The Face of Chromatin Variants

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Summary

A developmental programme affecting human face shape is shown by Greenberg et al. (2019) to hinge on the ability to distinguish a single methyl group between two histone variant isoforms and the action of the chromatin remodelling enzyme SRCAP. This challenges researchers to link atomic structure to a morphological defect.

Body

Jacques Monod quipped that "anything that is true of *Escherichia coli* must be true of elephants, only more so" to illustrate the universality of biological principles (Friedmann, 2004). Even ardent reductionists will be impressed by the work of Wysocka and colleagues in this issue of Cell which shows the shape of human faces can hinge on a methyl group distinguishing serine from threonine in two histone variant isoforms.

Floating-Harbor Syndrome (FHS) is a rare human genetic disorder resulting in anatomical development deficiencies, including characteristic craniofacial abnormalities leading to triangular face shapes. While previously exome sequencing identified the causative mutations as heterozygous truncations of the chromatin remodeller SNF2-Related CREBBP Activator Protein (SRCAP), how this deficiency resulted in the phenotypes of the disorder was unclear. (Hood et al., 2012). The mutations delete a C-terminal region of the protein containing AT hook domains, and now Greenberg et al., 2019 show that the primary consequence of the deletion is actually loss of nuclear localisation signals which overlap the AT hooks. This causes truncated SRCAP to remain in the cytoplasm of a variety of human cell lines including the relevant cranial neural crest cells (CNCCs).

The reduced abundance of nuclear SRCAP is correlated with changes in gene expression in CNCCs, including genes involved in cell migration and morphogenesis. Making the same SRCAP deletion in Xenopus leads to delays in neural crest migration. This causes craniofacial phenotypes in the frogs that resemble the face shapes observed in human FHS patients.

SRCAP is known to introduce H2A.Z into chromatin (Ruhl et al., 2006) and this histone variant affects gene regulation (Giaimo et al., 2019). H2A.Z is encoded by two isoforms in most vertebrates, and the human isoforms differ at only 3 amino acid positions. Surprisingly, knockdown of only the H2A.Z.2 isoform leads to the craniofacial phenotype in frogs. Furthermore, over-expression of H2A.Z.2, but not H2A.Z.1, can partially rescue the phenotype of SRCAP truncation. This implies that the FHS phenotypes result from insufficient H2A.Z.2 incorporation in chromatin due to reduced SRCAP abundance.

The two variants are found to be present at many genomic locations, but the minor H2A.Z.2 isoform is enriched at a subset of enhancers. Consistent with this distribution of H2A.Z isoforms promoting expression, these enhancers are adjacent to genes down-regulated in the SRCAP truncation mutants found in FHS. These enhancers also have elevated A/T nucleotide compositions, nicely implicating a possible targeting by the AT hook domains in functional SRCAP.

Finally, individual mutation of the 3 amino acid differences between the H2A.Z isoforms reveals that exchanging the serine at position 38 in H2A.Z.1 to the threonine in H2A.Z.2 enables H2A.Z.1 Ser38Thr to partially rescue SRCAP truncation mutants. This implies that the additional methyl group of the threonine sidechain is responsible for the distinctive contribution by H2A.Z.2 to normal face development.

Overall this is a stunning piece of work which leverages developmental observations using a Xenopus model system and genomic analysis using disease-relevant differentiated CNCCs. It provides a very satisfying explanation for how specificity and efficiency in incorporation of a histone variant isoform can drive a gene expression programme with influences at an anatomical scale, and adds an additional dimension to the identification of facial shape determining genes.

The work also provides a textbook example of two important challenges in chromatin biochemistry.

Firstly, the Snf2 family of molecular motors that monopolise chromatin remodelling have undertaken a remarkable expansion in eukaryotic evolution (Flaus et al., 2006). The minimalist fungal genome of *Encephalitozoon cuniculi* encodes only 6 Snf2 family proteins, yet the human genome has 32 members. SRCAP belongs to the Swr1 subfamily, along with a close paralog EP400 encoding the p400 protein. The duplication resulting in the SRCAP and EP400 pair appears to have occurred in the vertebrate lineage, and the authors point out that it parallels a duplication yielding the two isoforms H2A.Z.1 and H2A.Z.2.

This is a tidy example of subfunctionalisation to enrich regulatory potential, potentially arising from vertebrate whole genome duplications. It is reminiscent both of the radiation of the Chd subfamily that underlies the linkage of human CHD7 mutation with CHARGE syndrome, and the archetypal roles for the human SMARCA4 and SMARCA2 genes in cancer and neurological disorders respectively (Kadoch and Crabtree, 2015). There may be more such examples to be teased out by studying rare genetic diseases and Snf2 family phylogenomics.

More broadly, the ability of the Snf2 family ATPase motor to be duplicated and co-opted for new and specific roles may be one of the features that allowed eukaryotic chromatin to provide a rich “epigenetic” regulatory layer in humans (and elephants) which overlays the basal genetic encoding of regulatory circuits we share with *E. coli*.

Secondly, the identification of H2A.Z.2 at A/T rich enhancers as the fulcrum of the gene regulatory circuit being misregulated in FHS reminds us of the difficulty in providing a full mechanistic explanation for chromatin regulation.

The change at Thr38 affects the structure of this region of the nucleosome and its dynamics in cells as assessed by photobleaching (Horikoshi et al., 2013)(see figure). It remains to be determined whether this is a direct effect or an alteration to the ability of nucleosome binding factors to engage with nucleosomes bearing this H2A.Z.2-specific amino acid change.

Teasing out an explanation will be greatly aided by structural analysis exemplified by studies of yeast Swr1 activity on nucleosomes (Willhoft et al., 2018). Compared to yeast Swr1, human SRCAP and p400 both contain large but non-identical proline/threonine/serine-rich insertions within the Snf2 ATPase region that are likely to extend over the nucleosome surface.

That small changes to histone proteins should have disease phenotypes in humans is not without precedent. Histones are amongst the most highly conserved genes in nature, and point mutations at various sites within histone genes can promote cancer (Nacev et al., 2019). The constraints on histone evolution likely arise from multiple functions in genome packaging and as the substrate through which genes are controlled. Understanding how subtle alterations to histone signalling direct specific developmental changes is none-the-less a substantial and ongoing challenge.

Figure 1 - The histone isoform change associated with Floating-Harbor syndrome.

H2A.Z position 38 (yellow) with serine in H2A.Z.1 differs from threonine in H2A.Z.2 by a single methyl group (grey), resulting in rearrangements of L1 loop (white) that packs against DNA (red) based on PDB structures 3WA9 and 3WAA (Horikoshi et al., 2013). Reduced H2A.Z.2 levels at specific cell migration and morphogenesis enhancers leads to craniofacial abnormalities characteristic of FHS (Hood et al., 2012).

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