

Osamu Hayaishi—from the discovery of oxygenases in soil microorganisms to unraveling the enigma of sleep in mammals

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Introduction

We stand in awe of the breadth of important scientific contributions made by Osamu Hayaishi. In 1955, the research groups of Osamu Hayaishi, and the late Howard S. Mason, each independently discovered a new family of enzymes now referred to as oxygenases.^{1,2} These enzymes catalyze the incorporation of either one or 2 atoms of molecular oxygen into various substrates. Hayaishi and coworkers discovered and characterized more than 30 oxygenases, crystallized 4 of these and undertook extensive studies examining the structure and properties of these oxygenases (for an overview, see.^{3–5}) Professor Hayaishi's work also included oxygenases involved in the metabolism of biologically important compounds such as vitamin A and prostaglandins (PG). Regarding PG, Osamu Hayaishi and coworkers found that PGD₂ is the most abundant prostanoid in the mammalian brain, including humans and eventually made the serendipitous observation in 1981 that

PGD₂ induces sleep when microinjected into the brains of rats.^{6–8} This unexpected discovery was the start of Osamu Hayaishi's second career in the field of sleep research.

In addition to providing a brief overview of Osamu Hayaishi's career as a biochemist and sleep researcher, we will provide some examples of his lifelong role as a mentor who is revered for giving inspiration and advice to young scientists. Moreover, the legacy of Osamu Hayaishi for 21st-century Japanese bioscience is exemplified by the groundbreaking establishment of the Osaka Bioscience Institute, simply known as OBI, that was founded in 1987 and was operated using a system consistent with high-impact international scientific institutions, one that never existed prior to the founding of OBI but now this system is widely adopted by other institutions in Japan.

The discovery of oxygenases

Osamu Hayaishi made outstanding and pioneering contributions to biomedical sciences and enzymology by his discovery of oxygenases. These enzymes are widely distributed in nature and represent a unique group of enzymes that catalyze the direct incorporation of molecular oxygen into various substrates. Osamu Hayaishi started his research in Japan immediately after World War II under very difficult conditions, as only poor quality instruments and reagents were available for research. Osamu Hayaishi decided to look for microorganisms that could survive and grow in a simple medium supplemented with specific amino acids. By using tryptophan that was a gift from his mentor, Professor Yashiro Kotake, at Osaka Imperial University (now Osaka University), Osamu Hayaishi identified several microorganisms in soil and began experiments to clarify these mechanisms of metabolism.

Initially, Osamu Hayaishi found an enzyme that catalyzed the conversion of catechol to muconic acid by oxidative cleavage, and named the enzyme "Pyrocatechase," the first enzyme that cleaved the benzene ring under aerobic conditions. Prior to Hayaishi's discovery of the pyrocatechase in 1955, the only known mechanism of biological oxidation was the 'dehydrogenation' theory proposed by Heinrich Wieland in 1920, namely the transfer of hydrogen atoms or electrons.⁹ However, by examining the incorporation of heavy molecular oxygen (¹⁸O₂) into the substrate during catalysis, Osamu Hayaishi demonstrated the biological fixation of molecular oxygen. Thus, Hayaishi introduced a new concept of oxidation and termed the enzymes 'oxygenases', which catalyze oxygen-fixation reactions.

Since the original discovery of oxygenases, Hayaishi and colleagues found more than 30 oxygenases and undertook extensive studies concerning the structure and properties of these oxygenases. These enzymes are widely distributed in nature and play a vital role in the degradation of a variety of synthetic and natural compounds. A number of oxygenases characterized by Hayaishi play crucial roles in the metabolism of amino acids such as tryptophan, lysine, histidine and others. His studies on these amino acids resulted in the elucidation of several new and physiologically important metabolic pathways, both in mammals and microorganisms. Furthermore, studies on individual oxygenases involved in these metabolic pathways have yielded basic information on the regulatory mechanism that controls the activity of oxygenases. In addition to these studies, his work extends to oxygenases involved in the metabolism of other biologically important compounds, such as prostaglandins, vitamin A and carcinogenic hydrocarbons, that incite the

Keywords: Osaka Bioscience Institute, oxygen, prostaglandins, Tekijuku, vitamin A

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Submitted: 07/07/2015

Accepted: 07/08/2015

<http://dx.doi.org/10.1080/23328940.2015.1072658>

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development of malignancy. These results are summarized and reviewed in a number of monographs and proceedings.¹⁰⁻¹⁴ The importance of Hayaishi's discovery of oxygenases and characterization of their physiological, medical and environmental role is also reflected in a remarkable list of international awards and honors that were bestowed on Osamu Hayaishi, including: The Asahi Prize (Japan), 'The Grand Cordón of the Order of the Sacred Treasure' by the Emperor of Japan, The Japan Academy Prize, Elected Foreign Associate of the National Academy of Sciences of the USA and The Wolf Prize in Medicine (Israel).

The mystery of sleep

In the middle of the 1970s, Osamu Hayaishi began a new project on PG synthesis in the brain with Shozo Yamamoto and Takao Shimizu at Kyoto University. This bold step into neuroscience was originally met with skepticism from the neuroscience community concerning a possible role of PG in the brain. At that time PG represented a *different* type of messenger, for example one not stored in

vesicles but produced on demand. Nevertheless, the neurobiological project grew rapidly, mainly because they found PGD₂ to be a major PG produced in the rat brain. In addition, they quickly succeeded in purifying an enzyme for its synthesis—one in a group of PGD synthases—from rat brain, and identified a potential binding protein for PGD₂ in rat brain synaptosomes. In 1981, Ryuji Ueno, a PhD student at that time, investigated the effect of PGD₂ injections into the rat brain on body temperature whereby he linked a decrease of body temperature caused by central PGD₂ to lipopolysaccharide-induced hypothermia in rodents.¹⁵ However, the most surprising observation during these experiments was that the PGD₂-injected rats started to sleep on the test platform.⁸

Osamu Hayaishi immediately began to collaborate with Professor Shojiro Inoue of the Tokyo Medical and Dental University,⁷ who is a world-renowned pioneer in the study of a 'sleep substance' that accumulates in the brain during long periods of wakefulness. In 1998, Yoshihiro Urade took over the position of Department

Head from Osamu Hayaishi, who continued to concentrate on being Director of OBI (Fig. 1), and started a collaboration with Thomas Scammell and Clifford Saper at Harvard Medical School to demonstrate that PGD₂ mediates the activation of a key sleep center, namely the ventrolateral preoptic area (VLPO), and suppresses a key arousal center, that is the tuberomammillary nucleus (TMN), thus demonstrating a functional link between the VLPO and histaminergic arousal center in the TMN.¹⁶ Since the year 2000, Hayaishi, Urade and colleagues, including Zhi-Li Huang and Michael Lazarus (Fig. 2), have used several types of gene-manipulated mice, including: PGD synthase gene knockout (KO) mice and human PGD synthase transgenic mice, DP receptor-KO mice, histamine H1 receptor-KO mice, adenosine A2A receptor-KO mice, adenosine A1-receptor-KO mice and by the creative combination of genetic and pharmacological dissection of signal transduction pathways, elucidated the molecular mechanism of sleep-wake regulation by the PGD₂ and adenosine system (for details, see reviews by Urade and colleagues¹⁷⁻¹⁹ and in this issue, Fuller et al.).²⁰

In 1999 Osamu Hayaishi received the first 'Distinguished Scientist Award', together with Alexander Borbély who proposed a widely accepted model of sleep/wake regulation called the "two process model", at the 3rd Congress of the World Sleep Federation at Dresden, Germany. Standing on the stage with the memorial plaque in his hand, Osamu Hayaishi had a glowing smile on his face because his scientific contribution to the field of sleep research was officially being recognized in the world of sleep research. The legacy of Osamu Hayaishi for sleep research may be best summarized in his own words with elegant humor from a memorable lecture he gave at the age of 91 during the 6th Congress of the World Sleep Federation at Kyoto in 2011: "Sleep is perhaps one of the most important and yet least understood physiological functions of the brain. Sleep is essential for life, but we cannot answer even the simplest questions, such as 'what is sleep?', 'why do we need to sleep?', and most importantly, 'where and how are sleep and arousal regulated?'.



Figure 1. Osamu Hayaishi (left) and Yoshihiro Urade (right) at the library of Osaka Bioscience Institute (OBI), ca. 2005. Osamu Hayaishi was director of OBI from 1993–1998 and also served as the head of the Department of Molecular Behavioral Biology, which focused on elucidating the molecular mechanisms of sleep/wake regulation. In 1998, Yoshihiro Urade assumed the position of Department Head from Hayaishi who concentrated on being Director of OBI. Photo Credit: Yoshihiro Urade, Japan.

Moreover, the number of sleep-disorder patients has recently been increasing exponentially and now exceeds more than 30% of the total population in most countries. More than 107 different sleep disorders have now been described; but in most instances, their etiologies are not yet clearly understood, simply because basic sleep science research has really only just begun. This is the end of my swan song; but studies are still in progress in my own and other laboratories to attain our final goal of understanding in biochemical terms the entire mechanism of sleep-wake regulation. We realize that we have a long way to go, but hopefully, we are on the right track. Lastly, I hope that the PGD₂ level of you did not rise too high during my talk.”

The mentor Osamu Hayaishi

Osamu Hayaishi has shaped the careers of many eminent scientists, serving a life-long role as a mentor who is revered for giving inspiration and advice to young scientists. Hayaishi-sensei, which is the way he is respectfully addressed in Japan by his pupils and peers — the Japanese word *sensei* literally means “person born before another” and is used after a person’s name as a title to refer to a figure of authority, including teachers, doctors and professors —, always said, “You can do anything you want. I can introduce you to whoever you want to collaborate with. I can arrange travel to wherever you want to visit for experiments. Everything is up to you.” These messages were translated to mean that ‘Everything is up to *your* talent in science. I have already set up the scientific environment perfectly except for *your* talent. If the project is not successful, the only one logical reason for the failure is *you*’. This was and remains his strategy for educating young scientists and for example, Michael Lazarus benefited greatly from this policy when he, after 3 y of post-doctoral work with Osamu Hayaishi at OBI, joined Clifford Saper’s systems neurobiology group at Harvard Medical School to identify the brain site for fever²¹ and again when he, after returning to OBI as staff scientist, established successful collaborations and friendship with some of the world’s best scientists in sleep and temperature neurobiology that



Figure 2. Osamu Hayaishi, Clifford Saper, Zhi-Li Huang and Michael Lazarus at Osaka Bioscience Institute (OBI; left to right), ca. 2007. Clifford Saper (from Harvard Medical School) was a speaker at OBI’s Monthly Lecture series on several occasions and was a member of OBI’s Board of Advisors from 2010–2013. Osamu Hayaishi is a great admirer of Professor Saper’s work on the hypothalamic regulation of body temperature, sleep and circadian rhythms.^{32,33} Photo Credit: Michael Lazarus, Japan.

include, to name a few: Andrej Romanovsky at St. Joseph’s Hospital and Medical Center, USA; Pierre-Hervé Luppi, Lyon Neuroscience Research Center, France; Michael Duzsenko at the University of Tuebingen, Germany; and Kazue Semba at Dalhousie University, Canada. Needless to say that all pupils of Osamu Hayaishi and their students consider themselves as one scientific family that, to name but a few, include Professor Hayaishi’s former PhD students Shuh Narumiya at Kyoto University and Takao Shimizu at Tokyo University whose seminal work on prostaglandins and other lipid mediators and their receptors is closely related to temperature, fever and sleep.²²⁻²⁴

Osamu Hayaishi also very much enjoyed the journal club that he created in the Department of Medical Chemistry at Kyoto University and was later continued by many of his students at other institutions in Japan, including OBI. He had introduced a seminar style from the laboratory of Nobel-prize

winner Arthur Kornberg, Osamu Hayaishi’s mentor and life-long friend, to Kyoto University for practical training in scientific debate. The students and scientific staff referred to this seminar as “Hayaishi’s Dojo,” an analogy to *Dojo of Judo or Karate*, the Japanese word *dojo* meaning “exercise hall” in English. This liberal and democratic atmosphere was quite different from the conservative and hierarchical atmosphere prevalent at that time in many other laboratories in Japan.

Even after Osamu Hayaishi celebrated his 90th birthday he continued to come to the lab almost every day to enjoy scientific debate or squeeze the best out of young researchers. Although Hayaishi encouraged young scientists to choose their own area of research, he always reminded them to aim for the highest level of scientific innovation and originality. In Hayaishi’s opinion, “scientists are a curious species, and the harder the problem, the more interesting it looks.”

The Osaka Bioscience Institute

After mandatory retirement from Kyoto University at the age of 63 and a 5-year tenure as leader of a special government project, the 'ERATO Hayaishi Bio-information Transfer Project', Hayaishi became the founding director of OBI in

1988 (Fig. 3), which was established as part of the centennial commemorative project by the City of Osaka. Yasushi Oshima, the mayor of Osaka at the time, wanted OBI to serve as "a second and modern Tekijuku" to educate and nurture future leaders in bioscience. Tekijuku was

an exclusive private school in Osaka during the Edo Period in the 18th century, which provided the only source of Western scientific knowledge during Japan's isolationist era. Tekijuku attracted many ambitious and gifted young people from all over Japan, who later became leaders in major universities and government institutions.

Over 27 y Osamu Hayaishi guided, as director, department head, director emeritus, and chairman of the Board of Trustees of the OBI Foundation, the institute's development into a world leading center of scientific research.²⁵ From the beginning, Hayaishi wanted to build an innovative research institute that chooses to be great instead of big. In contrast to other research institutions in Japan at that time, which offered permanent tenured positions, Hayaishi's idea was to employ young scientists on fixed-term contracts ranging from 3 to 10 years, as he believed that a turnover of scientists is important for innovative research. The institute offered well-paid positions, with an excellent research environment hosting state-of-the-art facilities, and researchers were allowed to choose their own areas of research. Even by contemporary standards this was quite an unorthodox approach to research in Japan. In an unprecedented step for the Japanese academic community in the 1980s and 1990s, all researchers from students up to the head of the department were individually evaluated on an annual basis by an external committee composed of eminent scientists, including Nobel laureates. At that time, Hayaishi's approach with fixed-term contracts without internal evaluation was thought to be too idealistic by some administrators at Monbusho — the former Japanese Ministry of Education — and many doubted whether this approach would succeed. As if to disprove their critics, OBI scientists have made many major breakthroughs over the last 2 decades. Examples include discovery of the basic mechanisms of apoptosis by Shigekazu Nagata²⁶ and clarification of the control mechanism of sleep by Osamu Hayaishi (cf. Section 2 and the review by Fuller et al. in this issue²⁰). The quality of OBI research was highlighted in 2002 when the OBI was ranked at the top in scientific



Figure 3. Osamu Hayaishi was the driving force for the Osaka Bioscience Institute (operated between 1987 and 2015), simply known as OBI, to become a world leading center of scientific research. The unique and elegant building of OBI with metallic external walls (upper photo) was designed by the late Kenzo Tange, perhaps the most famous architect from Japan. At the entrance to OBI stands a sculpture entitled "La Porte d'Espérance" in French, meaning "Gate of Hope" in English. It is a masterpiece by the late Yasuo Mizui, an internationally renowned Japanese sculptor. The lower photo shows members of the Department of Molecular Behavioral Biology, also known as the "2nd Department," which includes Director Emeritus Osamu Hayaishi (center), Head Yoshihiro Urade (right) and Staff Scientist Michael Lazarus (upper left), in the marble entrance hall of OBI, ca. 2010. Photo Credit: Yoshihiro Urade and Yoan Chérasse, Japan.

impact factors for publications on molecular biology and genetics from 1991 to 2001.

OBI played a prominent role during Hayaishi's leadership in promoting career development for a new generation of scientists in Japan and leading Japanese universities have modeled their programs to be consistent with OBI. For example, Yoshihiro Urade and Michael Lazarus are now principal investigators at the World Premier International Research Center Initiative-International Institute for Integrative Sleep Medicine (WPI-IIS) at the University of Tsukuba, where globally prominent scientists from multiple research fields gather under the leadership of Masashi Yanagisawa to solve the mystery of sleep. The WPI program was launched by the Ministry of Education, Culture, Sports, Science and Technology of Japan with the aim of building globally visible research centers, like WPI-IIS and 8 additional centers across Japan, and is directed on the basis of the same principles that Osamu Hayaishi once defined and realized at OBI for the first time in Japan. Because OBI was directed by Osamu Hayaishi to be international, the legacy of OBI and its founding director also lives on in many laboratories around the world with principal investigators trained at OBI; most prominently in China at the Shanghai Medical College of Fudan University, where Zhi-Li Huang has established an OBI-style sleep laboratory, the biggest in China. There students and young scientists are trained by Zhi-Li Huang in the same way as he was taught by his mentor Osamu Hayaishi. Another trainee of Osamu Hayaishi is Bruno Kilunga Kubata who discovered the metabolism of arachidonic acid in parasitic protozoa at OBI²⁷ and is now a professor at Kinshasa University and the Chief Executive Officer of Biosciences Eastern and Central Africa Network of the African Union. As of April 1, 2015, OBI has

officially closed its doors over lack of financial support by Osaka City.

Concluding remarks

The authors of this article would like to express their deep gratitude to their mentor Osamu Hayaishi who has generously supported their research over many years. We hope that our past, current and future progress in the understanding of humoral and neuronal mechanisms of sleep/wake regulation^{17,19,28-32} will provide great happiness and satisfaction to Osamu Hayaishi.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Kristopher McEown for editorial help and WPI-IIS for administrative assistance with this manuscript.

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