, penta- or dodecanucleotide repeats [1–5]. The types of repeats associated with expansions are usually, although not always [6], capable of forming "hairpins" or other types of non-canonical DNA structures. For example, the triplet repeat associated with Friedreich's ataxia [2], (GAA·TTC), is capable for forming triplex DNA/H-DNA [4].

The effects of long (>100 repeats) trinucleotide tracts on genome stability have been examined in bacteria, yeast, and cultured mammalian cells. These tracts tend to have high levels of alterations, with deletions exceeding expansions in most yeast and bacteria studies [7]. These alterations are likely to reflect the processing of non-canonical DNA structures by various cellular repair proteins [8] or errors made during replicative DNA synthesis of these unconventional repetitive sequences [6].

In *E. coli*, plasmid-borne tracts of $(GAA{\cdot}TTC)_n$ exhibit length- and orientation-dependent instability [9]. The longer (>100 repeat) tracts tend to contract, particularly when oriented such that the GAA sequences are on the lagging strand template during replication; henceforth, we will refer to this orientation as the GAA orientation and the reverse orientation as the TTC orientation. Similar orientation- and length-dependence effects are observed in yeast [10]. In yeast, replication forks stall at the $(GAA{\cdot}TTC)_n$ tracts in the GAA, but not the TTC, orientation. This result argues that one cause of tract instability is likely to be related to stalled or broken DNA replication forks. In the experiments described below, all yeast strains had tracts in the GAA orientation based on our previous analysis of tract-associated replication fork blockage [11].

$(GAA{\cdot}TTC)_n$ tracts also stimulate recombination and chromosome rearrangements. Plasmid-borne tracts in *E. coli* elevate the frequency of both intra- and interchromosomal recombination [12]. Kim et al. [13] showed that $(GAA{\cdot}TTC)_n$ tracts increased the frequency of deletions, translocations, and ectopic recombination in the GAA orientation with a smaller effect in the TTC orientation. In strains with a $(GAA{\cdot}TTC)_{230}$ tract in the GAA orientation, a double-stranded DNA break (DSB) occurs within the tract; this break is not observed for tracts in the TTC orientation. The $(GAA{\cdot}TTC)_{230}$ tracts in both orientations act as strong mitotic recombination hotspots in diploid yeast cells [11]. In addition, in these strains, a tract-associated DSB was observed with $(GAA{\cdot}TTC)_{230}$ tracts in both orientations. Based on these results, it is likely that there are two types of tract-associated DSBs: those that occur in exponentially growing cells (orientation-dependent) and those that occur in stationary phase cells (orientation-independent).

In mammalian cells, DNA sequences capable of forming triplex DNA, including $(GAA{\cdot}TTC)_n$ tracts, can induce mutations of flanking sequences. Wang and Vasquez [14] showed that a triplex-forming sequence derived from the *c-myc* promoter stimulated mutations in an immediately adjacent reporter gene by about 20-fold; over 98% of these mutations were deletions or complex rearrangements. Wojciechowska et al. [15] found that $(GAA{\cdot}TTC)_{200}$ tracts stimulated deletions and rearrangements of an adjacent reporter gene

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3- to 10-fold. No significant tract-associated increases in point mutations were observed in either of these mammalian cell studies. In contrast, Bidichandani et al. [16] reported a three-fold elevation of point mutations induced in the *X25* gene by a (GAA·TTC) tract located within the intron in serially passaged lymphoblasts. In addition, point mutations could be induced about 10-fold in mammalian cells by transformation with triplex-forming oligonucleotides [17]. In yeast, Shishkin et al. [18] showed that (GAA·TTC) tracts located within an intron of the *URA3* stimulated point mutations and small deletions in the coding sequences flanking the intron; subsequent studies showed that these mutations were generated independently of the error-prone DNA polymerase zeta (K. Shah and S. Mirkin, personal communication). Large deletions that included the tracts as well as the flanking *URA3* coding sequences were also observed. In addition, expansions of the (GAA·TTC) tracts were observed. These expansions were much more frequent with long (≥125 repeats) than short tracts (≤100 repeats) and were not affected significantly by the orientation of the tract.

In both *E. coli* [19] and *Saccharomyces cerevisiae* [20–23], DSBs can elevate the frequency of mutations in sequences located near the break. These studies utilize site-specific endonucleases, HO or I-SceI, to efficiently generate DSBs. Strathern et al. [20] showed that an HO-induced DSB stimulated reversion of a point mutation in a tightly linked reporter gene ; this effect was largely dependent on Rev3p [24], encoding the catalytic subunit of DNA polymerase zeta. In a system that allowed detection of both point mutations and deletions, most of the mutations induced by HO were point mutations or frameshifts with large deletions representing a minor class [25]. Yang et al. [21] showed that long single-stranded regions adjacent to the site of an I-SceI-generated DSB were hypermutable by ultraviolet light and methyl methanesulfonate, and this hypermutability was dependent on DNA polymerase zeta. In contrast to the studies of Holbeck and Strathern [24] and Yang et al. [21], Hicks et al. [22] found that mutations introduced during *MATα-HMR* gene conversion events were independent of DNA polymerase zeta. Mutations introduced during break-induced replication in yeast were also only modestly (two-fold) reduced in cells lacking DNA polymerase zeta [23].

In the studies described in the paragraph above, site-specific endonucleases were used to generate the mutagenic DSB. In our study, we examine the mutagenic effects of sequences that are prone to DSB formation in the absence of a site-specific endonuclease. Below, we investigate mutations induced by a $(GAA{\cdot}TTC)_{230}$ tract located about 1 kb from the reporter gene. We show that long (230 repeats), but not short (20 repeats) significantly elevate the frequency of both large deletions and point mutations within the reporter gene. The large deletions usually delete part or all of the (GAA·TTC) tract in addition to part or all of the *URA3* gene, and sequence analysis demonstrates that these deletions are a consequence of non-homologous end-joining events. The tract-induced point mutations require Rev3p and are usually associated with reductions in the length of the $(GAA{\cdot}TTC)_{230}$ tract. We suggest that deletions and point mutations reflect two different outcomes of the cellular repair of tract-induced DSBs. In a related study in yeast, elevated levels of mutations were observed in a reporter gene located about 8 kb from DSB-prone sites generated by either a quasi-palindrome or a $(GAA{\cdot}TTC)_{230}$ tract (N. Saini, Y. Zhang, Y. Nishida, and K. Lobachev, personal communication). These induced mutations were also dependent on DNA polymerase zeta.

2. Materials and methods

2.1. Yeast strains

We used four haploid strains with two different tract sizes (20 and 230 repeats) of GAA·TTC tracts in two different orientations (termed "GAA" and "TTC"): WXT10 [$(TTC)_{20}$], WXT11 [$(TTC)_{230}$], WXT12 [$(GAA)_{20}$], and WXT13 [$(GAA)_{230}$] [11]. WXT10-13 had an insertion of the wild-type *URA3* gene located 1.2 kb centromere-proximal to the tract (Table S1; Fig. 1, Fig. S1). These strains are derived from the haploid strain PSL5 [26] that is derived from the YJM789 genetic background [27]. The DNA sequences flanking the tracts are shown in Fig. S2. The details of the constructions and genotypes of these strains are given in Supplementary Material (Text S1, Table S1, and Table S2). Standard yeast procedures were used for transformations [28]. Media were prepared as described previously [26,29].

2.2. Measurements of mutation rates and analysis of ura3 mutations

We measured the rate of *ura3* mutations in the WXT strains by measuring the frequency of 5-fluoroorotic acid (5-FOA) resistant derivatives in multiple independent cultures (>20). The methods used to determine the rates of *ura3* mutations in various strains were similar to those described previously [30] except for the drug used for selection. A detailed description of rate measurements is given in Supplementary Material (Text S1).

Independent 5-FOA^R resistant strains were analyzed by PCR to determine the ratio of *ura3* point mutations to deletions. In two

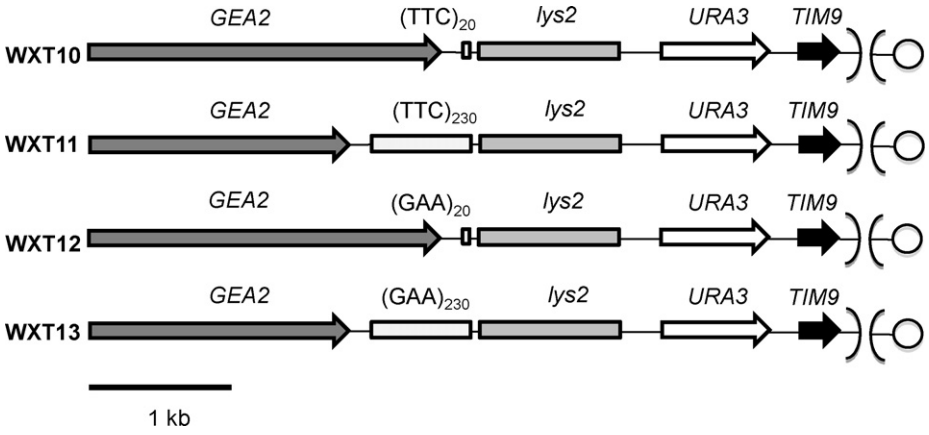

Fig. 1. Depictions of genes flanking the GAA·TTC tracts on chromosome V in strains used in the study (not drawn to scale). In all strains, the tracts (shown by white rectangles) are located about 1.2 kb from the *URA3* gene that is used as a reporter of mutagenesis. The *GEA2* and *TIM9* genes flank the tract and the *URA3* gene; the centromere is shown as a circle. A 1 kb spacer fragment containing a 3′ fragment of the *LYS2* gene is inserted between the tracts and the *URA3* gene. The nearest essential genes flanking the tract are *TIM9* and *SNU13* (located about 14 kb centromere-distal to the tract).