Japan Academy Prize to:

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for "Regulatory Mechanism of Calcium Concentration inside the Cell"

Outline of the work:

When cells are stimulated IP_3 is produced (inside the cell) and subsequently the intracellular Ca^{2+} concentration increases - the later initiates/modulates a plethora of diverse physiological responses. It is now known that the IP_3 receptor regulates Ca^{2+} release from the endoplasmic reticulum, a major intracellular Ca^{2+} store, and hence plays an important role in living systems. However despite its importance, the mechanism through which IP_3 increases intracellular Ca^{2+} concentration was still unknown 20 years ago. Initially the 'IP_3 receptor' was hypothesized to be a target molecule of IP_3, and presumed to release Ca^{2+} from an unknown intracellular Ca^{2+} store. Dr. Mikoshiba's contribution to unraveling the mechanism of IP_3 mediated Ca^{2+} increase, was the identification of the IP_3 target molecule and its role as a Ca^{2+} release channel. His work on this subject originated from an analysis of the protein P400 (now named the IP_3 receptor), which is greatly decreased in the cerebellum of ataxic mutant mice. Dr. Mikoshiba started his studies with the purification of P400 and the generation of specific monoclonal antibodies against it. He then went on to make a key finding, by demonstrating that P400 is an IP_3 binding protein and a functional Ca^{2+} release channel he underlined P400 as the IP_3 receptor. Notably, the IP_3 receptor was shown to be located on the endoplasmic reticulum. Dr. Mikoshiba went on to determine the entire sequence of the IP_3 receptor, by cDNA cloning (1989).

Now that he had identified the IP_3 receptor, Dr Mikoshiba and colleagues focused their attention on obtaining an in-depth description of the relationship between IP_3 receptor structure and function. Analysis of the detailed structure of the IP_3 receptor identified it as an allosteric protein that changes its form (from windmill to square form) reversibly in the presence and absence of Ca^{2+}. These studies revealed a unique property of the IP_3 receptor, that even though its covalent bond is cleaved by trypsin into several peptides, they can reassemble to form functional IP_3-induced Ca^{2+} release channel. Dr. Mikoshiba's group also succeeded in conducting a three dimensional crystallographic analysis of both the IP_3 binding core and the regulatory region of the IP_3 receptor, and from this clarified the IP_3 receptor pore gating mechanism. The fresh knowledge was further utilized to construct a new IP_3 sensor, developed around state of the art FRET (fluorescence resonance energy transfer) technologies.

Dr. Mikoshiba's analysis, based on developmental biology, demonstrated that the IP_3 receptor is a Ca^{2+} oscillator and essential in: fertilization; dorso-ventral axis determination at the 4- to 8- cell stage after fertilization; cell division; and neurite extension. Furthermore IP_3 receptor type 1 deficient mice showed: cerebellar ataxia, demonstrating that the type 1 receptor is important for neuronal plasticity; an abnormal secretion of the nerve growth factor, BDNF. Comparatively, the analysis of IP_3 receptor type 2 and type 3 double deficient mice revealed an absence of exocrine function and a phenotype similar to that of Sjögren syndrome (which is characterized by dry eyes and dry mouth). Interestingly Sjögren syndrome patients were recently shown to have antibodies (in

their blood) against the IP_3 receptors.

Dr. Mikoshiba recently expanded the known role of the IP_3 receptor by the discovery of an endogenous pseudo IP_3, IRBIT (IP_3 receptor binding protein released with inositol 1,4,5-trisphosplate) that binds to the IP_3 binding core and is released following IP_3 application. IRBIT not only regulates the amplitude and frequency of Ca^{2+} oscillation, but also acts as a tertiary messenger - activating the Na^+HCO^- cotransporter 1 and thereby regulating the acid-base balance. To re-iterate this important new finding, the function of IP_3 is not only to release Ca^{2+} but also to regulate acid-base balance. Furthermore, redox (oxydo-reduction) regulation was considered to be independent of Ca^{2+} signaling, but Dr. Mikoshiba linked the two signaling systems by discovering ERp44 as a new redox sensor that regulates the IP_3 receptor. In an effort to complete the list of IP_3 receptor interacting proteins, Dr. Mikoshiba has now screened the proteins associated with the IP_3 receptor and identified various molecules such as 4.1N, TRP channel, and glutamate receptors. These findings suggest that the IP_3 receptor is closely linked with many other signaling pathways and various diseases.

In conclusion, Dr. Mikoshiba and his collaborators, 20 years ago, discovered and sequenced the IP_3 receptor – an IP_3 gated Ca^{2+} channel that is located on the endoplasmic reticulum. He further revealed the detailed three-dimensional structure by concerted studies involving X-ray crystallographic analysis and cryo-electron microscopy. Based on Dr. Mikoshiba's expertise in developmental biology and biochemistry and his unique and original ideas, he and his collaborators developed and advanced research on "Regulatory Mechanism of Calcium Concentration inside the Cell."

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