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Review

The area code hypothesis revisited: Olfactory receptors and other related transmembrane receptors may function as the last digits in a cell surface code for assembling embryos

*William J. Dreyer**

Division of Biology, California Institute of Technology, Pasadena, CA 91125

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ABSTRACT **Recent evidence emerging from several laboratories, integrated with new data obtained by searching the genome databases, suggests that the area code hypothesis provides a good heuristic model for explaining the remarkable specificity of cell migration and tissue assembly that occurs throughout embryogenesis. The area code hypothesis proposes that cells assemble organisms, including their brains and nervous systems, with the aid of a molecular-addressing code that functions much like the country, area, regional, and local portions of the telephone dialing system. The complexity of the information required to code cells for the construction of entire organisms is so enormous that we assume that the code must make combinatorial use of members of large multigene families. Such a system would reuse the same receptors as molecular digits in various regions of the embryo, thus greatly reducing the total number of genes required. We present the hypothesis that members of the very large families of olfactory receptors and vomeronasal receptors fulfill the criteria proposed for area code molecules and could serve as the last digits in such a code. We discuss our evidence indicating that receptors of these families are expressed in many parts of developing embryos and suggest that they play a key functional role in cell recognition and targeting not only in the olfactory system but also throughout the brain and numerous other organs as they are assembled.**

The area code hypothesis helps explain how chromosomes sculpture living organisms. The DNA contained in the two cells that will form identical twins is able to choreograph the parallel development of two strikingly similar individuals through birth and through all of the stages of their lives. In a favorable environment, the twins will grow, rearrange their bodies at puberty, and go through the changes of maturity and aging in parallel. Even the MRI images of their brains will be strikingly similar and very different from other brain images. It was consideration of this extraordinary precision of cell and neural assembly that originally lead us to propose the area code hypothesis (1). The hypothesis was based on extensive genetic, molecular, and cellular studies of the immune system (refs. 2 and 3; see also refs. in ref. 1).

Key elements of the hypothesis are the following. 1. Large multigene families must exist that code for cell surface receptors providing highly specific cell–cell recognition functions. 2. Receptors must be used repeatedly in a combinatorial fashion so that a finite number of genes can provide enough information to generate the required large number of cellular addresses. 3. Programmed genetic switching similar in some respects to that seen during the development of the immune system is assumed to aid in the complex control of the expression of these address codes in specific lineages and cells (4). 4. Some classes of cell surface receptors are assumed to be widely expressed throughout the organism and code for large regions resembling the country codes of our telephone dialing system. Other classes of molecules would be more restricted in expression and are expected to code for multiple smaller regions of the embryo somewhat comparable, according to this metaphor, with the multiple regions specified by area codes and regional prefixes throughout the world. Finally, it is assumed that molecules exist that encode a specific cellular address comparable with the four digits used to code for a single, specific telephone in any one of the numerous, distinct topological regions specified by the earlier codes. Both the telephone digits and the genes and cell surface receptors that provide this last part of the code are of course expected to be used repeatedly in diverse physical locations. Studies in our laboratory (5–7) and many others (8) have succeeded in identifying a large number of cell surface molecules that are involved in cellular interactions and assembly and seem to play a role more or less analogous to county, area, and prefix codes. However, the predicted highly specific final part of the code has eluded us until now.

Data obtained by searching the genome databases have provided us with evidence suggesting that the very large families of seven-transmembrane receptors, including the olfactory receptors, may indeed be used in a combinatorial way during the assembly of many tissues in addition to the olfactory regions. Such molecules therefore have many of the properties expected for area code molecules. Experiments are suggested by these results that can be used to test the validity of the area code hypothesis.

METHODS

Internet GRATEFUL MED (National Library of Medicine; http://www.igm.nlm.nih.gov/) and SCISEARCH (Institute for Scientific Information; http://isanet.com) databases were used for retrieval of bibliographic information. Large numbers of references including abstracts were downloaded into PROCITE 4 (Institute for Scientific Information) for further searching and analysis locally as well as for formatting references.

The online resources available through The National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) include several databases that were used extensively in this work. The information that is reported in Table 1 was obtained by searching the dbEST database (Database of Expressed Sequence Tags) using the text string: olfactory AND receptor. Six hundred and one hits were returned as of the 2/18/98 update. The information included in Table 1 represents only a partial list. The quality of the sequence data varied

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Abbreviations: VNO, vomeronasal; dbEST, Expressed Sequence Tags database.

*To whom reprint requests should be addressed. e-mail: dreyer@caltech.edu.

Table 1. Search of dbEST for olfactory receptor expression in tissues

Tissue-source of RNA	dbEST ID
Liver/spleen	363864, 354217, 156098, 347337, 363860, 474480, 380307, 153228, 352182, 168933, 141689, 395357, 597381, 510649, 347787, 91639, 18454
Lung	954216, 498479, 498483, 939339, 1130457, 117342, 723746, 1644990, 1663526, 1574236, 1522628
Heart	491132, 887378, 461677, 733579, 887598, 235252, 588679, 114840
Placenta	196106, 193508, 206183, 197975, 432198, 1576745
Brain	233020, 283714, 188843, 331341, 42598, 64251, 151626, 712287, 306632
Pineal Gland	1426549, 345459, 1451432
Prostate	939790, 954402, 1670434, 1147520, 1218857, 868944, 869330, 1218648, 1152521, 1298671, 1298734, 2394196
Testis	1104389, 1414182, 1306625, 1425879, 1318566, 1039952, 1306468, 1326543, 1307226, 1478699, 1534360, 1537251
Breast	300904, 300902, 286374
Mouse mammary	1102549, 1516567
Pregnant uterus	659986, 660104, 790661
Lung	117342, 1130457, 528502, 723746
Mouse skin	1484894
Retina	937202
Testis	1481318566, 1478698, 1534360, 1537251, 1039952, 1306468, 1587477, 1480933, 1104389, 14780482, 1478698
Colon	1305576, 1320848, 1266055, 1224456, 1158847, 1654512, 1669804, 1644878, 1220796, 1604549, 1573063
Ovary	1551728, 1559748, 1551267, 1551288, 1100065, 1071934, 1122687, 1548667, 1551288
Embryo	938609, 1388065, 16700
Kidney	1698409, 1599751, 1645467, 1669570, 1558941, 1555887, 1645467

widely as is normal for the expressed sequence tags. Nevertheless, it is clear that this approach provides a great deal of useful information on the expression of olfactory receptor genes in a large number of different tissues. Many of the retrieved sequences were related to known olfactory receptors. Those found to code for other proteins were deleted from the study. Other informative searches used known amino acid sequences of specific olfactory receptors from various species to retrieve expressed sequence tags. For these studies, BLAST 2.0 (Gapped BLAST and Graphical Viewer) with the advanced BLAST option was used. The TBLASTN program was used to search the dbEST database.

RESULTS AND DISCUSSION

Olfactory Neurons Each Express a Single Receptor and Use that Receptor to Target a Specific Pair of Bilaterally Symmetrical Glomeruli. Recent research including the elegant experiments by Mombaerts *et al.* (9, 10) has shown that the olfactory receptors themselves do in fact play an important role in axonal targeting as their processes extend from the olfactory epithelium to specific glomeruli in the olfactory bulb. Neurons that express the same receptor gene but are dispersed in the olfactory epithelium target their processes to a single pair of bilaterally symmetrical glomeruli (11, 12; see Fig. 1). There are 1,000 or so different genes that code for olfactory receptors. Approximately the same number of glomeruli are arranged in a precise, topologically ordered array in each of the two sides of the olfactory bulb. These serve as highly specific targets for the growth cones of the olfactory neurons, each expressing a single receptor gene. Because these olfactory receptors bear the hallmarks of the proposed area code molecules, it seemed appropriate to ask whether they might be expressed in other parts of the developing embryo (and adult) as expected for such molecular codes. A search of the genome and literature databases revealed a remarkable number of examples of these genes expressed in tissues other than the olfactory system. Axons expressing vomeronasal (VNO) receptors are believed to target the accessory olfactory bulb with similar high precision, and they too are assumed to play a role in cell targeting.

Examples of the Expression of Members of These Families of Receptors in Tissues Other than the Olfactory Epithelium. Expressed sequence tags are being entered into the dbEST database at a rapid rate and now represent an important new resource for the study of gene expression. The cDNA samples used for these sequencing studies are obtained from a wide variety of tissues, developmental stages, and organisms. The data vary in quality but nevertheless provide a rich source of information. A search of dbEST revealed many examples of the expression of olfactory receptor genes expressed in tissues other than the olfactory system. A partial set of results from this study is summarized in Table 1. Surprisingly, these genes are expressed in liver, lung, colon, testis, ovary, uterus, prostate, thyroid, brain, and many other tissues and tumors. In addition, a search of the bibliographic databases revealed several publications dealing with the expression of olfactory receptors in a few tissues (13–15).

The original area code paper reviews a number of systems in which cell migration plays a role in organogenesis. The embryonic heart is a particularly interesting example of an organ that is assembled by using migrating cells that coalesce and construct the tissue with great precision. In pursuing the notion that olfactory receptors can act as receptors in an area code system we were therefore gratified to find in our searches of dbEST that specific olfactory receptors are indeed expressed in the embryonic heart. A publication also was found that provides further evidence for such expression (13). One olfactory receptor, OL1, was studied in detail and the data, including *in situ* hybridization studies, seem very convincing. The authors further state that other olfactory receptors are also expressed in the embryonic heart but give no data. It will be most interesting to learn the extent, timing, and topography of the expression of these receptors in the embryonic heart and also in the many other organs where they are expressed.

The widespread expression of members of the olfactory receptor family in numerous organ systems obviously supports the hypothesis that the receptors perform functions other than the recognition of olfactants. Because these receptors play a dual role as receptors for small molecules in the olfactory epithelium and as cell surface-addressing molecules that aid in the assembly of the olfactory bulb, one obvious notion is that they also may play a dual role in other parts of the embryo. The possibility that olfactory receptors mediate cell–cell recognition and organ construction and also function as cell surface receptors for many classes of small molecules, represents an extremely provocative concept when considering the roles of these very large families of genes. Another surprising consequence of this notion is that some of the very widely expressed receptors of the calcium sensing and metabotrophic glutamate families (found in the VNO/accessory olfactory system) also may have dual functions and thus play a role in cellular addressing during development. One would certainly not anticipate or postulate a dual role for these receptor classes if members of these families were not functional in the VNO olfactory system as receptors for pheromones and other small molecules and for targeting the accessory olfactory bulb (16–20).

Assembly of the Olfactory Bulb: A Model for other Parts of the Brain and Embryo. How is the topologically precise target of

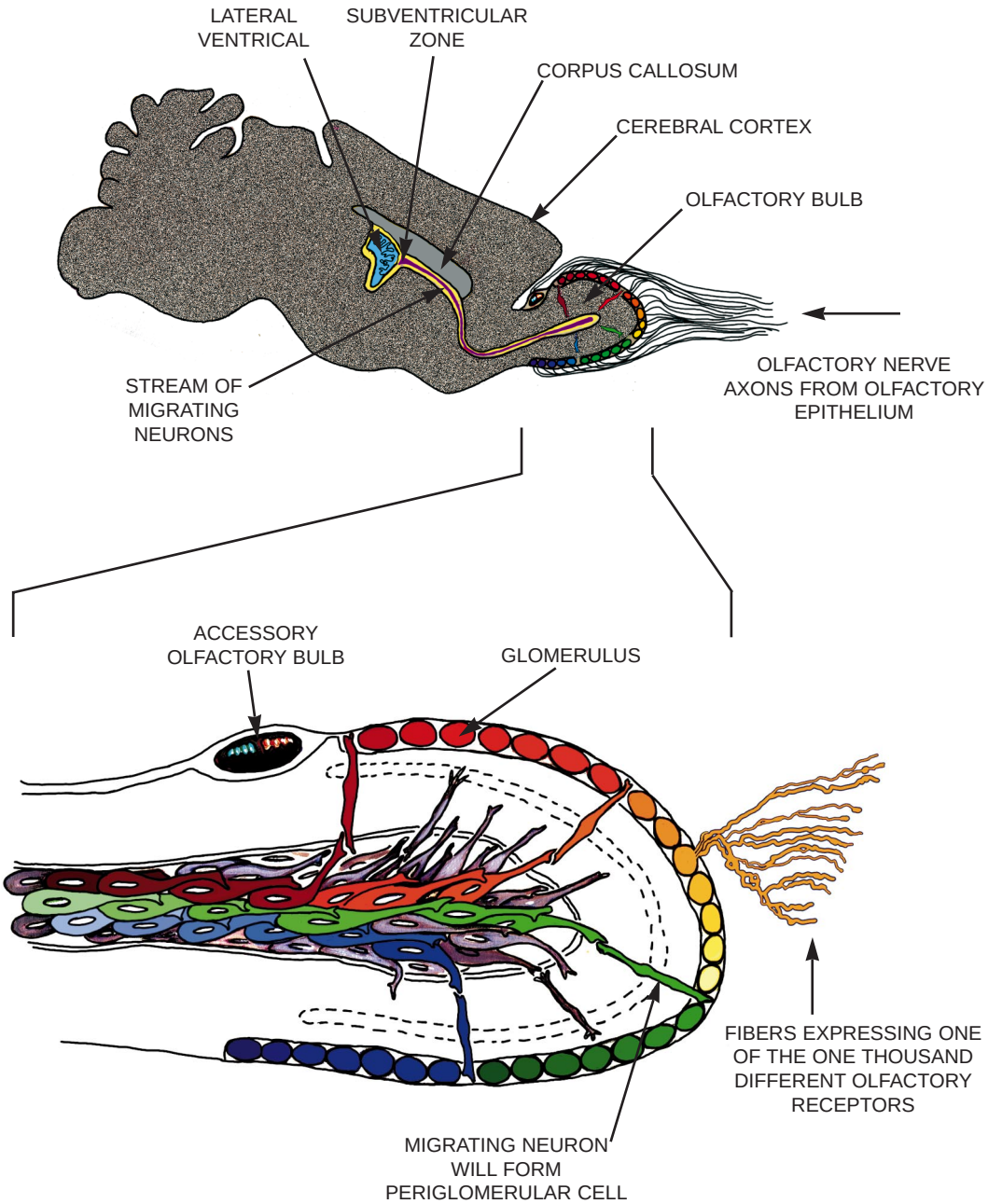

FIG. 1. Hypothetical mechanism for the assembly of the precise topological map of glomeruli. A gradient of molecular affinities of olfactory receptors. Approximately, 1,000 molecularly distinct glomeruli are arranged in a topologically precise map in the olfactory bulb. This map is bilaterally symmetrical, but only one side is illustrated here. There are four distinct zones of glomeruli in the bulb (47–50), illustrated here in various shades of red, yellow, green, and blue. Gradients of colors on glomeruli within each zone are used to suggest an orderly gradient of molecular affinities of the individual receptors. A stream of migrating neurons originates in a specific fate-mapped region of the subventricular zone (22). Cells migrate as streams with the growth cones of each contacting the cell ahead (21). Colors and gradients are used again to suggest that receptors on each cell differ in an orderly way so that neighboring cells have receptors that bind with the highest affinity to each other. After reaching the olfactory bulb, cells change their direction of migration and move toward the surface of the bulb where they generate periglomerular cells (22). The dendrites of these cells then form the targets for incoming growth cones of olfactory nerve axons. Hundreds of olfactory neurons bearing the same, specific, olfactory receptor converge on a single pair of bilaterally symmetrical glomeruli (10–12). Their growth cones synapse with the dendrites of the periglomerular cells presumed to express the identical receptor. These homophilic interactions occur with the highest affinity. According to this hypothesis, receptors on neighboring glomeruli have closely related but different structures, hence are bound with a slightly lower affinity. Mitral/tufted cells also synapse with glomeruli but are not illustrated here.

olfactory axons, the olfactory bulb, assembled? As discussed above, several research groups agree that olfactory neurons expressing the same olfactory receptor, from among the 1,000 or so total receptors, converge on a single pair of glomeruli in the olfactory bulb. A logical consequence of this fact is that each glomerulus in one of the bilaterally symmetrical olfactory lobes has a unique address on the fixed topological map of the olfactory bulb. There are ≈1,000 distinct addresses in each lobe. Furthermore, the maps are the same in each of the inbred individuals and they are believed to be "hardwired" by genetic programs that control development. It has been determined that the targets are established during embryogenesis. When the growth cones of olfactory neurons start entering the olfactory bulb, the targets await. It follows that the assembly of this target structure must, itself, use a very sophisticated molecular-addressing system during embryogenesis and then display molecules that provide the topologically precise, distinct targets for olfactory nerve growth cones.

The subventricular zone, a considerable distance posterior to the region where the olfactory bulb is formed, is the birthplace of neuronal precursor cells that are destined to form the olfactory bulb. Topological fate maps of this region reveal various specific positions of cells that are destined to generate distinct parts of the forebrain. A small region in the extreme anterior of the subventricular zone is the source of cells that will begin the migration to the region where the olfactory bulb is assembled (refs. 21 and 22; see Fig. 1). We assume that migratory cells are generated in an ordered fashion from these precursor cells and that the order of birth of daughter cells relates to their ultimate position in the topology of the olfactory bulb. As such cells are born, they begin migrating along a narrow tube-like pathway bounded by glial cells but, unlike other regions of the embryonic brain, no radial glial processes are seen. The migrating spindle-shaped cells remain in contact with neighboring cells in front, beside, and behind and migrate as a stream only a few cells in diameter (21). Cell division continues while they migrate and maintain contacts. As cells in this stream reach the inner region of the developing olfactory bulb, some form granule cells but many change directions and move outward toward their final positions near the surface of the bulb and become periglomerular cells. The dendrites of these cells become targets for the growth cones of olfactory cell axons that form synapses with them (22, 23). A required consequence seems to be that this pattern of cell generation and migration relates directly to the specificity of the target receptor(s) that each cell ultimately will express. This process forms the precise and bilaterally symmetrical topological map of future targets for the growth cones extending from olfactory neurons born in the olfactory epithelium to the glomeruli in the olfactory bulb.

Olfactory receptors play a key and proven role as address molecules targeting the glomeruli. But what molecules form the targets, and what known gene families might code for such receptors? Is it reasonable to suppose that a totally different mechanism is used as cells there migrate to form that extraordinarily precise target structure, the olfactory bulb? Why not use the same families of genes, again in a combinatorial code, for the formation of this neural structure? What molecular codes are used to assemble other parts of the brain by nearby cells in the fate map of the subventricular zone? What about other parts of the brain and, indeed, other regions of the embryo? It seems important to examine various regions of the brain and embryo to determine where and when olfactory and VNO receptors are expressed. Clearly, it is reasonable to consider molecules expressed throughout the developing embryo.

There are many molecules other than the olfactory and VNO receptors that have been shown to play an important part in cell surface recognition (8). These molecules fulfill many of the addressing functions needed in an area code system by providing the equivalent of the country codes, area codes, regional codes, etc. One such example is O-CAM, one of a large number of cell surface receptors in the Ig supergene family (24, 25). O-CAM is expressed on a subset of olfactory nerve axons that extend from the four zones of the olfactory epithelium to the specific zones of glomeruli in the olfactory bulb. This molecule is expressed on axons originating in three of the four zones of the olfactory epithelium and on one of the two zones from the VNO region. O-CAM thus seems to provide an excellent candidate for an area code molecule coding for geographic regions rather than for a specific cellular address. It is assumed that other, probably related, receptors will be found on zones in which O-CAM is absent and that these will form part of the combinatorial code.

Is it possible to conceive of genetic, molecular, and cellular mechanisms capable of accomplishing the assembly of the 2,000 or so target sites in the olfactory bulb? As discussed above, neuronal precursor cells migrate considerable distances along stereotyped routes to lay out a precise, bilaterally symmetrical target map in the olfactory bulb. The mechanisms responsible are completely unknown. The only other example of this extraordinary level of migratory specificity is seen in the targeting of the axonal growth cones as they extend to form synapses in the olfactory bulb. In the absence of any good alternative we shall consider the possibility that members of the olfactory and VNO receptors are expressed in the cells that form the target arrays in the olfactory bulb. In this scenario, molecular interactions of these receptors with each other provide the required specificity for both migration and targeting. How then could cells interact in such a way as to form the precise topological map of cells expressing target receptors? One intriguing possibility is suggested by the structure of the receptors themselves and by certain interesting patterns in which these structures are arrayed in the target maps. All of these receptors contain seven helical domains that traverse the membrane and arrange themselves so as to form a pocket at the cell surface. Studies have shown that these pockets provide specific sites for binding ligands. These receptors also display extracellular loops of varying size that provide additional specificity for interactions (26). Differences in the amino acid sequences within the domains forming the pockets and loops provide the individual specificity for ligand binding. There is speculation that this structure also might provide specificity for homophilic interactions (27).

Consider the notion that these combined binding sites provide the required specificity for both homophilic and heterophilic interactions of these receptors. Homophilic interactions could account for the target specificity known to occur as the olfactory axons seek specific glomeruli in the olfactory bulb. But how is the specificity of cell migration and bulb assembly explained? A possible hint derives from the observation that olfactory receptors with an unusual type of extracellular loop structure cluster together in both the olfactory epithelium and in the target bulb structure (28). Indeed, several studies suggest that glomeruli are arranged with receptors of similar structure displayed on adjacent glomeruli and within a specific region of the olfactory bulb (29). It seems possible that receptors differing only slightly in the amino acid sequence of the binding sites responsible for homophilic interactions could still interact with relatively high affinity. The binding-constant difference could serve to guide neighbors to each other. Other adjacent cells could again have receptors with close but lower affinity. In this manner, a type of affinity gradient could be established that, at least theoretically, could help explain the relationships maintained among cells as they migrate and assemble the target map in the olfactory bulb. Such a gradient of receptor affinities also would aid the growth cones of olfactory neurons as they seek their targets in the bulb.

What sort of orderly genetic programs are sophisticated enough to generate and maintain 1,000 or more cells, each expressing one receptor gene? Elaborate genetic controls must function to maintain the expression of a single, specific olfactory receptor gene in each of the olfactory stem cells and in its daughter olfactory neurons as they continue to be born throughout life. Furthermore, these controls must allow the expression of only one of the two alleles present in each cell (30). The complexity of this genetic problem is very reminiscent of the similar situation seen in the immune system where sophisticated alterations are made in the germline DNA as specific B or T cells are generated. There too only a single allele is expressed in each cell. The altered DNA sequences are replicated for the life of a stem cell thus accounting for the lineage memory. Genetic switching therefore remains an attractive aspect of the area code hypothesis, particularly for the control of the expression of the olfactory receptors discussed here. Indeed, it is extremely difficult to imagine that a mechanism using only transcription factors, etc. is capable of mimicking the immune system's single allele expression and stem cell-specific receptor expression.

Genetic Switches Known to Function in Various Organisms. The earliest proven example of developmentally controlled genetic switching occurred in large colonies of Cyanobacter over 2 billion years ago (31, 32). The same types of cyanobacteria exist today and form large colonies identical to those in the fossil record. In this organism, DNA rings are excised from the

germline cell's DNA to form somatic cells that can fix nitrogen for the use of the entire colony. There is good reason to believe that this type of genetic switch evolved very early and has been selected for use in numerous subsequent species because of its efficacy as a means of programming the formation of different cell lineages.

Numerous types of repeats and transposable elements also have been shown to play a role in chromosomal programs, wherein germline DNA is altered as specific cell types are formed. Ciliates, for example, use transposases to excise specific transposon-like elements from germline DNA as a part of the mechanism used to form the somatic macronucleus from the germline micronucleus (33, 34). Excision of specific transposable elements occurs in *Drosophila* as polytene chromosomes are formed from the germline (A. Gould and W. Dreyer, unpublished observations). In another example, it is now known that the telomeres in *Drosophila* are maintained by two different transposable elements (35). Ribosomal DNA, like telomeres, must be controlled and maintained during development. These chromosomal regions contain numerous tandem copies of rDNA. In *D. melanogaster* specific transposable elements (different from those that maintain telomeres) are associated with rDNA (36). It seems very possible that they aid in the recombination control required for the maintenance and amplification of these chromosomal regions. There are numerous other examples of DNA alterations during development of other organisms.

The mechanism by which DNA is excised during the development of the immune system is very closely related to many of the examples mentioned above. Indeed, the RAG-1 transposase is evolutionarily related to the enzymes responsible for transposable element rearrangements found in essentially all eukaryotes and even bacterial switches such as the invertrons (37–40). Ten to 20% of the DNA of most multicellular organisms is made up of mobile DNA elements; hence large numbers of genes coding for members of the transposase/recombinase family are found in these genomes, and according to our hypothesis, some may function in normal development.

The list of confirmed examples of programmed alterations in DNA is now so long that one is quite safe in stating that not all of the repeats and elements that make up a significant part of all chromosomes are "junk DNA." It therefore seems reasonable to examine the possibility that some of the transposon-related elements may play a role in programming the expression of such genes as the olfactory receptors. Again, no other known mechanisms that do not involve alteration of DNA seem adequate to perform the extraordinarily complex programming of gene expression that is discussed here.

One obvious ramification of developmentally programmed DNA alteration is that cells from fully differentiated tissues could not be used to clone new individuals. And in fact this prediction of the hypothesis seems to be true despite the two widely quoted examples of cloning from "differentiated" tissues. Neither the cloning of Dolly from the udder of a sheep (41) nor Gurdon's cloning of an adult frog from larval frog intestines (42) has been proven to have been accomplished from a differentiated cell type. The Dolly experiment has not been repeated and, even after 36 years, no successful repeat of Gurdon's result has been accomplished using confirmed differentiated cells from adult frogs (43). In both of these cases, the cloned individual was the very rare outcome of numerous experiments, and in both cases, an embryonic germ cell could have been the cell actually selected for cloning. This result is possible because the sheep that served as a donor for Dolly was pregnant and because the larval frog intestine is a known site of germ cell migration during development. In contrast to the above reports, the successful use of nuclei derived from blastula cells in the nuclear transplantation experiments pioneered by Briggs and King in 1952 (44) has been reproduced many times and similar procedures have been used by numerous scientists in a variety of species throughout the past 46 years. Nuclear transplantation from blastulas is compatible with the area code hypothesis because DNA switching has not yet occurred at this stage of development and the cells are therefore totipotential.

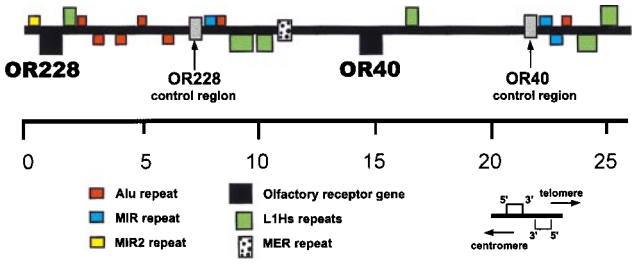

FIG. 2. Diagram of a region of human chromosome 17 that codes for two olfactory receptors. This figure, based on the work of Glusman *et al.* (46), illustrates one of many sequenced regions of chromosomes that code for olfactory receptors and also contain numerous mobile elements. Note the pattern of elements near the upstream control elements of the two olfactory receptor-coding regions (OR228 and OR 40). See the original publication for more details of this work. We hypothesize that some of these elements are used as genetic switches for the control of the expression of the 1,000 or more olfactory receptor genes. The mobile element-related and transposase mechanisms used could be evolutionarily related to those now known to control the expression of genes in the immune system. This would help explain how the olfactory system, like the immune system, expresses only one receptor gene in each stem cell. In both the olfactory and immune systems, such committed stem cells not only remember which receptor gene to express but also which one of the two alleles (30).

Are repeats and transposon-related elements present in the sequences of the multigene families of olfactory receptors? Fig. 2 illustrates one of many examples of the DNA sequences of regions containing genes coding for olfactory receptors. Two olfactory receptors are coded by the DNA sequence illustrated. Note the pattern of elements near both upstream control regions. We believe that careful consideration should be given to the possibility that repetitive elements, including some of those illustrated here, may play a role in programming the expression of the very large families of seven-transmembrane receptor genes that have been found.

Experiments Are Needed to Test the Hypothesis. The updated area code hypothesis presented here leads us to propose several specific experimental tests of its validity:

1. Where and when during organogenesis are specific olfactory and VNO receptors expressed throughout the embryo? The data discussed above provide strong support for the notion that such receptors are indeed expressed in numerous tissues other than the olfactory regions. However, the data available at this time do not provide topological details of the expression of these molecules over time and space in the developing embryo. We predict that each receptor will be expressed in a speckled pattern throughout the embryo similar to the locations of the last four digits of phone numbers in geographic locations where they are used repeatedly in combination with other digits to code for different telephone sites. This type of pattern might easily be mistaken for an experimental artifact. A possible example of this may have been already published (14). Close examination of the expression of olfactory receptors in chicken embryos before, during and after notochord formation (see Fig. 6 in ref. 14) reveals numerous such specks not seen in the control Fig. 4*B* in ref. 14. The notochord does indeed express an olfactory receptor, but the speckled appearance of other parts of these sections was not noted by the authors. Obviously, more experiments are needed. As one example, the transgenic mice used by Mombaerts *et al.* (10) would provide an excellent source of embryos for the study of the expression of olfactory receptors in tissues other than the adult olfactory system illustrated in their publication.

2. Do seven-transmembrane receptors interact with each other as is predicted by the above discussion? We have not yet uncovered any studies bearing directly on this aspect of the hypothesis, but

such experiments are feasible. Several of the available excellent methods were used by Yoshihara *et al.* (24) in their studies of homophilic interactions of O-CAM. We have used an additional method (45). If it can be shown that no homophilic or heterophilic interactions can occur among these receptors other molecules would have to be found to explain the known facts. However, we are not able to offer any reasonable alternative hypotheses at this time.

3. Is there a gradient of closely related receptors on the topological map of glomeruli on the olfactory bulb? Although several publications referenced above suggest that this may be true, more work needs to be done. Structural and functional studies of olfactory receptors expressed on neighboring glomeruli are needed to test this notion. Single-cell PCR techniques should facilitate testing of this "receptor gradient" hypothesis.

4. Is the control of the expression of the 1,000 or so different olfactory receptors due in part to DNA switches? By now there are so many confirmed examples of the role of DNA alterations in somatic cells of diverse organisms that this part of the hypothesis should be given serious consideration. Several experimental approaches are now capable of providing data relevant to this subject. PCR methods can be used to compare specific stretches of DNA in germ line and somatic cells. DNA libraries from both cell types also can be used to detect specific differences. Protocols are readily available because studies of such differences in cells of the immune system have become commonplace in recent years. We suggest that experiments be carried out to test the notion that the immune system is not alone in the use of mobile-element related genetic switches in developmental controls of cell lineages.

CONCLUSIONS

Our finding that olfactory receptors are expressed in a large number of different tissues has led us to suggest that they may play a central role in coding for cell positioning during embryogenesis. According to this hypothesis, these and other less specific receptors are used in a combinatorial strategy that provides molecular codes to cell surfaces. Cells use these cell surface codes to guide their assembly of complex three-dimensional structures. The genetic control mechanisms required for the control of these codes are so sophisticated that we suggest they use genetic switches related to mobile elements to aid in the control of the expression of codes on embryonic cells. Recombinases from the very large family encoded by mobile elements are candidates for a role in such DNA alterations. Rag-1, a member of this large recombinase family, plays a key role in the genetic events that use mobile element-related switches during the development of the immune system (37, 38). A homeodomain that also is found on some of these recombinases (including Rag-1) raises more intriguing questions (39, 40).

As a good heuristic model, this updated area code hypothesis makes predictions and suggests experimental tests that can be carried out by using currently available methods. The implications and potential applications of the knowledge to be gained from such experiments will be profound.

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