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Spotlight

BREACHing new grounds in fragile X syndrome: Trinucleotide expansion linked to genome-wide heterochromatin domains and genome misfolding

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In a recent study in *Cell*, Malachowski et al.[1] show that the trinucleotide expansion in the *FMR1* gene underlying fragile X syndrome triggers formation of large heterochromatin domains across the genome, resulting in the repression of synaptic genes housed within these domains.

A series of neurological diseases, such as fragile X syndrome (FXS) or Huntington's disease, are caused by genetic alterations due to trinucleotide expansions, where short sequences of three bases within a gene are abnormally repeated tens to hundreds of times. In cases where the repeat expansion occurs within the coding sequence of the gene, an abnormal protein is made, which is then thought to cause the disease. However, repeat expansions also occur in non-coding regions and will, in this case, not affect the protein sequence. One example is FXS, which results in intellectual disability and is caused by a CGG expansion (>199 repeats) in the 5′ untranslated region of the *FMR1* gene, encoded on the X chromosome. The classic textbook model is that *FMR1* gene expression is silenced in FXS patients, leading to loss of the protein it encodes, FMRP, and downstream defects at the synapse and the nucleus due to FMRP loss. Due to difficulties in engineering repetitive sequences, previous work often relied on a *Fmr1*/FMRP knockout mouse to model FXS. By using human cells and tissue derived from FXS patients with the mutation-length CGG expansion, Malachowski et al.[1] discover a new mechanism that might also contribute to the disease and is independent of FMRP.

The study uses a series of induced pluripotent stem cell (iPSC) lines with variable repeat length, profiled by long-read sequencing. Central observations are also confirmed in postmortem brain samples from patients. Upon iPSC differentiation toward the neural lineage, extensive chromatin profiling reveals that FXS cell lines carry a 5Mb heterochromatin domain on the X chromosome containing multiple genes, including *FMR1* (Figure 1). As a consequence, genes in the domain are silenced, affecting not only *FRM1*, but also two neuronal genes, *SLITRK2* and *SLITRK4,* located > 2MB away from the *FMR1* locus, which might also contribute to the disease phenotypes. Surprisingly, the appearance of large heterochromatin domains is not restricted to the region surrounding of the disease locus but is observed throughout the genome across many different chromosomes. Histones in these domains are tri-methylated at lysine 9 on histone 3 (H3K9me3), and multiple genes involved in synaptic functions and epithelial integrity tend to be repressed. These genes could be explored for their link to the clinical presentations in FXS linked to brain and connective-tissue defects. In addition, binding of CTCF, a central organizer of higher-order chromatin folding, is lost, which results in dissolution of topologically associating domains (TADs) and long-range chromatin loops. Interestingly, the heterochromatin domains form strong inter-chromosomal interactions in *trans*. The authors name these domains BREACHes for "beacons of repeat expansion anchored by contacting heterochromatin." Importantly, "repair" of the repeat expansion (cut-back to a shorter premutation length) reverses the X chromosome BREACH and lowers H3K9me3 signal on multiple BREACHes on autosomes. Repeat cut-back also reverses gene silencing and *trans* contacts. This is an important finding since it suggests that at least some of the downstream consequences of the FXS repeat expansion could be reversible and therefore potentially amenable to therapeutic interventions.

The discovery of BREACHes highlights the role for heterochromatin in FXS, which has been suggested previously as a disease mechanism for repeat expansion disorders.[2] Although H3K9me3 is typically associated with constitutive heterochromatin, e.g., at centromeres or at transposable elements, large (facultative) H3K9me3 domains have been observed in other contexts. An interesting example are olfactory receptor (OR) clusters in olfactory neurons. Here most OR alleles are silenced by H3K9me3, allowing expression of only a single allele per cell, which assumes a euchromatic state.[3] H3K9me3 tethers OR genes to heterochromatic foci in the center of the nucleus, resulting in strong *trans* interactions. Although the *trans* contacts observed between BREACHes appear to be less frequent since they are spread throughout the nucleus when visualized by microscopy, similar mechanisms might underlie their formation. Through read-write feedback loops, H3K9me3 can spread along the genome and also re-establish H3K9me3 domains after cell division.[4] Such systems can exhibit bistability, which allows a genomic region to switch between a repressed or an active state in response to a transient signal or perturbation.[5] This property is thought to enable epigenetic memory to allow different cell types or states to maintain distinct transcriptional profiles. Interestingly, a subset of BREACHes found in iPSC lines differentiated toward the neural lineage carry H3K9me3 in lymphoblastoid B-cells from healthy donors (Figure 1, right). This suggests that BREACHes

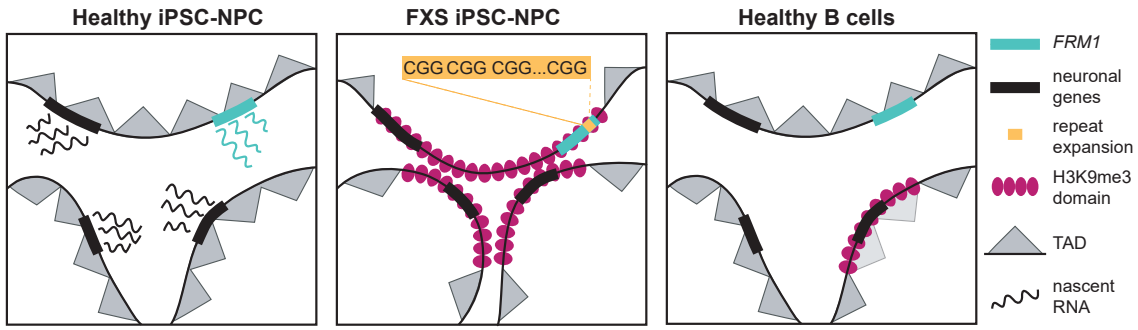

Figure 1. Heterochromain-hubs form in FXS iPSC-NPCs

The *FRM1* gene (blue) and other neuronal genes are expressed in iPSC-NPCs from healthy donors (left), but are silenced through large heterochromatin domains in FXS iPSC-NPCs, which carry a trinucleotide repeat expansion (yellow) within *FRM1* (middle). Heterochromatin domains, termed BREACHes, exhibit loss of TADs and increased contacts in *trans* (middle). Some of the FXS-specific heterochromatin domains are also found in B cells from healthy donors (right).

arise at regions which normally acquire H3K9me3 in other lineages to silence the contained (neuronal) genes. It appears as if an epigenetic memory system that has evolved for stable silencing of genes that should not be expressed in a given cell type has gone awry in FXS, leading to erroneous silencing of neural genes in the brain.

An exciting question that remains unanswered is how the repeat expansion in *FMR1* leads to the establishment or maintenance of BREACHes on autosomes. Given the observed *trans* contacts between the *FMR1* locus and autosomal BREACHes, one compelling hypothesis for future testing is that such physical proximity might facilitate the spreading of heterochromatin in *trans*. However, as *trans* contacts are not observed when BREACHes are absent, increased inter-chromosomal contacts might also be a consequence of heterochromatin formation.

Intriguingly, BREACHes can also be found beyond FXS in other perturbative and disease scenarios involving genome instability. Specifically, the authors find BREACHes in iPSCs created with knockdown or dominant-negative expression of p53, which is a known established cause of genome instability in iPSCs.[6] BREACHes are enriched for several forms of genome instability, including replication-stress-induced double-strand breaks and stepwise short expansions of other repetitive sequences. Moreover, the authors found that nearly all FXS iPSCs show reproducible silencing of genes linked to the p53-mediated DNA damage response. An intriguing open question for future exploration is the potential link between p53-mediated genome instability pathways and repeat expansion disorders.

Taken together, this recent study from the Phillips-Cremins lab proposes a new molecular mechanism underlying FXS that acts on the level of heterochromatin domain formation. The very detailed and comprehensive dissection of the newly discovered BREACHes now provides exciting avenues for future mechanistic and preclinical studies. Moreover, the work could open opportunities for identifying previously unrecognized disease mechanism in other contexts.

DECLARATION OF INTERESTS

The author declares no competing interests.

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