氏　　名	佐々木　真理
学位（専攻分野）	博士（理学）
学位記番号	総研大甲第965号
学位授与の日付	平成１８年３月２４日
学位授与の要件	生命科学研究科　生理科学専攻 学位規則第６条第１項該当
学位論文題目	Identification and characterization of two novel voltage-sensing proteins : voltage-regulated phosphatase and voltage-gated proton channel
論文審査委員	主　査　教授　久保　義弘 教授　岡村　康司 教授　岡田　泰伸 教授　山下　直秀(東京大学)

論文内容の要旨

Identification of two novel voltagesensing proteins : voltage-regulated phosphatase and voltagegated proton channel

It is known that common structure is conserved among all known voltage-gated ion channels : the fifth and sixth transmembrane segments (S5 and S6) and the loop region form the hydrophilic pore and the first four transmembrane segments (S1-S4) constitute the voltage-sensor domain. The typical feature of the S4 segment is that positively charged amino acids are periodically located at every third position.

In systematic survey of ion channel genes from *Ciona intestinalis* genome, several genes showed homology to ion channel genes but could not be categorized into any family of known ion channels. One such gene was named Ci-VSP (Ciona Voltage-sensor containing Phosphatase). Ci-VSP consists of four transmembrane segments, and in the second and third transmembrane segment (S2 and S3) there are negatively charged amino acids, and in the fourth transmembrane segment (S4), four positively charged amino acids were periodically aligned at every third position, which is signature sequence of voltagesensor of the voltage-gated ion channel. However, in spite of having homology to the voltage-sensor domain of the voltage-gated channels, Ci-VSP lacked pore domain. C-terminal cytoplasmic domain of Ci-VSP has homology to PTEN (Phosphatase and Tensin homolog deleted on chromosome Ten, which has been known as PIP_3 phosphatase. In this study, she cloned Ci-VSP and characterized its function focusing on the voltage senor. In case of voltage-gated ion channels, gating currents are observed based on the conformational change of the S4 segment. At first, to test whether Ci-VSP can sense the membrane potentials or not, I tried recoding the gating current from Ci-VSP by the the two-electrode voltage-clamp method, and obtained the current traces similar to those of the gating currents of voltagegated ion channels. Quantitative analysis was performed by the cut-open voltage-clamp method with which intracellular part can be perfused to eliminate the intrinsic outward currents. The charge (Q)-voltage (V) relationship (Q-V curve) from these currents was fitted with Boltzmann function, saturating at high membrane potentials. Q-On and Q-Off were identical. These results prove that Ci-VSP induced currents are 'gating currents' based on the conformational change of Ci-VSP protein. When two out of four positive charges in S4 were mutated to neutral ones, gating currents were fully eliminated, suggesting that positively charges in S4 are important for sensing the membrane potential change. Some of the other S4 mutants replacing the positively charged arginine to neutral glutamine showed different voltage-dependency or different kinetics from wild type. These results suggest that positive charges in S4 are critical for sensing the membrane potential change and the positive charges in S4 contribute to gating currents. SHE also show that only four transmembrane segments (S1~S4) of Ci-VSP are enough to sense the membrane potential. This also suggests that only four transmembrane segments can function as an independent functional unit of the voltage-sensor.

During studying the molecular function of Ci-VSP, other genes were found from mouse EST having homology to voltage-sensor domain. One such case was the mouse cDNA, RIKEN cDNA 0610039P13, putatively encoding a protein that consisted of four transmembrane segments with homology to the voltage-sensor domain. However, this gene lacked any structure similar to the pore domain and moreover, it had almost no C-terminal cytoplasmic region. So this gene was named mVSOP1 (mouse Voltage-sensor domain Only Protein). In tsA201 cells expressing mVSOP1, depolarizing step pulses induced slowly-activating outward currents accompanied by tail inward currents at repolarization. Tail currents measured under different pH conditions were reversed at voltage levels corresponding to equilibrium potentials for protons that are predicted from Nernst equation, indicating that these currents are through proton-selective channels. Imaging analysis demonstrated that pH_{in} recovers after an acid load and recovery was faster after membrane depolarization in mVSOP1-transfected cells. These results proved that VSOP1-induced currents were voltage-dependent proton currents. VSOP1-induced currents showed hallmark features of native voltage-dependent proton channel; shift of threshold and kinetics of activation occurred dependent on the pH difference across the cell membrane. VSOP1 shares other properties with native voltage-dependent proton currents, including sensitivity to polyvalent cations such as Zn^{2+} and Cd^{2+} and relatively high temperature sensitivity. A neutralizing mutation of a positively charged residue in S4-like segment rendered negative shift of activation by about 50 mV, causing significant inward current. This indicates that this site is critical for the channel to be kept close at the membrane potential where the direction of the driving force for proton flow is inward, consistent with the previous view that outward rectification of voltage-dependent proton channel is based on its gating nature. VSOP1 was expressed predominantly in blood cells including macrophage exhibited by quantitative RT-PCR. This expression profile is consistent with previous studies in which native proton currents have been reported in many blood cells. She therefore propose that VSOP1 constitutes the main molecular component of voltage-dependent proton channel.

Before Ci-VSP and VSOP1 were identified, the voltage-sensor is the molecular structure exclusively found in the voltage-gated ion channels. Discovery of VSP and VSOP suggest that membrane potentials and voltage-sensor play more important and diverse roles in physiological conditions.

論文の審査結果の要旨

膜電位依存性チャネルは、興奮性細胞等の重要な機能素子であり、多数のメンバーを含む大きなファミリーを構成している。一次構造的には、ひとつのユニットに６つの膜貫通部位を持ち、その中の陽電荷を持ったアミノ酸に富む４番目の部位 S4 が、膜電位センサーとして機能していること、S5, S6 を含む後半部位がチャネルの穴(ポア)を形成していることが知られている。申請者の所属する研究グループは、ユウレイボヤゲノムデータベースの探索によって、S4 を含む４つの膜貫通部位のみを持ち、ポア領域を欠くという極めて興味深い一次構造を有し、機能が全く未知のふたつの遺伝子を見いだした。申請者佐々木真理氏は、アフリカツメガエル卵母細胞等の in vitro 発現系を用い、これらの蛋白の持つ分子機能の電気生理学的解析を行った。

第一の分子 Voltage Sensor Protein (VSP) は、S4 直後の細胞内領域に、phosphatase 活性を持つ PTEN 分子に類似したドメインを有しており、申請者の所属するグループの共同研究者により、膜電位依存的に phosphatase 活性が調節されることが示された。申請者は、VSP のゲート電流の測定を行い、特異な一次構造にも関わらず、他の膜電位依存性チャネルと同様なゲート電流があることを示した。さらに、S4 の陽電荷を持つアミノ酸を系統的に変位させた実験の結果に基づいて、VSP においても、S4 が膜電位センサーとして機能していることを結論した。

第二の分子 Voltage Sensor Only Protein (VSOP) は、S4 の後に、短い細胞内領域のみを有するもので、チャネルポア領域は存在せず、その機能は未知であった。申請者は、ホヤおよびマウスの VSOP の遺伝子を培養細胞に発現させて、その機能にパッチクランプ法によりアプローチし、ポア領域がないにもかかわらずイオン電流があることを示した。そして、この電流は、種々の陽イオンや陰イオンの置換により変化せず、細胞内外の pH に依存して変化することから、プロトン電流であることを結論した。さらに、申請者は、ゲートが、絶対な膜電位や、絶対的な pH で決定されるのでなく、細胞内外の pH の勾配を感知していること、および S4 領域の陽電荷を持ったアミノ酸の変異により、膜電位依存性チャネルの場合とは非常に異なる変化がみられることから、そのゲート機構が特異なものであることを示した。また、S1 領域の点変異によりプロトン電流が消失したため、この部位が、プロトンのイオン透過に関わっている可能性を考察した。最後に、VSOP が血球系の細胞等に高発現しているに基づいて、その生理的意義について推察した。

このように、本研究は、これまで機能が知られていなかった、VSP, VSOP という分子の機能解析を行ったものである。VSP については、S4 が膜電位センサーとして機能していることが明らかにされ、VSOP については、分子実体が未同定で、その解決が待ち望まれてきたプロトンチャネルであることが明らかにされた。イオンチャネルの研究分野の新たな展開を開くことが期待されるすぐれた研究である。審査委員会は、全員一致で、本論文が学位論文として相応しいものであると判断した。