PERSPECTIVE

Anatomic Demarcation of Cells: Genes to Patterns

Howard Y. Chang

An organizing principle of the diverse cell types in multicellular organisms is their anatomic location. In turn, anatomic location is patterned by the positional identities of cells along developmental axes. Recent progress in functional genomics and chromatin biology illustrates how cells use specific gene expression programs to encode location. Dynamic chromatin states of key genes, notably the *Hox* loci, serve as the internal representation in cells of their positional identity within the animal.

The same genetic blueprint gives rise to thousands of cell types that make up the human body, and these different cell types must be correctly arranged in spatial patterns to make functioning tissues and organs. In continuously regenerating tissues, the genome faces an additional challenge of ensuring the faithful transmission of information throughout a lifetime—in humans, over decades. An important organizing principle, based on the anatomic location of cells in the animal, guides many cell fate choices and is set in place during embryonic development (*1*). Thus, the key question is how cells in multicellular organisms "know" where they are located in the body. Recent studies have provided insight into how mammalian cells represent, remember, and act upon their positional identities.

Gene Expression and Positional Identity

Cellular identities are organized on the basis of position along developmental axes, for instance along the anterior-posterior (head to tail) axis or proximal-distal axis (close or far away from the trunk along limbs or other appendages) (Fig. 1A). Homeotic genes (*Hox* genes, encoding transcription factors) are expressed in a nested pattern along these developmental axes. *Hox* genes are master regulators of body morphogenesis in development and evolution, and mutations in *Hox* genes transform one body segment into another (*2*, *3*). But fundamental questions still remain. If a finger is defined by a combination of *Hox* genes that indicates anterior and distal positional identity, do those *Hox* genes together turn on one or more unique "finger" genes, or alternatively, does the combination of anterior and distal *Hox* target genes together shape a finger?

This conundrum started to be resolved with the advent of full-genome sequences and the technology to interrogate gene expression patterns genome-wide. Comparison of global gene expression patterns of cell types thought to be homogenous, but derived from different anatomic sites—including fibroblasts, endothelial cells, smooth muscle cells, fat cells, and bone—showed that each of these cell types retained extensive and stereotypic differences in their gene expression patterns based on their anatomic sites of origin (*4*–*8*). Many of these differences were retained with ex vivo passage, which suggested a "transcriptional memory" of positional identities. Specifically, cells from each anatomic site had a unique combination of gene expression patterns that reflected the site's location along three developmental axes—anterior-posterior, proximal-distal, and cutaneous versus internal organs (*9*). Like a map based on longitudes and latitudes, cells reflect their anatomic origin by a digital combination of gene activities based on positional identity of cardinal axes from development. Indeed, position-specific gene expression programs are directly controlled by Hox proteins, which mediate anatomic-specific inductive effects (*10*).

Encoding Positional Identity: The Genome as a GPS Device

In a way, the genome functions as a global positioning system (GPS) device. Because ongoing *Hox* activity in adult cells is required for proper expression of genes denoting positional identity (*10*), a key challenge of cellular positional identity is keeping the right *Hox* genes turned on and off. In mammals, 39 *Hox* genes are clustered on four chromosomal loci, and their order along the chromosomes is roughly correlated with their spatial pattern of expression along the anterior-posterior and proximal-distal axes, a property termed colinearity (*11*). At the most fundamental level, positional identity is encoded in cells by the biochemical state of chromatin, the DNA-protein complex where *Hox* genes reside (*12*–*14*).

Eukaryotic DNA is wrapped around histone proteins, and this DNA-protein complex is subject to a large array of chemical modifications and protein interactions, which are believed to dictate whether the chromatin is accessible to gene activation or condensed for gene silencing (*15*). Comparison of chromatin states of cells from distinct anatomic sites has revealed salient features of how the chromatin states of *Hox* loci reflect the cells' positional identities (Fig. 1B) (*12*–*14*). Notably, in differentiated cells, the chromatin of the *Hox* loci can be programmed in either the ON or OFF state that extends across multiple *Hox* genes and intervening regulatory sequences, with the particular sequences in the ON or OFF state dependent on the positional identity of the cell along developmental axes. Extending our analogy of the map, each *Hox* locus can be considered a cardinal axis, like a longitude or latitude, and the chromatin state is the cellular mechanism for marking where on each axis the cell is located (Fig. 1B).

The ON state of the *Hox* loci is characterized by extensive occupancy of RNA polymerase II, intergenic transcription of long noncoding RNAs, and interaction with the trithorax group of proteins, which catalyze trimethylation of histone H3 on lysine 4. The OFF state is characterized by interaction with the Polycomb group of proteins, trimethylation of histone H3 on lysine 27 (H3K27me3), and also DNA methylation. Long noncoding RNAs, transcribed from within and outside of the *Hox* loci, also regulate chromatin states and gene expression by interfacing with trithorax and Polycomb complexes (*13*, *16*, *17*). Further, active *Hox* genes loop out from the remainder of the condensed chromosome territories in some tissues, which implies that the chromatin state of *Hox* genes can be reflected by—but does not strictly require—higher-order reorganization of the chromosomes (*18*). These chromatin states and corresponding *Hox* expression patterns of cells create a stable, cell-intrinsic memory of their positional identities. Chromatin patterns of tissues in vivo and of isolated cells ex vivo appear quite similar (*13*, *14*), even after extensive ex vivo passage (*10*), and *Hox* expression patterns are not altered by heterotypic cell-cell interactions (*10*). Nonetheless, trithorax, Polycomb, and long noncoding RNAs are continually required to reinforce positional identity (*13, 19*). A recent finding that Polycomb proteins remain bound to DNA even during DNA replication provides another possible explanation for the persistence of the transcriptional memory of positional identity (*20*).

How did these chromatin states representing positional identities arise? It is known that enhancers, regulatory elements that stimulate gene expression, are located at both ends of the *Hox* loci. When pieces of the *Hox* loci were either deleted or inverted, the distance between the enhancers and each specific *Hox* gene was critical for the timing and spatial pattern of *Hox* expression (*21*, *22*). For instance, reducing the distance between a 3′ enhancer and a *HoxD* gene typically led to premature and more proximal expression of that *HoxD* gene. Surprisingly, the establishment of *Hox* chromatin states follows a more complex strategy. Inversion of a portion of the *HoxD* locus—moving several genes millions of bases away from their cognate enhancer—modestly

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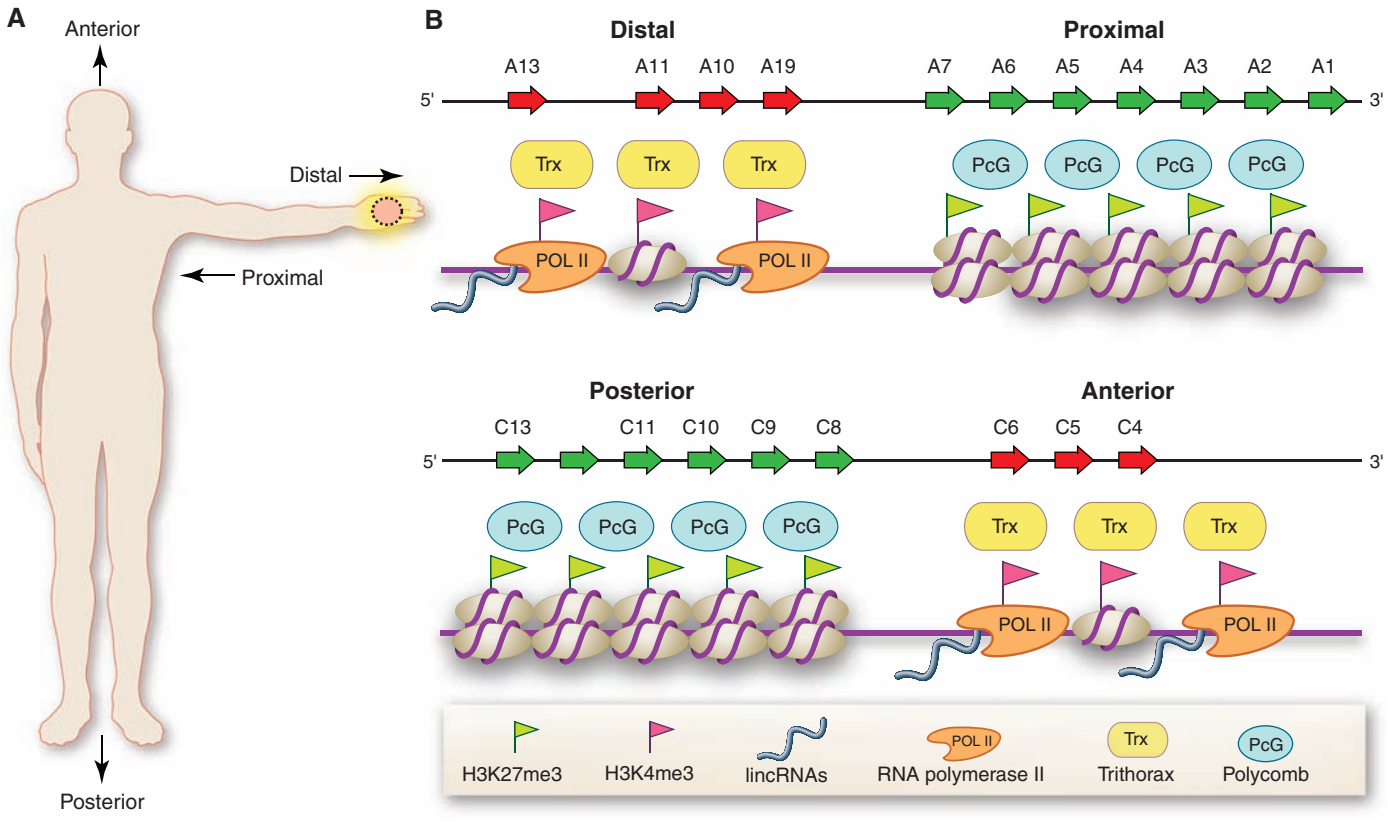

Fig. 1. The genome as a GPS device. (**A**) Developmental axes relative to human anatomy. The proximal-distal axis applies to all appendages such as upper limbs, lower limbs, and external genitalia. For instance, cells from the finger have anterior and distal positional identities. (**B**) Encoding positional identities in chromatin. Domains of open chromatin progress from 3′ to 5′ on all four of human *HOX* loci according to anterior-posterior position, but in different tissues. *HOXC* locus is strongly reflective of anterior-posterior identity in skin fibroblasts; whereas *HOXB* locus is active in cells from internal organs. Chromatin state on *HOXA* and *HOXD* loci demarcate proximal-distal identity. Thus, for cells in the finger, their positional identity is encoded by distal identity, which is reflected by active 5′ *HOXA* genes, and anterior identity is reflected by active 3′ *HOXC* genes. Active genes are shown in red; silent genes shown in green. Abbreviations: lincRNAs, long noncoding RNAs; H3K4me3, histone H3 lysine 4 trimethylation; H3K27me3, histone H3 lysine 27 trimethylation; Trx, trithorax group proteins; PcG, Polycomb group proteins.

affected chromatin marks associated with transcriptional activation in parallel with altered gene expression near the inversion breakpoint, but notably, had little effect on the ground state of the locus, characterized by broad occupancy of H3K27me3 (*14*). These and other findings imply the existence of parallel regulatory systems for encoding the transcriptional and chromatin states of *Hox* genes in relation to positional identity (*23*). The existence of multiple layers of regulatory sequences and extensive chromatin domains encompassing multiple *Hox* genes are likely to be driving forces for *Hox* genes to stay together and become more compact during vertebrate evolution (*24*).

Implications of Positional Memory

These insights into the cellular encoding of positional identity also point to gaps in our knowledge. How do cells of different positional identities sense each other and allocate cell fates to achieve proper proportion? This emergent property of large groups of cells certainly occurs in development (*25*) and implies a plasticity of positional identities and signaling mechanisms to link cell-cell interactions to changes in chromatin state. Plasticity of positional identity may be particularly important in wound repair when cells need to integrate their positional identity with the existing identities in the wound bed for proper regeneration (*26*). Cancer cells may also commandeer mechanisms involved in positional memory for their progression (*27*). Ultimately, these challenges need to be traced to understand how regulatory sequences specifically program chromatin states in the *Hox* loci and other master regulators of cell fate.

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I thank J. Helms and A. Oro for discussion and critiques. Supported by California Institute for Regenerative Medicine. H.Y.C. is an Early Career Scientist of the Howard Hughes Medical Institute.

10.1126/science.1175686

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Science **326**, 1206 (2009);
DOI: 10.1126/science.1175686