Poised for development

Lauren A Choate & Charles G Danko

A new study tracks the distribution of bivalent H3K4me3/H3K27me3 chromatin in male germ cells of six vertebrate species. The results have big implications for understanding the mechanisms that specify animal development.

Chromatin state has a central role in gene regulation, with specific histone modifications marking the promoters of transcriptionally activated or repressed genes. A bivalent chromatin state occurs when activating and repressing histone modifications—specifically, trimethylation of histone H3 at lysine 4 (H3K4me3; activating) or lysine 27 (H3K27me3; repressing)—mark the same nucleosome near the promoter of a transcriptionally repressed gene[1]. These bivalent histone modifications are hypothesized to 'poise' silenced genes for rapid activation, wherein the bivalent H3K4me3$^+$H3K27me3$^+$ chromatin state shifts to an active H3K4me3$^+$H3K27me3$^-$ state[2]. Poising occurs in germ cells at genes that are silenced during gamete development and become activated as embryogenesis begins[3]. Thus, the chromatin state in the germ line is hypothesized to poise important genes for activation at specific stages of embryonic development, providing an epigenetic layer of control over somatic tissue patterning. On page 888 of this issue, David Page and colleagues[4] systematically identify H3K4me3$^+$H3K27me3$^+$ poised genes in six vertebrate species during two distinct stages of male germ cell development—prophase of meiosis I (pachytene spermatocytes) and after the completion of meiosis (round spermatids). Overall, they find that, whereas poised genes are often deeply conserved during animal evolution, lineage-specific poising correlates with evolutionary innovations.

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Poised genes as ancient regulators

Genes classified as poised in five mammalian species, termed 'core poised' genes, were enriched for conserved developmental regulators that are responsible for initiating the differentiation of specific organ systems (**Fig. 1**). As evidence for this idea, the authors found that core poised genes were expressed during early stages of mouse embryogenesis more often than expected. Core poised genes frequently encoded homeodomain transcription factors that have central roles in development and body patterning[5]. Moreover, the promoters of core poised genes were more highly conserved at the DNA sequence level than those with conserved retention of the H3K27me3 silencing mark, suggesting that core poised genes represent an evolutionarily conserved mechanism for specifying the development of somatic tissues in an embryo.

Additional experiments in a non-mammalian outgroup, chicken, and data from *Drosophila melanogaster*[6] point toward ancient conservation of germline poising in animal species. Mammalian core poised genes that encode transcription factors had orthologs in chicken that were more likely to be poised than genes having other biological functions. This observation suggests that poising of transcriptional regulators that initiate somatic tissue differentiation is at least as old as the amniote common ancestor. A comparison of core poised genes to their *Drosophila* orthologs showed an enrichment for binding of the repressive chromatin modifier Polycomb, which is responsible for depositing the H3K27me3 mark[7], suggesting that the repressive half of poising may have already been present at some amniote poised genes before the emergence of the bilaterian ancestor. Taken together, the analyses by Lesch *et al.* demonstrate that a poised chromatin state

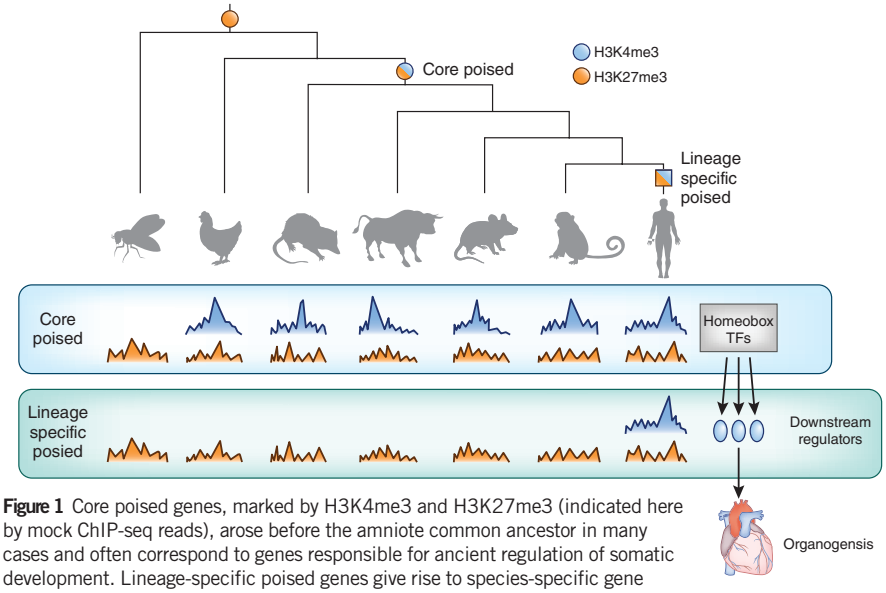

Figure 1 Core poised genes, marked by H3K4me3 and H3K27me3 (indicated here by mock ChIP-seq reads), arose before the amniote common ancestor in many cases and often correspond to genes responsible for ancient regulation of somatic development. Lineage-specific poised genes give rise to species-specific gene expression and are, in some cases, correlated with new traits. TFs, transcription factors.

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arose in germline cells early during animal evolution and has become a central feature of genes that specify somatic tissue development[4].

Poising changes correlate with new traits

As poising marks genes that will undergo transcriptional changes between germ cells and embryos[3], evolutionary changes in poising may affect organism development. Intriguingly, analyses by Lesch *et al.* suggest that lineage-specific changes in poised marks may contribute to changes in gene expression and, ultimately, to the acquisition of new traits. Genes that were poised in only one species, termed 'differentially poised', correlated with differences in gene expression during embryonic development. *ZSWIM4*, for instance, was uniquely poised in the human male germ line, and its transcript is uniquely expressed in the placenta of humans but not mice or cows[8]. Similarly, Lesch *et al.* report examples in which a single-species loss of poising correlates with changes in transcript abundance. Additionally, chicken-specific loss of poising was further correlated with trait differences between mammals and birds in at least five cases. *TPBG* is one example of such a chicken-specific loss in poising that is consistent with *TPBG* expression in a mammalian-specific developmental structure, the trophectoderm[9]. Therefore, species-specific changes in poised chromatin state correlate with changes in transcript abundance and may ultimately contribute to phenotypic differences between species.

Species-specific differences in gene poising were also accompanied by differences in regulatory DNA sequences. Lesch *et al.* identified transcription factor binding motifs enriched in the promoters of species-specific poised genes in the five mammalian species. Interestingly, in some cases, these motifs were canonical DNA-binding sequences recognized by transcription factors encoded by core poised genes. Taken together, these results suggest that species-specific changes in poising may facilitate the activation of genes that are immediately downstream of core poised transcription factors in developmental gene regulatory networks (**Fig. 1**). These interactions might fine-tune species-specific traits while maintaining deeply conserved gene expression programs across animal species that specify particular tissues.

Overall, the data and analyses presented by Lesch *et al.* suggest that core poised genes initiate evolutionarily conserved gene regulatory programs that direct somatic organ patterning in embryos. Likewise, changes in the repertoire of poised genes may establish morphological differences between species. How ancient of an evolutionary innovation is the poised chromatin state? Although additional H3K4me3 and H3K27me3 ChIP-seq experiments must be performed in more distantly related species, especially non-amniotes, the analyses by Lesch *et al.* provide the first evidence that a poised chromatin state may be much more ancient than the common amniote ancestor—with a tantalizing possibility that, given its central role in development, poised chromatin may represent a key innovation during the evolution of animals.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Decoding germline *de novo* point mutations

Anne Goriely

Analysis of a large whole-genome sequencing data set of 36,441 high-quality *de novo* mutations (DNMs) that arose in 816 family trios provides an unprecedented view into the landscape of DNMs in the germ line. This work both refines and challenges some of the views previously held on the nature and origin of DNMs.

Mutations are the source of all sequence variation in the human genome. Considering how important they are to species evolution and disease, it is surprising how little is known about the mechanisms controlling the occurrence and molecular spectrum of mutations, or even the rate at which they are introduced in the genome. A study of the largest set of germline *de novo* point mutations available so far, reported by Christian Gilissen, John Niederhuber and colleagues[1] in this issue, makes some strides toward bridging this gap in knowledge.

We are all mutants

With the advent of next-generation sequencing, it has become possible to directly measure the intergenerational mutation rate across the human genome. In 2012, a landmark study by Kong *et al.*[2] presented whole-genome sequencing data for 78 family (father–mother–child) trios that indicated that each newborn carries 30–100 (average of 60) sequence alterations, also known as DNMs, that are not present in the DNA of their parents. Because DNMs arise in the parental germ line (sperm or egg) before fertilization, their occurrence is intimately linked to the biology of germ cells. Whereas, in females, all eggs are produced during fetal development and do not divide after birth, sperm are continuously produced over a man's reproductive lifetime. Because the number of stem cell divisions increases as men age, it was postulated that DNMs must occur as a result of DNA replication errors that progressively accumulate in aging males[3] (**Fig. 1**). This was precisely what the study by Kong *et al.*[2] described: the total number of DNMs in a child strongly correlated with the age of the father at conception, increasing by ~1 or 2 DNMs for every additional year of paternal age, whereas mothers contributed a small fraction (~10 DNMs), regardless of their age[2]. As similar results have been obtained by several other studies[4,5], the consensus with regard to germline DNMs—and therefore to sporadic disease risk—is that the main culprit is the error-prone process of DNA replication cycles associated with advanced paternal age.

Sexing DNMs

The strength of the study by Goldmann *et al.* is undoubtedly its size, as these authors report the analysis of whole-genome data from 816 family trios, making this data set ten times larger than the study by Kong *et al.*[2]. In total, 36,441 DNMs were identified, corresponding to an average of ~45 mutations per child (a rate lower than that previously described

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