平成２３年度大阪大学医学部医学科２年次９月学士編入学試験問題

英語

答は、すべて解答用紙に記入すること。

第２問

次の文章は嗅覚の分子メカニズムの解明について述べた文章である。これらを読んで以下の問１から問７に答えよ。

INTRODUCTION

The subject of my lecture is the sense of smell, one of ①the five senses through which we ②perceive the world. Through the sense of smell, humans and other mammals can perceive a vast number and variety of chemicals in the external world. It is estimated that humans can sense as many as 10,000 to 100,000 chemicals as having a distinct odor. All of these "odorants" are small, ③volatile molecules. However, they have diverse structures and somehow those different structures are perceived as having different odors.

The sense of smell is mediated by the olfactory system, a system that is characterized by exquisite sensitivity and discriminatory power. Even a slight change in the structure of an odorant can change its perceived odor. For example, the close relative of a chemical that is perceived as pear can have the scent of an apple. In addition to odorants, the olfactory system detects pheromones, chemicals that are released from animals and act on members of the same species, stimulating hormonal changes or ④instinctive behaviors, such as mating or aggression. The olfactory system also detects ⑤predator odors, which can elicit innate fear responses.

Over the past 16 years, our work has focused on two questions. First, how do mammals detect so many different environmental chemicals? And second, how does the brain translate those chemicals into diverse odor perceptions and behaviors?

Odorants are initially detected by olfactory sensory neurons, which are located in the olfactory epithelium lining the nasal cavity. These neurons transmit signals to the olfactory bulb of the brain, which then relays those signals to the olfactory cortex. From there, olfactory information is sent to a number of other brain areas. These include higher cortical areas thought to be involved in odor discrimination as well as deep limbic areas of the brain, which are thought to mediate the emotional and physiological effects of odors. In contrast to odorants, pheromones are detected primarily in the vomeronasal organ, or VNO, a separate olfactory structure in the nasal septum. From VNO neurons, signals are relayed through the accessory bulb to the medial amygdala and then the hypothalamus, areas implicated in hormonal and behavioral responses to pheromones.

The olfactory epithelium contains millions of olfactory sensory neurons. It also contains supporting cells and a basal layer of stem cells. Olfactory sensory neurons are short-lived cells that are continuously replaced from the stem cell layer. At the surface of the epithelium, each neuron extends cilia into the nasal lumen, allowing it to come in contact with odorants dissolved in the nasal mucus. Each neuron communicates with the brain via a single axon that it extends to the olfactory bulb.

ODORANT RECEPTORS

In our initial experiments, Richard Axel and I asked how it is that these neurons detect odorants. Beginning in 1965 with the work of Robert Gesteland, numerous electrophysiological studies had shown that different olfactory sensory neurons are depolarized, or activated, by different odorants. John Amoore proposed that these neurons had odorant receptor proteins that varied in their affinity for different odorants. In the mid 1980s, hints started to emerge about signal transduction in the cilia of the olfactory neurons. Doron Lancet and Sol Snyder and their colleagues showed that odorants induce GTP-dependent increases in adenylyl cyclase activity in the cilia, suggesting the involvement of intracellular G proteins, and Randy Reed identified G_{olf}, a G protein that could mediate this response and was highly expressed in olfactory sensory neurons.

In 1988, Richard Axel and I embarked on a search for odorant receptors. The strategy we devised was based on three assumptions. First, odorant receptors would be selectively expressed in the olfactory epithelium. Second, since odorants vary in structure, there would be a family of varied, but related receptors, and those receptors would be encoded by a multigene family. And third, odorant receptors would be related to other types of receptors that interact with intracellular G proteins. By 1989, molecular cloning had revealed the structures of about 20 of these G protein-coupled receptors, or "GPCRs". All of these receptors had seven potential transmembrane domains and they shared a few amino acid sequence motifs.

On the basis of these assumptions, we set out to search for a family of GPCRs expressed in the rat olfactory epithelium. To do this, we first used PCR (the polymerase chain reaction)* to look for receptors expressed in the olfactory epithelium that were related to known GPCRs. We designed 11 oligonucleotide primers that matched amino acid sequences in transmembrane domains 2 and 7 of known GPCRs. We then used these primers in all 30 pairwise combinations to amplify related sequences in cDNA prepared from rat olfactory epithelium RNA. From the 30 PCR reactions, we obtained 64 different PCR products in the appropriate size range. Each of these appeared as a distinct band using agarose gel electrophoresis.

We then asked whether any of the 64 PCR products contained multiple members of a multigene family. To do this we cut the DNA in each PCR product with a restriction enzyme. Most of the bands were cut into a small number of fragments that added up to the original in size. However, one band, #13, was cut into a large number of fragments, suggesting that it might contain multiple members of a multigene family. When we cloned and sequenced five of the DNAs in #13, we found what we had been looking for. All five encoded novel proteins with the

hallmarks of GPCRs. Moreover, all five were related, but each one was unique.

Using these DNAs as probes, we isolated a series of related cDNAs from an olfactory epithelium cDNA library. We initially examined the proteins encoded by ten of the cDNAs. All ten proteins had the seven potential transmembrane domains characteristic of GPCRs. In addition, they had several amino acid sequence motifs seen in other GPCRs. However, the ten receptors all shared sequence motifs not seen in any other GPCRs, indicating that they were members of a novel receptor family.

（中略）

COMBINATORIAL RECEPTOR CODES FOR ODORS

In later studies, we asked how the OR (olfactory receptor) family encodes the identities of different odorants. To explore this question, we searched for ORs that recognize specific odorants. This work was done by Bettina Malnic in the lab in collaboration with Takaaki Sato and Junzo Hirono at the Life Electronics Research Center in Japan. We first exposed single mouse olfactory sensory neurons to a series of odorants, using calcium imaging to visualize their responses. We then isolated each responsive neuron and used RT (reverse transcriptase)-PCR to determine the OR gene it expressed. In every case, we identified only one expressed OR per neuron, confirming that each neuron expresses a single OR gene.

For test odorants, we used four different classes of n-aliphatic** odorants with different functional groups and carbon chains ranging in length from 4 to 9 carbon atoms. Each neuron was imaged as it was exposed sequentially to different odorants. If a response was seen, the neuron was retested with a lower concentration of the same odorant.

The data from this experiment make three important points. First, each OR can recognize multiple odorants, something previously shown by Stuart Firestein for one rat OR. Second, each odorant can be detected by multiple different ORs. And finally, and most importantly, different odorants are recognized by different combinations of ORs.

These results indicated that ORs are used combinatorially to encode odor identities. Different odorants are detected and thereby encoded by different combinations of ORs. However, each OR serves as one component of the codes for many odorants. Different odorants have different "receptor codes". Given the number of possible combinations of 1000 different ORs, this combinatorial coding scheme could allow for the discrimination of an almost unlimited number of odorants. Even if each odorant were detected by only three ORs, this scheme could potentially generate almost one billion different odor codes.

These studies also provided insight into several puzzling features of human odor perception. Changing the structure of an odorant even slightly can alter its perceived odor. Sometimes the change in odor can be dramatic. The aliphatic acids and alcohols that we used in our studies are excellent examples of this phenomenon. All of the acids have unpleasant odors, such as rancid***, sour, or sweaty. In contrast, all of the alcohols have pleasant odors, such as herbal, woody, or orange. In our studies, pairs of acids and alcohols that differed by a single

functional group invariably had different receptor codes. This indicates that even a slight change in the structure of an odorant can alter its receptor code, and thereby change its perceived odor.

Our studies showed that a change in the concentration of an odorant can also change its receptor code. At higher concentrations, additional ORs were invariably recruited into the odor response. This may explain why changing the concentration of an odorant can alter its perceived odor.

（Nobel lecture, Dec.8, 2004 by Linda Buck から抜粋、一部改変）

注）

PCR (the polymerase chain reaction)*：ポリメラーゼ連鎖反応。数十個の核酸を並べて作るプライマー（primer）と呼ばれる短い線状の DNA の鎖を、タンパク質の配列を表す cDNA（相補的 DNA）などといった長い DNA 鎖のそれぞれ別の場所に結合させ、その間の DNA を多量に増やすことが出来る反応

n-aliphatic**：脂肪族の

rancid***：悪臭のする

問１

本文の INTRODUCTION の下線部①～⑤の英語を和訳せよ。

問２

におい物質（odorant）とフェロモンは異なる経路で脳に至るが、その経路を下に示した。

（　　）部を適切な英語で埋めよ（the は付けなくてよい）。

におい物質

Olfactory sensory neuron →（　①　）→（　②　）→様々な脳の部位

フェロモン

Vomeronasal organ →（　③　）→（　④　）→（　⑤　）

問３

（ ）を英語で埋めよ。

嗅上皮（olfactory epithelium）に含まれる細胞は olfactory sensory neuron,（　①　）,（　②　）の３種類ある。また olfactory sensory neuron は（　③　）を鼻腔に出してにおい物質と接触し、その刺激を１本の（　④　）を通じて（　⑤　）に伝える。

問４

ODORANT RECEPTORS の章で、筆者らが「嗅覚の研究を開始する前、１９６５年以降に分かっていた事実」４つと「筆者らの研究を進める上での仮定」３つを本文中から英語で抜粋せよ。

問５

筆者らが「嗅覚受容体が GPCR であることを予測して行った実験」と「多くの良く似た遺伝子の集

まり（ファミリー）からなることを予測して行った実験」を示す１文を各々英語で抜きだして示せ。

問６

COMBINATORIAL RECEPTOR CODES FOR ODORS の章で、筆者らは嗅覚受容神経細胞を単離して、それににおい物質をかけて反応をみる実験を行ったが、その結果、におい物質とそれに反応する嗅覚受容体（OR）の間にはどのような関係があることが判明したか？３つ日本語で記せ。

問７

①“receptor code”とは何か、下の（　）内を日本語で埋めよ。

各々のにおい物質に対して、それに応答する嗅覚受容体の（　　）を指す。

②「比較的少ない遺伝子で非常に多数のにおい物質を動物が識別できる理由」、「においの物質のわずかな違いを識別できる理由」を“receptor code”という言葉を用いて日本語で記せ。