A Bad Case of Acne

Problem-Based Learning

Class of 2019

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Case contributed by:
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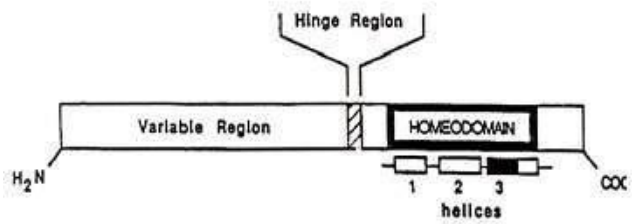
APPENDIX B

1) A primer on homeobox genes and accutane:

Accutane is 13-cis-retinoic acid which is a transcription factors that activates many of the same receptors as retinoic acid (RA). RA is a morphogen. (A morphogen is a substance that forms a continuous concentration gradient; there are different cellular responses, via receptors, reflecting specific thresholds to the morphogen.) An important group of genes for which RA is a transcription factor are the HOX genes.

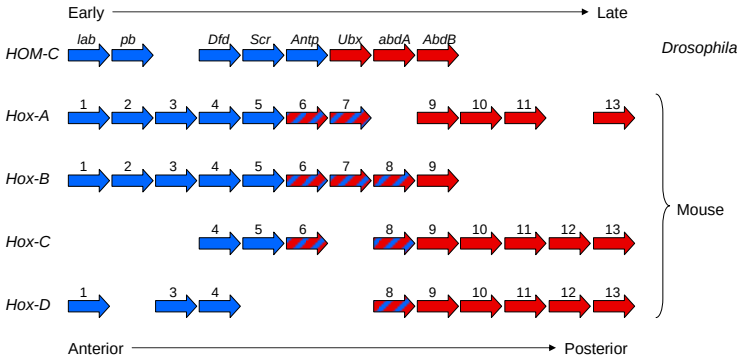


Homeobox genes code for proteins that bind DNA. The proteins are composed of a variable region, which can differ greatly in its amino acid sequence from one species to another, and a region called the homeodomain (coded for by the homeobox in chromosomes) that chemically binds to specific sequences of DNA and is very similar (evolutionarily conserved) in all multicelled animals. The homeodomains of these proteins consist of 60 amino acids and are composed of three segments (helices) of which helix 3 binds to the major groove of the DNA of chromosomes. Because most homeodomains are very similar in this segment, all bind to similar sites in DNA.

(From DeRobertis notes 2003)

Homeobox genes are conserved across species, but there have been duplications of the clusters across evolution; whereas Drosophila had one cluster of 9 genes, vertebrates have 4 clusters (HOXA, HOXB, HOXC and HOXD) each containing a set of paralogous genes. Each cluster lies on a different chromosome arranged in the same order as in Drosophila, but not all of the genes are present on all of the chromosomes. Mammals have a total of 38 HOX genes arranged in a 5' to 3' sequence in each cluster with the highest numbered gene (13) at the 5' end. Paralogous genes on each cluster have the same number (1 – 13) but no one cluster has all 13 genes.

***Hox* Genes**



Homologues of *Drosophila HOM-C*

Four partial clusters – *HoxA-2* and *HoxB-2* are ***paralogues***

Expressed from 3' (anterior) to 5' (posterior)

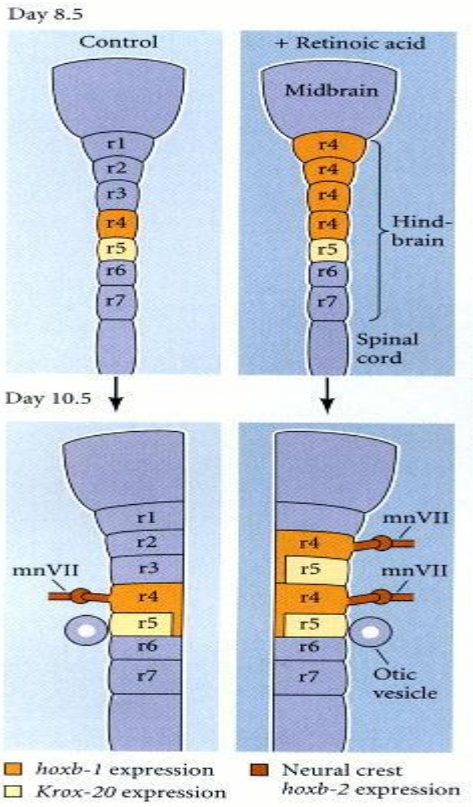
During development, the genes are expressed sequentially, starting with the 3'-most genes. The earlier the gene expression (e.g., the more 3' the gene location) the more rostral is the body segment that it controls. HOX gene expression is most like determined by some common transcription factors, such as retinoic acid (RA, discussed

below), in addition to local factors. Expression of each gene also affects the subsequent expression of neighboring genes. The sensitivity to RA differs according to the 5' – 3' position of the HOX gene with the HOX1 paralogues being the most sensitive and HOX 13 least.

The four clusters of HOX genes in vertebrates are expressed in the rhombomeres and spinal cord in overlapping patterns. Individual rhombomeres, and their neural crest cells, express unique combinations of HOX genes, providing a genetic code that directs their differentiation and ultimate fate. The HOX genes actually are master transcription factors that dictate segment identity by regulating target genes (which can also be transcription factors); some of these have so far been identified ((e.g. the TGF -family).

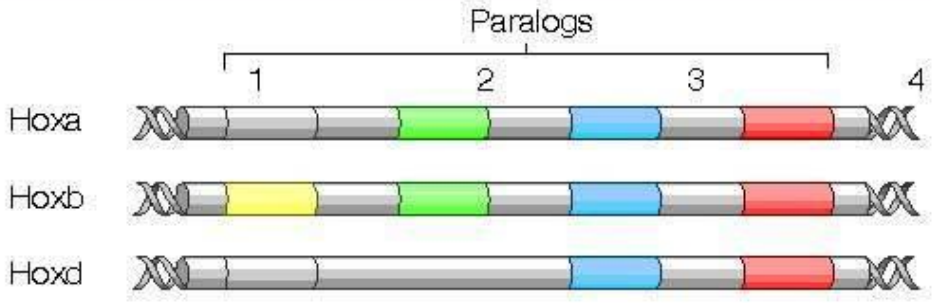
The importance of the HOX code in specifying the development of the head, neck and trunk, and the role of retinoids in controlling HOX expression, has been confirmed by manipulating levels of HOX expression in transgenic mice. Certain principles have emerged from these studies. First, the anomalies observed in animals in response to retinoids are dependent upon developmental stage. Early postimplantation exposure [gestational days (GD) 8–10] in mouse typically leads to craniofacial and CNS defects, whereas exposure on GD 12–14 in mouse is often associated with limb and genitourinary defects. Second, the teratogenic response for each retinoid is dose dependent. Higher doses increase the frequency and severity of malformations and are more likely to be lethal to the embryo. Thus, relatively lower doses of retinoids are toxic early in the critical phase of development, whereas higher doses are required later in the critical phase of organogenesis. Third, some retinoids are more potent than others. In mice, for example, 13-*cis*-RA (Accutane) and retinol are 20 and 4 times less potent teratogens than RA, respectively. Furthermore, the potency of the retinoid can vary with different animal models. Humans, for example, are more sensitive to 13-*cis*-RA than monkeys and rabbits, whereas mice and rats are relatively insensitive to this retinoid. (Species differences in response to the teratogenic potency of 13-*cis*-RA can be explained by variation in pharmacokinetics.) Interestingly, Accutane was tested on rats prior to approval for use in humans; however, doses that showed no significant effects in rats were teratogenic in humans because of this species difference. 13-*cis*-RA is a metabolite of vitamin A, and there is some evidence that retinol, in sufficiently large doses, is also teratogenic. Whether or not tretinoin, which is the form of retinoic acid that is applied topically, is teratogenic when applied topically is controversial.

"The activation of HOX genes explains the teratogenenicity of Retinoic Acid (Isoretinoin, Accutane), a vitamin A derivative used for the treatment of cystic acne. Despite label warnings, Accutane has been taken by at least 160,000 women of childbearing age. The most common abnormality found is cleft palate and other head and neck malformations. Formation of the palate roof through fusion of the palate is a complex process, and the cells in this region of the anterior head do not express genes of the HOX complexes. Exposure to RA causes the border of HOX gene expression to be displaced anteriorly. The expression of HOX genes in the wrong place is the cause of RA-induced congenital malformations. In mouse, it has been documented that the RA is a potent teratogen that acts through the activation of HOX genes." (From DeRobertis Biol Chem notes, 2003)

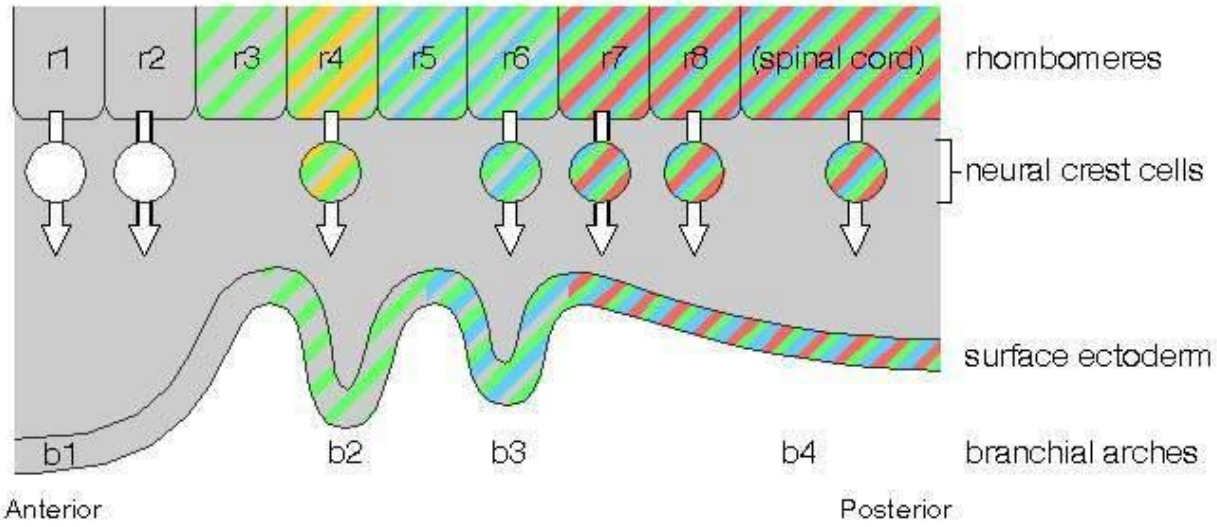


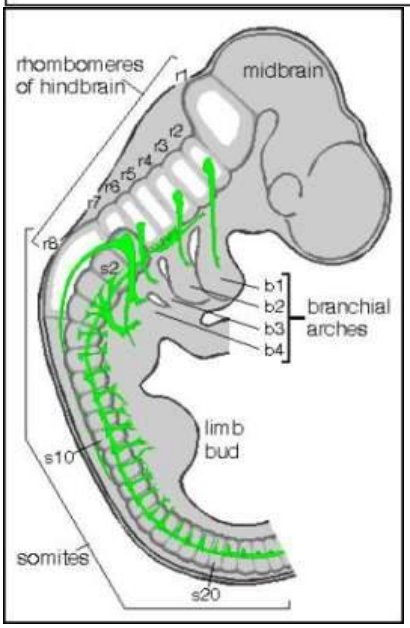
Note in the figure that excess RA at day 8.5 in a mouse embryo causes anterior movement of rhombomere 4 (r4) so that the structures associated with r1, r2, and r3 are abnormal (i.e. “posteriorized”) or not expressed.

Four paralagous groups of Hox genes in three Hox complexes



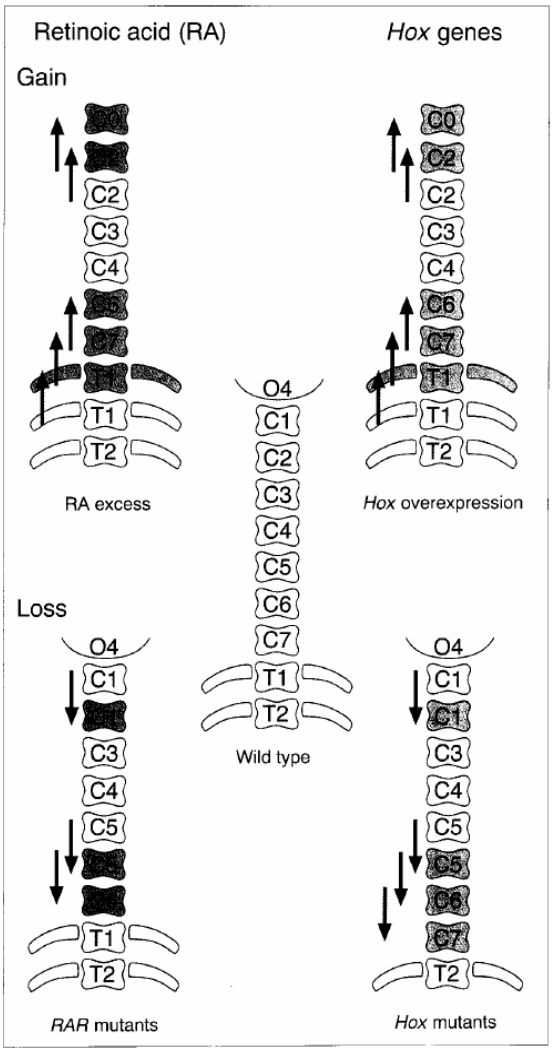
Expression of paralogs 1 to 4 in the hindbrain (r1 to r8), migrating neural crest cells, and the surface ectoderm of the branchial arches (b1 to b4)





http://www.mun.ca/biology/desmid/brian/BIOL3530_W2003/DB_Ch04/DBNVert2.html

Note that multiple HOX paralogs are expressed in a given segment. Setting up the segments and maintaining them are a result of intricate interactions among the paralogs.



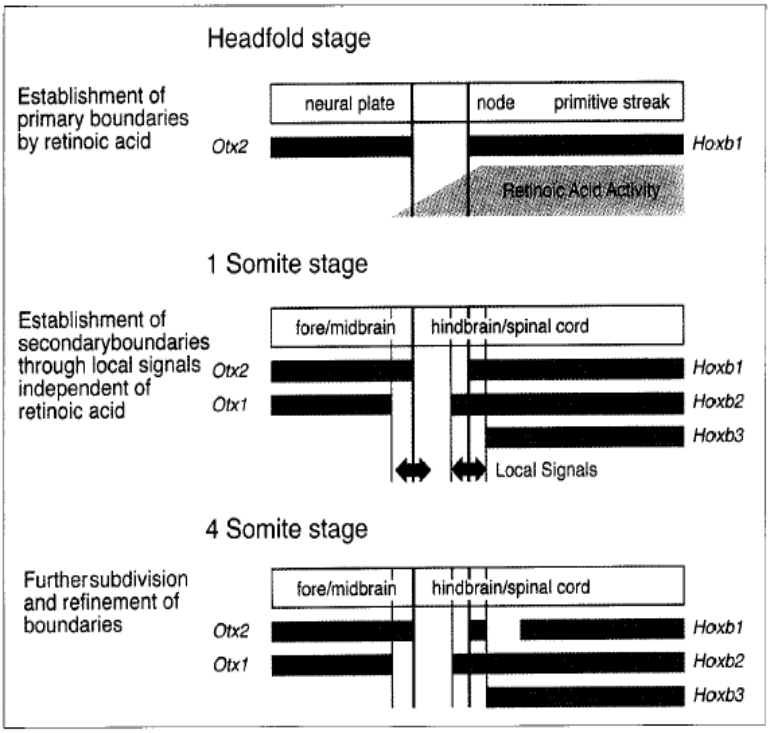
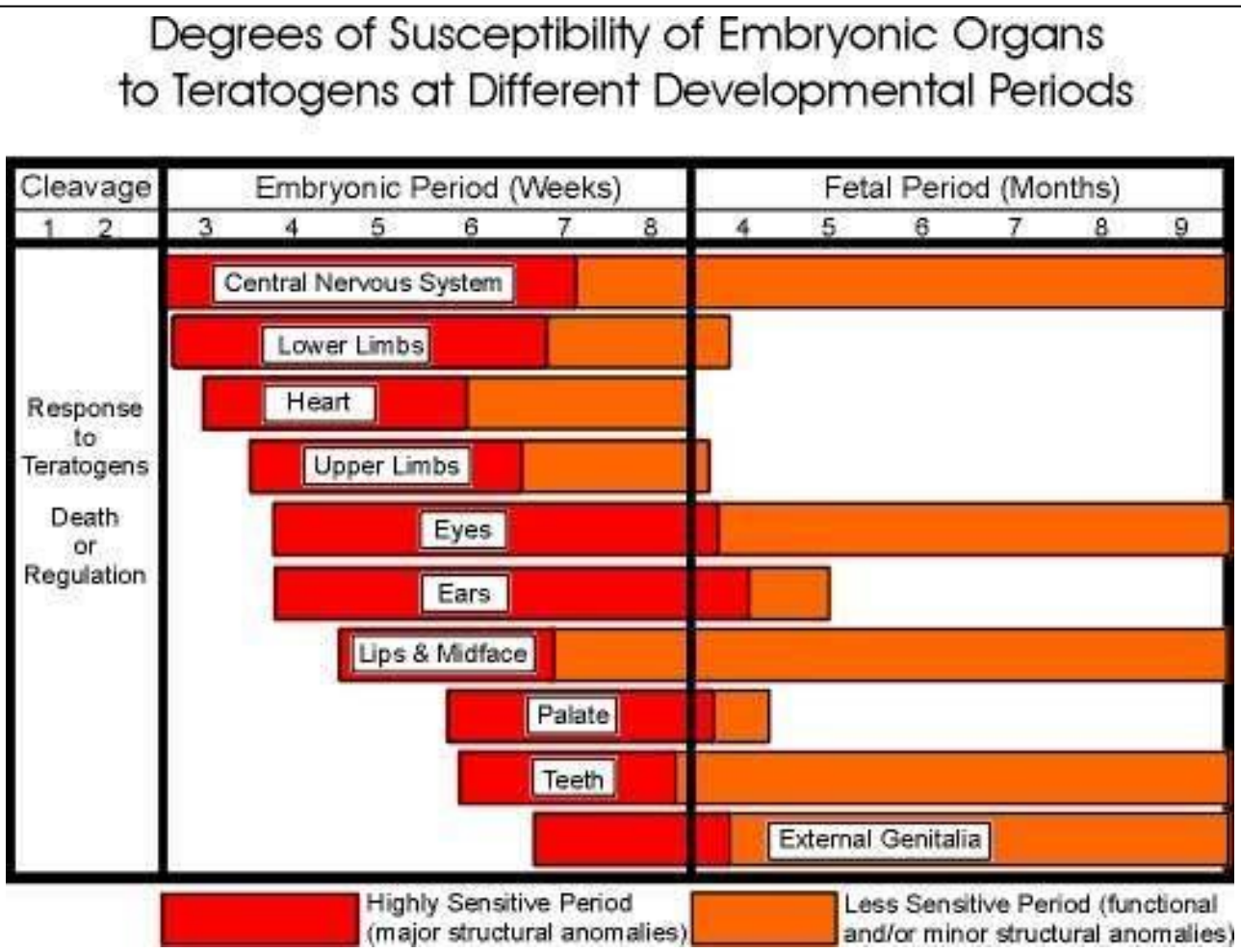


FIGURE 3. A model for the role of retinoic acid (RA) in axial patterning. It is hypothesized that RA released from the node promotes *Hox* paralog 1 expression in the posterior, and represses *Otx2* in the anterior, and by so doing, sets two primary embryonic boundaries (thick vertical lines). Furthermore, it is proposed that these primary boundaries become fixed or stabilized, and are necessary for the generation of further homeobox gene expression boundaries in the same vicinity (thin vertical lines), by signals (red arrows) distinct from RA. Because the secondary boundaries are dependent on the primary boundaries, disruption of RA signaling would be predicted to affect patterning in adjacent regions. The pattern and sequence of homeobox gene expression shown are derived from published *in situ* hybridization data for the mouse[4,5,18,19,22] and unpublished observations (for early expression of *Hoxb3*).

Note the effect of excess RA in moving the HOX boundary forward consistent with the creation of a 13th rib in the present case. It has a similar effect in the pharyngeal arches (PA) where HOX gene boundaries normally only extend to the 2nd PA but which extend up into the 1st PA under the influence of RA, thereby disrupting structures derived from the 1st PA (also known as the mandibular arch). NB: Because the correct migration of the external ear (auricle) depends upon growth of the mandible, disruption of this growth by posteriorizing this arch leads to malformation or absence of the external ear. In the spinal cord and primitive brain, HOX expression normally extends to the anterior boundary of the hindbrain (the 4th rhombomere) but this boundary is extended anteriorly under the influence of RA, thereby disrupting mesencephalically derived structures.

Critical Periods:
Cells are changing and following specified developmental programs
Interference with one stage prevents the proper sequence of events
There is a limited time frame in which sets of cells can complete their tasks
During this time cells can be very sensitive to disruptive agents
This is the "critical period" for organ or tissue, e.g. disruption of neural tube closure leads to spina bifida, incomplete palate formation leads to cleft palate, etc.



Abnormal development of the pharyngeal arch system can result in diverse craniofacialanomalies. Craniofacial anomalies account for about one-third of all human congenital defects. Abnormal fusion of the 5 facial swellings results in facial cleft defects: cleft lip occurs when the maxillary swelling fails to fuse with the medial nasal process, and cleft palate results from the failure of fusion of the palatine shelves across the midline. These are of variable severity, sometimes bilateral, and have a number of causes including exposure to retinoids during pregnancy. In the present case, it is important to note that no cleft palate was observed. The students should recognize (by the end of the week) that development of the palate begins at about the time that Deena stops taking Accutane. Development of the eyes, themselves, is not under control of the HOX genes, and any visual abnormalities observed were more likely due to effects of RA in anteriorizing the rostral boundary of HOX expression, thereby disrupting structures of mesencephalic origin including innervation to the eye and extraocular muscles. Other anomalies, such as cardiac abnormalities, might well have been expected given the period of exposure however there is significant individual variability in the abnormalities observed (possibly because of differences in individual pharmacokinetics as well as pharmacogenetics) and the sensitivity to RA might be less for HOX genes controlling their development. When Accutane was heavily prescribed in the ‘80s, before its teratogenic effects were known, a large percentage of live births had birth defects although the majority did not. (However, many may have spontaneously aborted as well.) Finally, the sensitivity to RA as a transcription factor declines greatly with increasing paralogue number so one would not expect to see effects in more caudal structures during this period (e.g. the limbs).