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A SCA7 CAG/CTG repeat expansion is stable in *Drosophila melanogaster* despite modulation of genomic context and gene dosage

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Abstract

CAG and CTG repeat expansions are the cause of at least a dozen inherited neurological disorders. In these so-called “dynamic mutation” diseases, the expanded repeats display dramatic genetic instability, changing in size when transmitted through the germline and within somatic tissues. As the molecular basis of the repeat instability process remains poorly understood, modeling of repeat instability in model organisms has provided some insights into potentially involved factors, implicating especially replication and repair pathways. Studies in mice have also shown that the genomic context of the repeat sequence is required for CAG/CTG repeat instability in the case of spinocerebellar ataxia type 7 (SCA7), one of the most unstable of all CAG/CTG repeat disease loci. While most studies of repeat instability have taken a candidate gene approach, unbiased screens for factors involved in trinucleotide repeat instability have been lacking. We therefore attempted to use *Drosophila melanogaster* to model expanded CAG repeat instability by creating transgenic flies carrying trinucleotide repeat expansions, deriving flies with SCA7 CAG90 repeats in cDNA and genomic context. We found that SCA7 CAG90 repeats are stable in *Drosophila*, regardless of context. To screen for genes whose reduced function might destabilize expanded CAG repeat tracts in *Drosophila*, we crossed the SCA7 CAG90 repeat flies with various deficiency stocks, including lines lacking genes encoding the orthologues of flap endonuclease-1, PCNA, and MutS. In all cases, perfect repeat stability was preserved, suggesting that *Drosophila* may not be a suitable system for determining the molecular basis of SCA7 CAG repeat instability.
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1. Introduction

The CAG/polyglutamine repeat diseases are a family of nine devastating neurodegenerative syndromes, including spinobulbar muscular atrophy (SBMA), Huntington’s disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA), and six spinocerebellar ataxias (SCA1, 2, 3, 6, 7, and 17; Zoghbi and Orr, 2000; Nakamura et al., 2001). These diseases result from mutations that change tandem CAG repeats, typically 10–30 units long, into expanded CAG tracts of as few as 35 repeats to as many as 300 repeats or more. As the CAG repeats are found within the coding sequences of genes, they produce abnormally long stretches of polyglutamine which disrupt the normal function of the surrounding gene and/or produce a toxic gene product, resulting in the neurodegenerative pathology (La Spada and

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Taylor, 2003; Michalik and Van Broeckhoven, 2003). One very intriguing aspect of these diseases (that often facilitated their mutation detection) is the phenomenon of anticipation—which may be defined as an earlier age of disease onset with more rapid disease progression in successive generations of a family segregating an inherited disorder (La Spada, 1997). As disease severity correlates with increasing CAG repeat expansion size, the molecular explanation for anticipation is the tendency of the disease-causing trinucleotide repeats to expand further when transmitted from an affected parent to an affected child. While the mechanistic pathways by which expanded trinucleotide repeats cause disease are being uncovered, the molecular events that underlie trinucleotide repeat instability remain rather poorly understood (La Spada et al., 2004).

An effective method for understanding polyglutamine disease pathology has been to introduce CAG repeat expansion mutations into model organisms. This approach has also been applied to the problem of "dynamic mutation" in order to identify *trans*-acting factors and *cis*-acting sequence elements that regulate the repeat instability process. In bacteria and yeast, the direction of replication through a repeat unit determines whether expansion or contraction will occur (Kang et al., 1995; Freudenreich et al., 1997; Miret et al., 1998). Another consistent observation has been that expanded trinucleotide repeats cause the replication machinery to stall in response to encountering unusual DNA conformations, such as hairpins, slipped-strands, and triplexes (Samadashwily et al., 1997; Sakamoto et al., 1999; Sinden, 2001). Subsequently, further studies suggested that Okazaki fragment initiation and processing at sequences consisting of expanded triplet repeats might account for their instability (Cleary et al., 2002; Mirkin and Smirnova, 2002). In support of this model, the flap endonuclease (FEN-1) has been shown to cause CAG repeat instability when hemizygous in mice and when absent in yeast (Lee and Park, 2002; Spiro and McMurray, 2003). As mutations in mismatch repair genes cause expansions and contractions of normal length dinucleotide and trinucleotide repeats in yeast, mice, and humans (Aaltonen et al., 1993; Peltomaki et al., 1993; Strand et al., 1993; de Wind et al., 1998), a role for mismatch repair enzymes in expanded triplet repeat instability has also been examined. Surprisingly, certain mismatch repair enzymes actually appear to be required for CAG repeat instability to occur (Manley et al., 1999; Kovtun and McMurray, 2001; van den Broek et al., 2002). In addition to *trans*-acting factors, considerable evidence exists for the importance of *cis*-acting genomic DNA sequences in driving repeat instability (Cleary and Pearson, 2003). For example, at the SCA7 CAG repeat locus, sequences 3′ to the repeat render expanded SCA7 CAG repeats intergenerationally and somatically unstable, suggesting that sequence-specific DNA structural alterations and/or binding proteins may influence stability (Libby et al., 2003). Although these studies provide tantalizing clues as to potential pathways that regulate trinucleotide repeat instability, a comprehensive model for repeat instability is yet to be elucidated.

While most studies of repeat instability have taken a candidate gene/pathway approach, unbiased screens for genes and factors involved in trinucleotide repeat instability have been lacking. As the mouse is still an unwieldy and low throughput organism for modifier screens, we reasoned that *Drosophila melanogaster* might be a useful system for the identification of *trans*-acting factors involved in

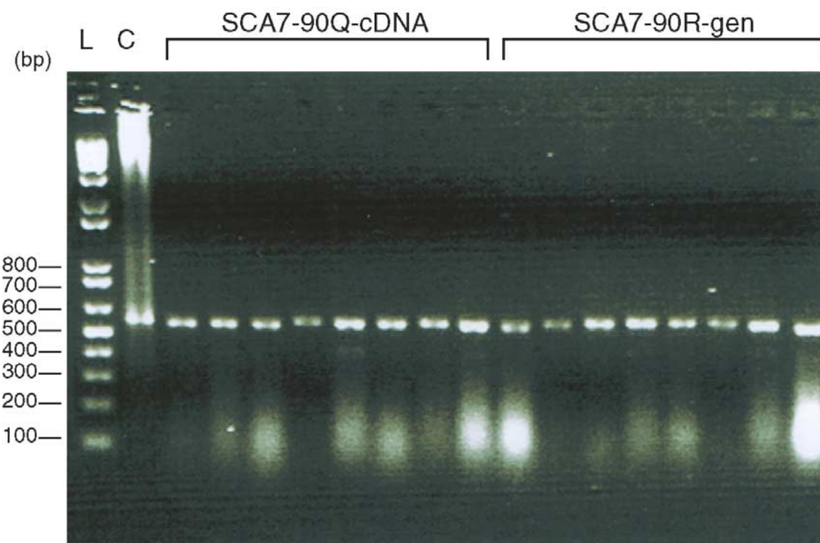

Fig. 1. CAG repeat expansions are stable in *Drosophila*. Repeat lengths were first screened by agarose gel electrophoresis. For each analysis, we PCR-amplified SCA7 CAG90 repeat-containing DNA fragments derived from pools of 20 adult flies using human ataxin-7-specific PCR primers. A typical agarose gel result is shown. Size marker is in the far-left lane (L), plasmid SCA7 cDNA is positive control (C; next lane); then results for 8 sets of *SCA7-90Q-cDNA* samples (next 8 lanes) and finally 8 sets of *SCA7-90R-gen* samples (right-most lanes). Capillary gel electrophoresis was also performed on each DNA pool, and yielded identically sized fragments in all cases (data not shown).

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