Impact Objectives

- Integrate our understanding of the many temperature-dependent phenomena that affect mammalian biological systems, so as to better understand how high precision regulation of temperature in cells may be used as a therapeutic treatment of common illnesses
- Further develop the less locally destructive hyperthermia treatment – the increasing of cell temperature – as a safer treatment of choice for many cancer patients
- Continue investigations into the application of capsaicin – the active component of chillies – as a local heating affect

Understanding the significance of temperature in living organisms

__Professor Makoto Tominaga__ and his team at the __Okazaki Institute for Integrative Bioscience__ (National Institute for Physiological Sciences) in Japan, are passionate about harnessing the potential therapeutic properties of temperature control

Can you share a little about your own background and how you came to take up your current position at the Okazaki Institute for Integrative Bioscience (National Institute for Physiological Sciences)?

My lab is working on thermosensitive TRP (Transient Receptor Potential) channels. There are 27 TRP channels in six sub-families in humans (TRPC, TRPV, TRPM, TRPML, TRPP and TRPA), and surprisingly, 11 of those are reported to be activated or modulated by temperature. Therefore, they are called thermosensitive TRP channels. I was an electrophysiologist working on some cation or anion channels. When I joined the David Julius Lab at the University of California in San Francisco, US in 1996, I became involved in the project of cloning the capsaicin receptor. The isolated gene encoding of an ion channel with six transmembrane domains in each subunit, and termed VR1 or vanilloid receptor 1. Surprisingly, the channel was found to be activated by heat over 43°C, a temperature known to cause pain in humans and monkeys. It was the first ion channel activated by heat directly since the channel activity was observed in the excised membrane patches. I believe I am the first person to look at the channel opening by heat. As many people know, the pungent sensation caused by chemical capsaicin, a main ingredient of hot chili peppers, is not a taste but a kind of pain. Therefore, VR1 (later called TRPV1) can be viewed as a receptor for nociceptive stimuli. It is called 'hot' for both spiciness and thermal hotness. And the wording was proven correct because both spicy stuff and high temperature activate the same protein TRPV1.

Could you explain more about the 'temperature sensing' strand of your work? Why is addressing temperature sensing mechanisms by focusing on plasma membrane molecules, intracellular molecules and intracellular metabolic pathways, an area which needs to be explored?

Temperature sensing can be done either by plasma membrane molecules, intracellular molecules or intracellular metabolic pathways. In my opinion, plasma membrane molecules, such as thermosensitive TRP channels, are the best option. And it is easy to imagine the relation of the functions of plasma membrane molecules and cell functions. Since thermosensitive TRP channels are non-selective cation channels with high calcium permeability, Na^+ influx can cause depolarisation necessary for action potential generation and Ca^{2+} influx leads to a lot of Ca^{2+}-dependent events in the cells including transcription factor regulation.

Could you explain more about the temperature-responding side? What information is so far known about how temperature affects metabolic function?

Na^+ influx leads to depolarisation of the neurons, and Ca^{2+} entering the cells through channels is involved in many cellular events through binding to a lot of Ca^{2+}-binding proteins. The events occurring in the cells vary depending on the channels expressed and cytosolic cell conditions.

What major diseases will benefit from

“The project will clarify the physiological significance of the uneven temperature distribution from the cellular level to the whole body level”

further investigation into thermal biology?

Body temperature regulation is a key issue in the medical field, especially during systemic infection. Now, physicians don’t strictly regulate body temperature because body temperature increase is often beneficial to control our immune system. However, we still have a lot to learn about the mechanisms for body temperature regulation. Little is known about the mechanisms of febrile convulsions. In the case of brain injury, patients experience high fever, however brain temperature regulation is not established. Hyperthermia is one option of cancer treatment, yet its mechanisms and a better way to control cancer temperature without side effects are not well established.

Could you explain more about how your work may be able to help tackle the obesity problem in the developed world?

Sympathetic nerve activation leads to the thermogeneration in brown adipose tissues followed by a reduction of body weight. Taking capsaicin is a simple way to induce afferent sensory nerve activation through TRPV1 activation, which causes efferent sympathetic nerve activation followed by thermogenesis. However, capsaicin is really pungent and we cannot take much. Capsiate, obtained from CH-19 sweet pepper, has the ability to cause activation of TRPV1 and TRPA1 and is now available from Ajinomoto Company as a supplement. TRPM8 activation and cold stimulation are also known to cause thermogenesis. We can make an ideal way for thermogenesis through the research of thermal biology.

What information do you hope to gauge from your research that will be useful to investigations into diabetes?

Warmth-sensitive TRPM2 channels are involved in insulin secretion in pancreatic beta-cells. It has long been known that measurement of insulin secretion in rodents should be done at 37°C, but the reason is unknown. We have proposed the importance of body temperature for insulin secretion. The first step in treatment of mild diabetes is exercise. People believe exercise increases blood glucose uptake to muscles. At the same time, exercise increases body temperature. We think small body temperature increases are important for insulin secretion from the pancreas through TRPM2 activation. We should soon have more information about TRPM2 involvement in pancreatic functions.

What role has collaboration played in the project’s success?

Our big research group of ‘thermal biology’ started with an expectation of dense collaboration with researchers from different fields, such as engineering (making a device to measure temperatures), molecular biology, cellular biology, behavioural analysis, human diseases, electrophysiology and biophysics. Such chaotic conditions will hopefully lead to a new science. Indeed, the research group member demonstrated the first intracellular temperature mapping and uneven intracellular temperature distribution. The project will clarify the physiological significance of the uneven temperature distribution from the cellular level to the whole body level.

Thermal Biology: Investigating the effects of temperature sensation on living organisms

Understanding how temperature-sensation fluctuations impact organisms, and mammals in particular, has become a key field in biology. ***Professor Makoto Tominaga*** *and his team are investigating how thermal changes in the local environment drive physiological behaviour in animals*

Professor Makoto Tominaga is passionate about his work. His investigations into the extremes of heat and cold sensation have raised interest in how the receptors in our cells may be used to combat many issues in the body. Transient Receptor Potential (TRP) channels are a group of ion channels – pore forming proteins – located through the walls of the cytoplasmic membranes of animal cell types. It is these that mediate many of the sensations we feel, including warmth or coldness, pheromones, osmolality, touch and pain.

After the discovery of the first thermoTRP channel – TRPV1 – in rats, it was established that animals have evolved sophisticated physiological systems for sensing ambient temperatures (thermosensation) since changes in environmental temperature affect various biological processes that affect them. Following this finding, research into other thermosensitive ion channels was conducted, resulting in the identification of 11 thermoTRP channels in rodents and humans, and with that came the realisation that ThermoTRP channels are an evolutionary aspect which drives much of the behaviour in many of Earth's life forms.

In all, there are 27 known TRP channels in humans, which are divided into six sub-families, termed TRPC, TRPV, TRPM, TRPP, TRPA and TRPML. Several sub-sets of TRPs have high sensitivity to changes in the local environment and are the drivers behind the thermosensitivity of those cells. One sub-set, TRPV1 – often referred to as the capsaicin receptor – has been shown to be particularly relevant when considering mediating hyperalgesia responses to inflammatory pain. There is now much interest in how TRPV1 and possibly other thermosensitive TRP channels may help provide new and novel therapeutic options used to treat many areas of clinical pain.

Different TRP channels are driven towards different thermosensitive directions, with TRPV3 low down on the heating side of the range and TRPV2 several degrees higher up. Conversely, TRVM8 is at the start of the transition to cooler sensed temperatures, while TRPA1 potentially activates much lower down the cooling side of the scale. TRPV1 is commonly referred to as the capsaicin receptor, while TRPM8 is called the menthol receptor. However, these receptors are species dependent and seem to respond differently depending upon which biological system they are sited in. While the actions of these receptors is well known, the extent of their possible application is less understood, and that it where Tominaga and his team come in.

> ***"There is much interest in how TRPV1 and other thermosensitive TRP channels may help provide new and novel therapeutic options used to treat many areas of clinical pain"***

Tominaga is Division head of the Okazaki Institute for Integrative Bioscience (National Institute for Physiological Sciences), Japan, and is running joint projects on temperature sensing in cells, and temperature responding systems. The ultimate goal of these two projects is to integrate our understanding of the many temperature-dependent phenomena that affect mammalian biological systems.

TRPV1, also known as the capsaicin receptor, is found in hot chili peppers

This connection will become the starting point for further research into the high precision regulation of temperature in cells, and how it may be used in a number of therapeutic systems. This is important research for the sufferers of many common illnesses, but is becoming increasingly important as an adjuvant treatment to chemotherapy and radiation treatment for certain forms of cancer. Since hyperthermia – the increasing of cell temperature – is far less locally destructive than either of the other treatments, it may become a therapy of choice.

TWIN-PRONGED APPROACH

Tominaga's interdisciplinary research project is set to investigate both hot and cold sensing across a range of biological systems including simple molecular and whole organism levels, and will consider how they respond to temperature alongside how temperature is sensed. The first aspect of the investigation, referred to as A01, will be achieved by focusing on molecules of the plasma membrane, intergranular molecules and intergranular metabolic pathways, seeking to develop means of detecting and regulating local temperatures at a cellular level. The second part of the research – termed A02 – will examine the neural pathways that amalgamate information on ambient temperature, how temperature affects metabolic functions, and the biological rhythms and mechanisms that are used in emotion formation. The team also hope to clarify communications between temperature-responding systems and further develop methods of detecting and regulating local temperatures in cells and organs.

CONTROLLING TEMPERATURE

Generally, body temperature in mammals ranges from 36°C to 40°C, with very little variance found under normal bodily circumstances. Fever and infection can drive the human body up to around the 40°C mark, but this as a whole body condition can be highly detrimental, and temperatures that high should be quelled as quickly as possible. While it is thought only skin and sensory neurons are able to gauge high or low temperatures, Tominaga's team are investigating whether cells deep in the body, and those unusually only exposed to a constant temperature can also sense, and may be stimulated by local changes in temperature. It has been shown that even small changes in temperature to the TRPM2 of pancreatic beta cells can stimulate the secretion of insulin, and elevated temperatures in mammalian bodies is a trigger for the immune system. The team have also reported that TRPM2 expressed in macrophages has been shown to be involved in the release of cytokines – the small proteins associated with cell signalling – and phagocytosis.

This has real-world applications, as temperature regulation on a cellular level can have profound effects. Hyperthermia has been shown to be effective against some kinds of cancer cells, and with the ability to be able to target specific cell areas and change their temperature in

> *'The ability to regulate and apply minute stimulation to different cell areas of the body has become a key issue in medicine'*

respect to the body as a whole, becomes increasingly important. Temperature is a fundamental physical driving force behind every biological reaction within living cells. Thus, temperature distributions inside a living cell reflects the thermodynamics and functions of cellular components. Previous research has demonstrated that the intracellular temperature distribution between the nucleus and centrosome of COS7 cells, both showed a significantly higher temperature than the cytoplasm and that the temperature gap between the nucleus and the cytoplasm differed depending on the cell cycle. Therefore, intracellular temperature mapping of living cells should promote better understanding of cellular events and the establishment of novel diagnoses and therapies.

temperature as a whole to defeat an infection and boost the immune system, and that gives an indication that temperature stimulation may have significant positive affects when applied to small areas. The possibilities for cancer treatments that target almost individual cells are obvious and heat treatment is unlikely to exhibit the damaging side effects associated with traditional cancer treatments.

As well as a new tool in the fight against cancer, thermal stimulation may be key to new weight loss and weight management treatments. Work by Tominaga and others has shown that activation of sympathetic nerves using capsaicin can lead to thermogeneration of brown adipose tissue, which in turn can aid weight loss. One of the main barriers to this becoming an acceptable treatment has been the means by which it is introduced into the body. Applying raw capsaicin is both painful and potentially dangerous to the patient, so an alternative means was needed. This has led to interest in refining lower-scoville alternatives, and capsiate obtained from CH-19 sweet peppers has now been successfully extracted and made into tablet form in a commercial venture. This has been found to be effective at activating the TRPV1 and TRVA1 channels, and with cold stimulants for the TRPM8 channel also being researched, that too may be used in therapy.

> *"As well as a new tool in the fight against cancer, thermal stimulation may be key to new weight loss and weight management treatments"*

But how is it practical to raise cell temperature without affecting the body in general? Previous research has shown that hot water is an effective means of activating TRPV1, but this is impractical to use on small areas of the body. However, similar results can be achieved by the application of capsaicin, the active component of chillies that produces a succinct sensation of burning pain, and it is this that Tominaga's team hope to employ to instigate a local heating affect.

The mechanism of heating has been subject to much research, and because the TRPV1 channel is a non-selective cation channel it was thought that a positive sodium ion flux (Na^+) through the pore caused depolarisation. However, further investigation has shown that calcium ions (Ca^{2+}) transferring through the TRPV1 channel cause a chloride efflux through the activation of the CA^{2+}-activated chloride channel. This transport mode works because intracellular chloride concentrations tend to be high in sensory neurons, leading to depolarisation.

THE BENEFITS OF TEMPERATURE CONTROL

The ability to regulate and apply minute stimulation to different cell areas of the body has become a key issue in medicine. We know the body will increase

Thermosensitivity in the medical world is really only in its infancy, and with further work also looking at how manipulation of the channels might help in the fight against other illnesses such as diabetes, this is a field that is going to see a lot of input. With Tominaga's team at the forefront of research, it is a field that we will be hearing a lot more of in the near future.

Project Insights

FUNDING

- A Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology in Japan (#15H02501)
- A Grant-in-Aid for Scientific Research on Innovative Areas 'Integrative understanding of biological phenomena with temperature as a key theme' (#15H05928)

COLLABORATORS

Okazaki Institute for Integrative Bioscience, Japan

CONTACT

Professor Makoto Tominaga

T: +81-564-59-5286
E: tominaga@nips.ac.jp
A: Division of Cell Signaling, Okazaki Institute for Integrative Bioscience (National Institute for Physiological Sciences), National Institutes of Natural Sciences, Higashiyama 5-1, Myodaiji, Okazaki, Aichi 444-8787 JAPAN

PROJECT COORDINATOR BIO

Professor Tominaga worked as a cardiologist for several years after graduating Ehime University School of Medicine, Japan in 1984. He then became a physiologist in Kyoto University, Japan. He joined the Dr Julius Lab at UCSF in the US in 1996 and became a professor of Physiology at Mie University School of Medicine, Japan in 2000, and then moved to Okazaki Institute for Integrative Bioscience, Japan in 2004.