How enzyme complex DCC recognizes the X chromosome

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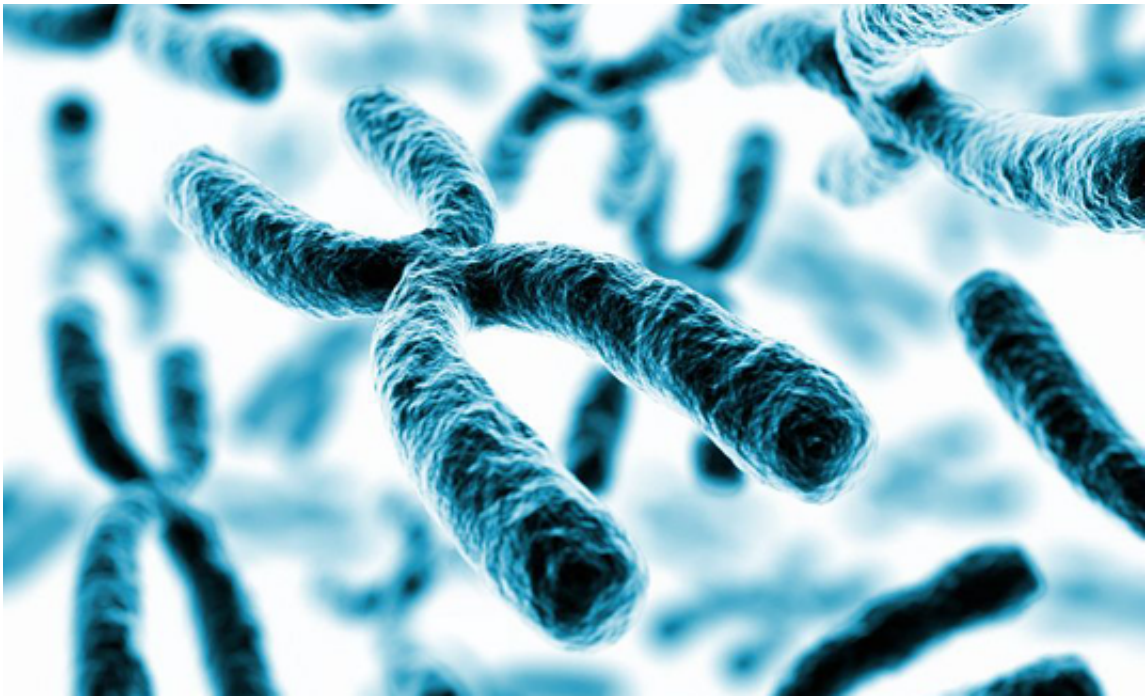

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In male cells of the fruit fly Drosophila, the X chromosome is twice as active as in female cells. Researchers at LMU Munich have now discovered how the enzyme responsible recognises the chromosome.

In many species, the sex chromosomes are unequally distributed: in humans as well as in the model organism Drosophila melanogaster male cells only possess one X chromosome, unlike female cells, which contain

two Xs. Male fruit flies compensate for this short-coming by doubling the activity of their single X chromosome. This vital process is controlled by the enzyme complex known as DCC (dosage compensation complex). "How this regulator distinguishes the X chromosome from all the other chromosomes has remained unsolved for a long time", says LMU biologist Professor Peter Becker from the Biomedical Center (BMC) at the LMU. Becker's team has now reported on an important conceptual and methodological breakthrough: the researchers demonstrate that a key role in the process is played by the fine detail of DNA shape. In addition, they have also identified the part of the enzyme complex that binds to the X chromosome. The insights gained from Drosophila are not only important for understanding the gene regulation in flies, but also illustrate fundamental mechanisms that affect all life forms in similar ways. The scientists have reported their results in the prestigious journal *Nature*.

Some 300 binding sites for the DCC enzyme complex to the X chromosome are known to date. From their DNA sequences, researchers have calculated the recognition sequence (known as the consensus sequence), in which each position is occupied by the particular DNA building block, which occurs most frequently in comparison with all binding sites. "The problem is that the consensus sequence signature that can be robustly identified at most DCC binding sites is also present some thousands of times on all other chromosomes", states Becker. "For this reason, we have previously been unable to predict whether a particular DNA sequence is actually a functional DCC binding site or not."

A novel strategy Becker describes as 'genome-wide biochemical analysis' has now provided a major step forward. The researchers were able to demonstrate that one specific building block from the DCC regulator – the MSL2 protein – is sufficient to reliably bind the consensus sequence. Furthermore, the MSL2 protein actually possesses two DNA binding domains, of which one binds to a DNA sequence, which extends the

previously known consensus sequence. "We called this new signature 'PionX', because it turns out that these binding sites represent the first DCC contact points to the X chromosome. There are, however, some 2,700 sequences in the fly genome that resemble the PionX signature a lot, of which only 57 function as genuine MSL2 binding sites", relates Becker.

"The decisive breakthrough was achieved by BMC bioinformaticians, first and foremost Tobias Straub, who calculated how the sequence of the base pairs affected the intricate structure of the DNA, also known as 'DNA shape'", states Becker. The researchers identified a particular shape shared by PionX sequences that is preferably recognised by the MSL2 protein. This structure makes the vital difference: it distinguishes the binding sites on the X chromosome from all others, enabling a selective interaction and regulation by the dosage compensation complex. "Our work has decisively advanced the understanding of chromosome-wide regulation during the process of X chromosome dosage compensation", states Becker. "However, our current progress only explains part of the X chromosomal recognition in vivo and we still have to improve our ability to distinguish correct DCC binding sites from 'false-positive' and false-negative' sites identified by our algorithm." In the future, the researchers intend to further refine the genome-wide biochemical analysis strategy, in order to better understand the recognition of the X chromosome by the DCC.

More information: Raffaella Villa et al. PionX sites mark the X chromosome for dosage compensation, *Nature* (2016). DOI: 10.1038/nature19338

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