FAN1 Prevents CRISPR-Cas9 Nickase-Induced Contractions of CAG/CTG Repeats

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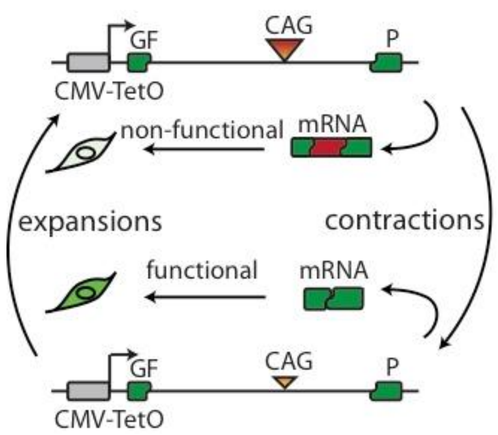
Abstract

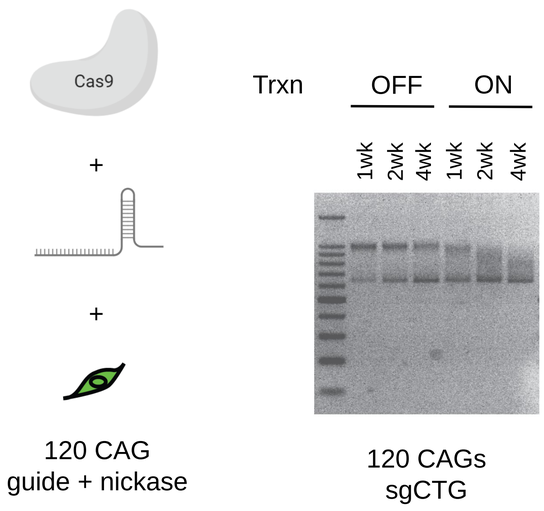
To date, CAG/CTG expansions at fifteen different loci cause neurodegenerative and neuromuscular disease, including Huntington's disease (HD). In HD, similarly to other expansion diseases, a longer expanded CAG tract causes a more severe phenotype and lowers the age at onset (AAO). Therefore, contracting expanded repeats to non-pathogenic lengths may provide a therapeutic solution. Our lab has developed a method to contract expanded repeats in human cell lines using a modified CRISPR-Cas9 nickase, although currently this mechanism of contraction is poorly defined[1]. Identification of modifiers of CRISPR-Cas9 nickase-induced contractions is important for both improving contraction efficiency and stratifying patients that would benefit from this therapy. Here we tested the hypothesis that a loss of Fanconi-associated protein 1 (FAN1), which has previously been identified as a modifier of HD AAO and somatic instability[2,3], impacts nickase-induced contractions.

GFP reporter assay for repeat instability



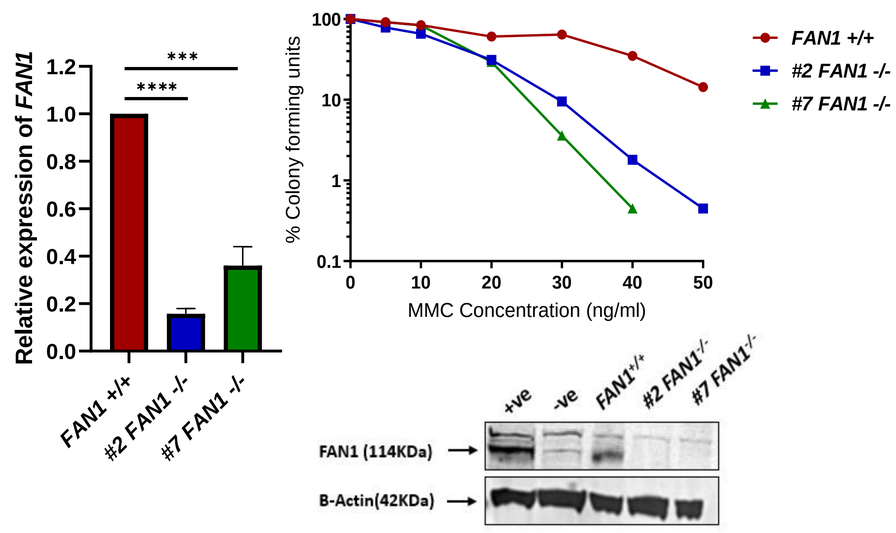
Changes in the expression of the GFP reporter is indicative of changes in repeat length.

Transcription is necessary to induce contraction events



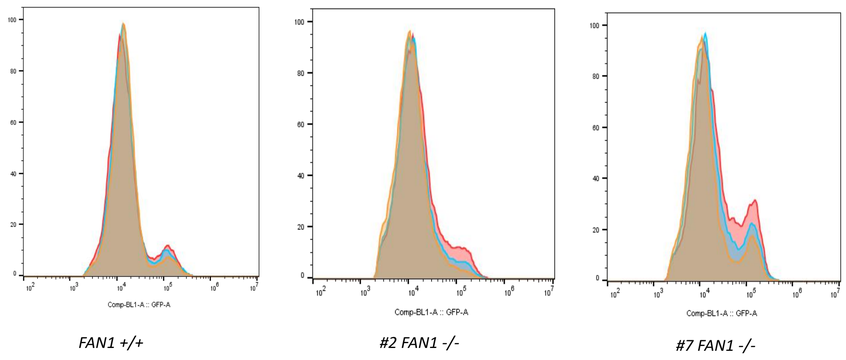
Generation of stable lines expressing both Cas9 D10A and sgCTG. Inducing transcription leads to contraction in these lines.

Generation of *FAN1* knockout lines



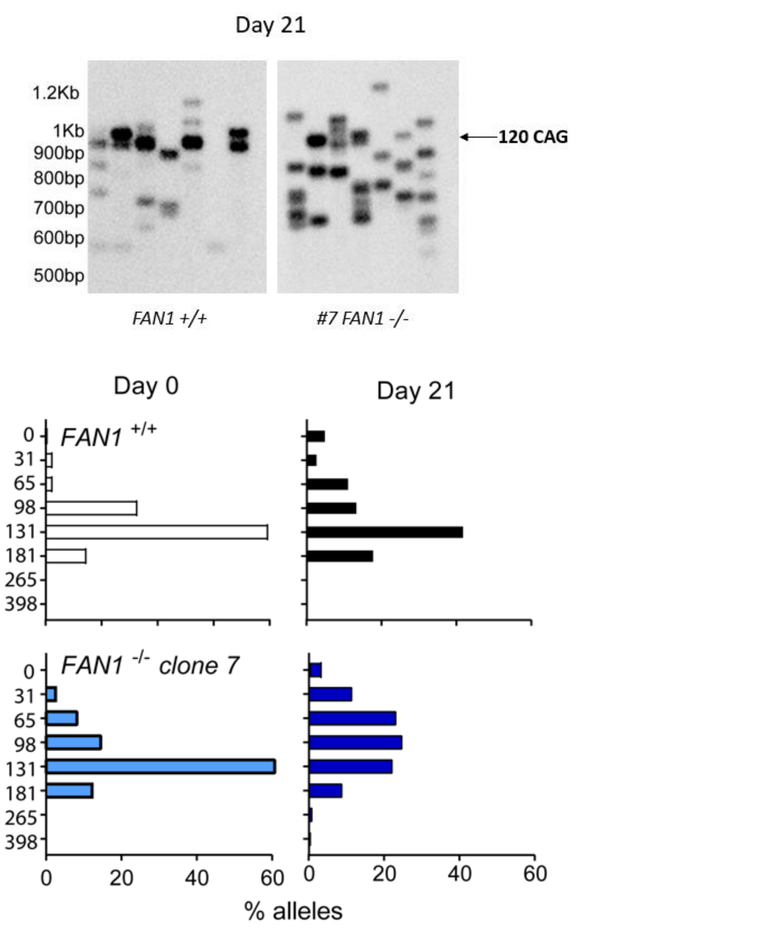
FAN1 knockout clones demonstrate reduced mRNA levels, an absence of detectable protein by Western Blot and are sensitive to crosslinking agent Mitomycin C.

FAN1-/- lines demonstrate increased GFP expression over 21 days



Histograms depict GFP expression which was monitored for 7 (orange) , 14 (blue) and 21 days (red) to observe how contraction rates change over time. In both FAN1 -/- clones there was a significant increase in GFP expression compared with wild type.

Small pool PCR confirms a loss of FAN1 increased contraction efficiency



Example small pool PCR image and allele frequency data demonstrates an increase in contractions of CAG repeats in FAN1 -/- line compared with WT.

Conclusions

- Contractions require transcription through the repeat tract
- We have generated two independent *FAN1* knockout clones in a system with stably integrated sgRNA and Cas9 nickase
- Both *FAN1* knockout clones show enhanced frequencies of nickase-induced contractions
- FAN1's impact on contractions occurs without impacting expansion events

References:

1. Cinesi, C., Aeschbach, L., Yang, B. & Dion, V. (2016) Contracting CAG/CTG repeats using the CRISPR-Cas9 nickase. *Nat. Commun.* **7** (1).
2. Gem-HD Consortium (2015). Identification of Genetic Factors that Modify Clinical Onset of Huntington's Disease. *Cell* **162** (3).
3. Gem-HD Consortium (2019). CAG Repeat Not Polyglutamine Length Determines Timing of Huntington's Disease Onset. *Cell* **178** (4).

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