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EDC exposure in 3D genome memory in transgenerational obesity

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A new study reveals that exposure of pregnant mice to an endocrine disrupting chemical, bisphenol A, induces multi-transgenerational (up to six) inheritance of obesity in their descendants. This effect depends on CTCF-dependent chromatin reorganization in the sperm, a synergistic outcome of altered DNA methylation, RNA modification and long-range chromatin interactions.

Refers to Jung, Y. H. et al. Recruitment of CTCF to an *Fto* enhancer is responsible for transgenerational inheritance of BPA-induced obesity. *Proc. Natl Acad. Sci. USA* **119**, e2214988119 (2022).

Bisphenol A (BPA) is one of the most widespread and well-studied endocrine disrupting chemicals (EDCs) worldwide. Epidemiological evidence and animal studies have demonstrated that BPA can interfere with mammalian reproduction and induce transgenerational inheritance of various diseases, including metabolic disorders and obesity[1]. The molecular mechanisms of ancestral BPA-induced transgenerational effects are not thought to be mainly due to alterations of the primary DNA sequence. Instead, these effects are thought to involve epigenetic mechanisms, including DNA methylation, histone modifications and non-coding RNAs. However, how BPA-induced epigenetic changes escape multiple rounds of germline epigenetic reprogramming (for example, genome-wide DNA demethylation) to induce phenotypes across multiple generations was a mystery. In a recent paper, Jung et al.[2] present compelling evidence that ancestral BPA exposure in mice drives ectopic long-range chromatin rearrangements between the *Fto*, *Irx3* and *Irx5* gene loci, which are maintained for up to six generations in sperm and are responsible for BPA-induced transgenerational inheritance of obesity.

Among various known actions, BPA can bind to transcription factors, such as nuclear hormone receptors (including oestrogen receptors and the androgen receptor), which regulates their DNA binding and transcription activities[1]. Interestingly, oestrogen receptors and the androgen receptor are retained in mature mouse sperm[3], and their occupancy on DNA might interfere with nuclear events, such as DNA methylation[4], which provides a potential mechanism by which BPA exposure can alter DNA methylation in the sperm.

Moreover, evidence showed that mammalian gametes contain specific 3D genome interaction patterns that are mediated by CCCTC-binding factor (CTCF)[3]. CTCF is an architectural protein that enables long-range interactions between gene loci that are lineally located at a distance, creating physical interactions of regulatory DNA elements, such as an enhancer (DNA sequence that can be bound by proteins to increase transcription) and a promoter (upstream of a gene where transcription factors bind to initiate transcription), located kilobases or megabases apart (Fig. 1). Importantly, CTCF preferentially binds to unmethylated DNA sequences[2], which means the change of DNA methylation at one CTCF binding site might exert long-range regulation of gene expression through CTCF-mediated interactions.

Intriguingly, previous work by Jung et al.[3] showed that CTCF-dependent 3D genome interaction patterns in sperm and oocytes can be passed on to the zygote and early embryos in mice. This finding suggests that environment-induced changes in gametic 3D genome organization might persist in the embryo and thus influence fetal development.

In the current study, Jung et al.[2] exposed pregnant mice (F0) to BPA during embryonic days 7.5–13.5, a critical time window when primordial germ cells develop and during which genome-wide demethylation happens. The resulting F1 male and female mice appear normal in terms of their body weight, but the F2 offspring from F1 cross-mating start to show obesity. Continuous breeding showed that the obesity phenotype persists in the F3–F6 generations (with penetrance decline from F5) and that mice in the F7 generation return to a normal body weight. The authors also found that the BPA-induced transgenerational obesity is correlated with increased food consumption in mouse generations that show an obesity phenotype.

To explore the molecular mechanisms, the authors examined the CTCF binding sites and the chromatin accessibility of control sperm versus the ancestrally BPA-exposed sperm[2]. They found an ectopic CTCF binding site in the enhancer in the *Fto* locus from ancestrally BPA-exposed F3 sperm, but not in control sperm. This ectopic CTCF binding site can interact with the promoter of *Irx3* and/or *Irx5* from a distance, which might enhance their transcription via enhancer–promoter interactions (Fig. 1). As *Irx3* and *Irx5* are known to regulate the function of appetite-controlling neurons[2], their overexpression in the brain might explain the overeating phenotype and the resulting obesity. Moreover, the authors found that this ectopic CTCF binding site is hypomethylated in F3 sperm but remethylated in F6 sperm[2]. These data suggest a 'self-induction loop' model in which BPA-induced hypomethylation of the CTCF binding site triggers the CTCF-dependent ectopic interaction between *Fto* and *Irx3* and/or *Irx5* loci. This ectopic interaction might further prevent the loci being remethylated and thus maintain the ectopic interactions for multiple generations. Yet, when the binding site becomes remethylated in F6 sperm and the ectopic interactions no longer exist, the F7 mice do not develop an obesity phenotype.

In a final crucial experiment, the authors deleted the identified ectopic CTCF binding site in a mouse model, which prevented the ancestral BPA-induced transgenerational obesity[2]. This finding clearly demonstrated that the CTCF-dependent chromatin interaction is required for triggering and passing on the obesity phenotypes to the following generations.

The implications of the work are far-reaching, for understanding both obesity and transgenerational epigenetic inheritance. First, this study not only reiterates the causal importance of long-distance

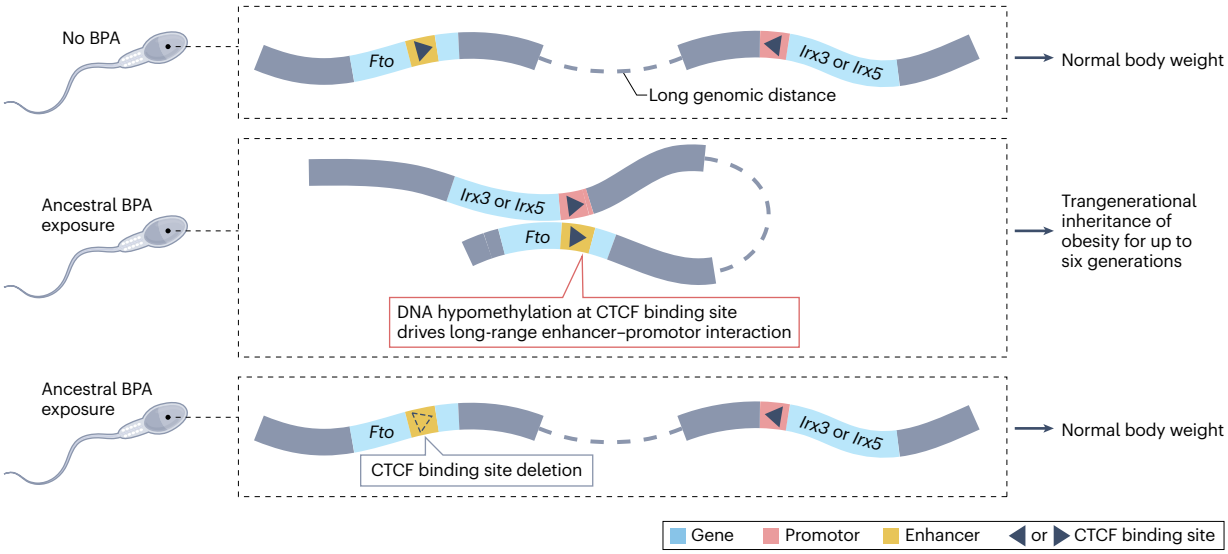

Fig. 1 | EDC-induced long-range control of gene expression based on ectopic CTCF binding. An illustrative model showing that DNA hypomethylation induced by endocrine disrupting chemicals at the CCCTC-binding factor (CTCF) binding site drives physical interactions between the enhancer sequence in *Fto* and the promoter sequence of *Irx3* or *Irx5*, increasing gene expression of *Irx3* and *Irx5* and causing obesity. Deletion of the CTCF binding site prevents the ectopic long-range interaction and prevents the obesity phenotype. BPA, bisphenol A.

chromatin interactions between *Fto*, *Irx3* and *Irx5* in obesity development[5], but also highlights that the trigger of such ectopic interactions does not necessarily require DNA sequence variation (such as single nucleotide polymorphisms) and could be due to environment-induced DNA methylation changes. Second, the study raises a new paradigm in understanding EDC-induced transgenerational effects from the perspective of 3D genome memory, providing a viable explanation for the long-standing question of why the exposure effect is most prominent at the window of primordial germ cell development during pregnancy. That is, it is probably because the global DNA demethylation during this time facilitated the stabilization of CTCF-based interactions between *Fto*, *Irx3* and *Irx5*. Finally, the study shows the power of using genetic mouse models to solidify the mechanisms of epigenetic inheritance, providing causal evidence for the correlative observations. Similarly, a knockout mouse model has been used in confirming sperm RNA-mediated epigenetic inheritance of paternal metabolic disorders, specifically regarding the involvement of small non-coding RNAs and RNA modifications[6].

However, several important questions remain unresolved. For example, how the overeating phenotype developed in the descendent mice was not addressed. The observations might suggest the maintenance of ectopic interactions between *Fto*, *Irx3* and *Irx5* throughout embryo development that penetrate specific appetite-controlling neurons, yet the specific cell lineages or cell types that preferentially maintain such ectopic interactions during development remain unknown, and the mechanisms await exploration.

Another puzzle concerns how the 'self-induction loop' of ectopic interactions between *Fto*, *Irx3* and *Irx5* in the sperm are gradually breached and how the F7 descendent mice do not develop obesity. This effect could involve stochastic events; however, part of the answer might be hidden in the function of FTO, an RNA demethylase with various RNA targets, including mRNAs, tRNAs and retrotransposon RNAs such as LINE1[7]. Indeed, Jung et al.[2] have identified an enhancer RNA with hypomethylated m^{6}A that correlates to a CTCF binding site in BPA-ancestrally exposed sperm, suggesting that levels and/or activity of FTO are increased. Interestingly, as m^{6}A RNAs in the nuclei direct DNA demethylation[8], FTO-mediated m^{6}A hypomethylation might be involved in facilitating DNA remethylation of CTCF binding sites to destabilize ectopic interactions between *Fto*, *Irx3* and *Irx5*. Moreover, altered sperm RNA modifications due to increased FTO activity can affect other RNA functionalities, including stability, subcellular compartmentalization and change of binding partners as part of the 'sperm RNA code'[9], which could affect the phenotype of offspring.

Finally, other EDCs[10], including products used to replace BPA (such as bisphenol S), can also induce adverse effects that persist for multiple generations. The molecular mechanisms presented by Jung et al.[2] might also apply to these EDCs, as many of these EDCs also bind to various transcription factors and their effects might accumulate with exposure to mixtures of EDCs.

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Competing interests

The authors declare no competing interests.