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学位論文題目	Control of growth and assembly of influenza virus: Role of viral proteins
論文審査委員	主　査　教　授　堀内賢介 教　授　瀬野悍二 教　授　廣瀬　進 助教授　藤山秋佐夫 教　授　水本清久（北里大学）

論文内容の要旨

Roles of viral NS and M proteins on the influenza virus growth were examined. The NS proteins of influenza virus, NS1 and NS2, are encoded by RNA segment 8. The NS1 protein is encoded by a colinear mRNA transcript, whereas the NS2 protein with the molecular weight of 14.2 kilodaltons (kDa) is synthesized after splicing of NS1 mRNA. Up to now, these NS proteins are believed to exist only in virus-infected cells. The NS1 protein, which localizes in nuclei of virus-infected cells, recognizes the *cis*-acting sequence on NS1 mRNA and controls its splicing to NS2 mRNA. NS2 is also present mainly in the nuclei. At present, however, little is known on the function of this protein. This study indicates that the NS2 protein, previously considered as one of the two nonstructural proteins (NS1 and NS2), exists in virus particles as a structural component. By immunochemical method, the number of NS2 molecules in a virus particle was estimated to be 130-200 molecules. After solubilization of viral envelope, NS2 was still associated with ribonucleoprotein (RNP) cores, but was later dissociated from RNP upon removal of the membrane M1 protein. A filter-binding assay *in vitro* indicated direct protein-protein contact between M1 and NS2. Following chemical cleavage of the M1 protein, NS2 was found to bind only a C-terminal fragment of M1. By an immunoprecipitation method, NS2-M1 complexes were also detected in virus-infected cell lysates. These observations altogether indicate specific molecular interaction between M1 and NS2, suggesting that NS2 regulates the function of M1 or vice versa.

The M gene of influenza viruses encodes 2 proteins, M1 and M2. The M1 protein is tightly associated with virions forming a matrix, which associates with RNP at its internal surface but interacts with envelope at its external surface. M1 interacts with both NP, thereby interferes with the function of RNP-associated RNA polymerase, and NS2 in virions. In virus-infected cells, M1 is involved in both early (uncoating and import of RNP into infected nuclei) and late (assembly of virions during maturation and export of RNP from nuclei into cytoplasm) stages of virus growth. On the other hand, M2 forms an ion channel and is considered to control the transport of hemagglutinin (HA). M2 may also control uncoating step to release RNP in the early phase of virus infection. Genetic studies described in this report suggested that one or both of the M proteins have a regulatory role(s) of the rate of virus growth. Influenza virus A/WSN/33 forms large plaques (>3mm diameter) on MDCK cells whereas A/Aichi/2/68 forms only small plaques (<1mm diameter). Fast growing reassortants (AWM), isolated by mixed infection of MDCK cells with these two virus strains in the presence of anti-WSN antibodies, all carried the M gene from WSN. On MDCK cells, these reassortants produced progeny viruses as rapidly as did WSN, and the virus yield was as high as Aichi. Pulse-labeling experiments at various times after virus infection showed that the reassortant AWM started to synthesize viral proteins earlier than Aichi. To determine which of the two M proteins, M1 or M2, is responsible for the fast rate of virus growth, an attempt was made to make recombinant viruses possessing the chimeric M gene between WSN and Aichi. For this purpose, I employed a newly developed RNA-transfection method into helper virus-infected cells. The ts-mutant derived from WSN, ts51, carrying the ts lesion

only in the M gene, were used as a helper virus to rescue the chimeric M gene RNA. A transfectant virus carrying a chimeric M gene consisting of WSN-M1 and Aichi-M2, CWA20, was generated by using an improved reverse genetics system. The CWA20 virus formed large-sized plaques, indicating that the M1 protein, but not the M2 protein, was responsible for this rapid growth of WSN-type. Taken together, I conclude that the reassortant viruses entry into growth cycle faster than the parent Aichi strain, presumably due to rapid uncoating of the M1 protein from RNP cores. As a result of rapid uncoating, the reassortant RNP should be transported into host nuclei faster than Aichi RNP, ultimately leading to an early onset of transcription of the viral genes.

論文の審査結果の要旨

安田二朗氏は、インフルエンザウイルスがコードする蛋白質の内、従来最も解明されていなかったＮＳ２及びＭ１蛋白の機能を明かにする目的で研究を進め、いくつかの重要な知見を得た。先ず、ＮＳ２がウイルス粒子の構造蛋白であり、粒子当り 130－200 分子含まれることを、免疫化学的方法を用いて見いだし、これが粒子中においても、また感染細胞溶出液中においても、ウイルスの主要な構成蛋白の一つであるＭ１と挙動を共にすることを明かにした。また試験管内において、ＮＳ２が特異的にＭ１に結合すること、その際Ｍ１のカルボキシ末端側に結合することを示した。一方、Ｍ蛋白については遺伝学的研究を行い、増殖速度の異なる２種類のインフルエンザウイルスの組み換え体を巧妙なセレクションを用いて分離し、それらのゲノム解析の結果から、Ｍ蛋白がウイルスの増殖速度を制御していることを見いだした。また、この増殖速度の違いはウイルス蛋白の合成開始時期の違いによることを、パルスラベル実験によって示した。Ｍ蛋白にはＭ１とＭ２の２種類があるが、Ｍ１とＭ２のキメラウイルスを構築することにより、増殖速度を制御するのはＭ１であることを示した。Ｍ１はウイルス粒子の殻膜を構成し、その内面ではリボヌクレオ蛋白（ＲＮＰ）から成る核に結合し、外面では外被蛋白に接している。Ｍ１は感染初期においてはＲＮＰの遊離及び細胞核への移行に、後期においてはＲＮＰの成熟及び細胞質への移行に関与すると考えられており、安田氏はこれらの結果に基づき、Ｍ１がＲＮＰの遊離速度に影響することによってウイルスの増殖速度を制御する機構を提唱している。安田氏の提出した論文は博士論文としての条件を充分に満たすものであることを、審査員全員一致で認めた。