exosomes. Proposed functions of these vesicles include roles in cell-cell signalling, removal of unwanted proteins, and the transfer of pathogens between cells. One such pathogen which exploits this pathway is the prion, the infectious particle responsible for the transmissible neurodegenerative diseases such as Creutzfeldt-Jakob disease (CJD) of humans or bovine spongiform encephalopathy (BSE) of cattle. Here we have investigated the generation of infectious prions in a cell culture system and characterised the forms of prion protein packaged into exosomes. We find that exosomes from these infectious cells are capable of transmitting infection to further uninfected cells suggesting cellular co-factors necessary for the generation of prion infectivity are contained within these vesicles. This work is mirrored by our studies on another protein involved in neurodegenerative disease, the amyloid precursor protein (APP) which is associated with Alzheimer's disease (AD). These results provide further evidence for a novel pathway in which PrP and APP fragments are released from cells and has implications for the analysis of APP processing and diagnostics for prion and Alzheimer's disease.

1SA2-04 プリオン感染における株多様性の存在と株間干渉現象の意義 Meaning of Strain diversity and Interference Seen in Prion Infection

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Each strain among infectious agents responsible for transmissible spongiform encephalopathies (TSE) has distinct biological characteristics. Because the pattern of histopathological changes including deposition of abnormal prion protein (PrP^{Sc}) in the brain is noticeably different and reproducible, it has been suggested that each strain has a unique cell tropism, however, the mechanism underlining this phenomenon are unknown. Mouse-adapted scrapie agents, 22L and Chandler isolate could infect Neuro2-a cells overexpressing mouse-PrP (N2a58) cells more efficiently rather than a neuroprogenitor cell line (1C11). Contrary, a human TSE agent, Fukuoka-1 (FK-1) could efficiently infect 1C11 and a hypothalamic neuronal cell (GT1-7 cells) but not to N2a58. In addition to the cell-tropism, virus-like "interference" between strains has also observed in pure cell culture system, and this virus-like phenomenon was independent from PrP-res. Furthermore, we found the innate immune responses to the prion-infection could be involved in latent infection of prion. These data suggest that infectious agent has quit similar properties to virus and transmission could be controlled by unknown factors other than PrP.

1SA2-05 強い神経細胞死活性を持つアルツハイマー病患者に由来する アミロイドβ凝集体

High-mass amyloid beta-protein assembly with a unique toxic surface from Alzheimer's disease brains

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Alzheimer's disease (AD) is the most common human neurodegenerative disease causing progressive dementia in the elderly. Conversion of amyloid β -protein ($A\beta$), a physiological derivative of amyloid precursor protein (APP), into neurotoxic supramolecular $A\beta$ assemblies is suggested to be a key event initiating AD. Extensive preclinical data suggested that $A\beta$ assemblies acquire a surface structure that is not present in a physiological $A\beta$ monomer and thereby induces synaptic impairment and eventual neuronal loss by interacting with neurons through their surface. Besides $A\beta$ fibrils, soluble forms of $A\beta$ assemblies with neurotoxicity have been produced in vitro or isolated from APP-expressing hamster cells and AD-model mice. These studies all support the toxicity of nonfibrillar $A\beta$ assemblies. However, although dimers that induce synaptic impairment without neuronal loss were recently isolated from AD patient brains, $A\beta$ assemblies that cause neuronal loss have not been isolated from AD patient brains. Therefore, it is essential to isolate the actual $A\beta$ assemblies present in human AD brains and to characterize their native properties in order to understand the mechanisms underlying $A\beta$ neurotoxicity and to develop more effective and safe therapies. We report here isolation and characterization of patient-derived, toxic, high-mass $A\beta$ assemblies.

1SA2-06 Implication of endogenous retrovirus in prion diseases

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Prion diseases are the unconventional fatal neurodegenerative disorders in the central nervous system (CNS). The conversion process induced by conformational change from cellular prion protein (PrP^C) to abnormal isoform (PrP^{Sc}) is the key molecular event in the pathogenesis of prion diseases, but the mechanism still remains unknown. Related to the conversion, previous evidences suggested that various molecules in host can function as cellular cofactors PrP^{Sc} formation. In here, we summarize our current studies about involvement of RNA molecule and endogenous retroviruses in prion diseases. In order to identify RNA function in prion diseases, we employed chemicals, Lithium aluminum hydride (LiAlH₄), that can cleave phosphodiester bonds of RNA and then assess the effects on the infectious agent. LiAlH₄ destroyed PrP^{Sc} and extended the scrapie incubation period in mice, and the results suggest RNA molecules can be important in maintaining the structure of PrP^{Sc}. To clarify implication of endogenous

retrovirus in prion diseases, we utilized mouse model expressing high titer of murine leukemia viruses (MuLVs) that is endogenous retroviruses in mouse. We analyzed the expression of MuLVs after scrapie infection and examined incubation period and the levels of PrP^{Sc} in scrapie infected mice. Results of the study showed that scrapie infection influenced the expression of MuLVs and the progression of scrapie pathogenesis was accelerated by MuLV. Subsequently, we established in vitro models with high concentration of MuLV for the intensive study between endogenous retrovirus and prion diseases. In addition, to verify the study, we are investigating the prevalence of human endogenous retroviruses (HERVs) in patients with human prion diseases.

1SA2-07 鳥、ブタ、そしてパンデミックインフルエンザ

Avian, swine, and pandemic influenza

Hiroshi Kida (1) ((1) Hokkaido University)

Since 2003, the H5N1 highly pathogenic avian influenza virus (HPAIV) has seriously affected poultry in Asia. Well over 500 million birds have died from infection or been killed for the control purposes. A HPAIV is generated when a nonpathogenic virus brought in by migratory birds from nesting lakes in the north is transmitted to chickens via domestic ducks, geese, quails, turkeys, etc. and acquires pathogenicity for chickens with repeated multiple infections in the chicken population. This pathogenicity does not mean for other species of birds and mammals, nor does it for humans. It was confirmed that more than 420 people have been infected with the H5N1 virus, 60% of whom died in Asia, the Middle East and Africa since 2004. Against this backdrop, it is assumed that the H5N1 virus will acquire the ability to transmit among humans and wreak havoc as a new virus. The WHO has warned for the past decade that this avian influenza virus is in the final stages of acquiring the ability to infect humans. In response, some of the nations have formulated emergency plans, including measures such as stocking an inactivated H5N1 vaccine for humans.

Now, swine-origin H1N1 influenza viruses transmit to humans more than 50,000 in over 100 countries, and WHO declared that the situation is now phase 6, stage of pandemic influenza. This must become a member that cause seasonal influenza. No one is interested in avian influenza any more although the situation of HPAI in the world.

I would like to stress the importance of surveillance of avian and swine influenza, and that preparedness for pandemic influenza should be based on the measures how to control the seasonal influenza.

1SA2-08 Avian influenza virus: from migratory bird infection to peptide inhibitor discovery

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Highly pathogenic avian influenza virus (HPAIV) H5N1 has spread around the world and causes worldwide concern about a possible human pandemic, either by HPAIV or a new virus variant with some segments from the HPAIV. In 2005, the reservoir hosts of the influenza virus became the victim of the HPAIV H5N1 in population, occurred in northwestern China, Qinghai Lake. In the following years until 2008, the unique group of viruses was isolated in the Qinghai Lake wild birds, Clade 2.2. In 2009, a new clade of viruses was isolated in the same area. Both of these clades are not the major viruses causing human infections in China or elsewhere. The phylogenetics and the impact to the environment of these outbreaks are discussed. In light of the increase of current drug-resistant strains of the influenza virus, we hypothesized that HIV T-20 like peptides derived from HA2 of influenza virus might be used as fusion inhibitors. One peptide was demonstrated to be a potent inhibitor for influenza virus.

1SA2-09 ウイルスペクターと非ウイルススペクターの遺伝子発現効率の定量的比較解析：細胞取込から転写・発現まで

Quantitative analysis of transfection efficiency between viral and non-viral vector: From cellular uptake to transcription/translation

Hideyoshi Harashima (1) ((1) Hokkaido University)

To improve transfection efficiency of non-viral vectors, quantitative and mechanism-based analysis of transfection efficiency between viral and non-viral vectors is essential for improving the effectiveness of non-viral vectors. We performed a systematic and quantitative comparison of the cellular uptake and subsequent intracellular distribution of exogenous DNA transfected by viral and non-viral vectors in living cells. As a model, adenovirus (Ad) and LipofectAMINE PLUS (LFN) were used for comparison since they are highly potent and widely used viral and non-viral vectors, respectively. Once taken up by a cell, Ad exhibited comparable endosomal escape and slightly more higher nuclear transfer efficiency compared with LFN. In contrast, LFN requires three orders of magnitude more intra-nuclear gene copies to exhibit a trans-gene expression comparable to the Ad, suggesting that difference of transfection efficiency principally arises from differences in nuclear transcription efficiency, and not from a difference in intracellular trafficking between Ad and LFN. We have further examined the contribution of transcription and translation processes to the overall differences in the trans-gene expression efficiency of nucleus-

delivered DNA by quantifying mRNA. As a result, transcription efficiency (Etranscript) of LFN was about 16 times less than that for adenovirus. Furthermore, translation efficiency (Etranslate) was approximately 460 times less in LFN. Collectively, an improvement in nuclear decondensation and reduction of interaction between vector and mRNA is essential to establish a new generation of non-viral vectors.

1SA2-10 プリオン病 – 感染メカニズムとダイナミクスに基づく創薬

Prion diseases - Biophysical approach to the infection mechanism and the structure based drug design -

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Conformational conversion of the normal form of prion protein (PrP^C) to a scrapie form (PrP^{Sc}) plays a central role in the pathogenesis of prion diseases. However, the infection mechanism of prion has been still unclear. Although some synthetic compounds which can interfere with the PrP^{Sc} production have been discovered, their inhibitory mechanisms still remain unknown. Objectives of the research is to elucidate the infectious mechanism of prion and the inhibitory mechanism of anti-prion compounds, based on the structural dynamical information on prion protein, i.e. characteristics of an intermediate state PrP^{*}, and further to optimise the chemical structures of compounds. Initially to identify the PrP^{*} specific sites, the chemical shift alterations under various PrP^{Sc} producing conditions have been observed using various NMR methods. For the rational design of molecules targeting these sites, we have developed a unique software, 'NAGARA', in which from quantum (fragment MO) to classical simulations (all atom & coarse grained dynamics) are included.

PrP^{*} specific sites are existent from the C-terminal region of the helix B to the central region of helix C. Based on the simulated results, we have synthesized a series of compounds with strong anti-prion activities, and identified the essential scaffolds necessary for the activity. Intriguingly, the amplitudes of chemical shift perturbations upon binding with these anti-prion compounds dramatically increased upon decrease of IC₅₀ of compounds, indicating the local stability of the prion-ligand interaction sites is crucial for their inhibitory action. Application of these findings may well lead to the way for the development of therapeutics for prion diseases.

1SA3-01 インフルエンザウイルスの RNA ポリメラーゼにおけるサブユニット間相互作用

Structural insight into essential subunit contacts of the influenza virus RNA polymerase

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The recent outbreak of a new, swine-related H1N1 influenza virus in Mexico has affected the entire world economy, even though it is not as pathogenic as imagined at first. Standard medication, such as NA inhibitors Tamiflu and Relenza, have proved effective against this viral strain, but present vaccines are not. This current pandemic shows that there is still danger of the emergence of new type influenza viruses. New viral strains against which humans have no immunity are spread rapidly from person to person. Furthermore, the swine-related H1N1 influenza virus is a hybrid of human, swine and highly pathogenic avian strains. This means that it is only a matter of time before a new highly pathogenic and Tamiflu-resistant influenza virus emerges, and we have to prepare for it.

The viral RNA-polymerase is not yet a target of any approved pharmaceutical, but has recently become a focus for the development of new anti-influenza drugs since it is highly conserved in avian and human influenza. It carries out a number of essential processes in the viral life cycle, many of which remain poorly understood. The three subunits, PB1, PB2 and PA play different roles within the polymerase, and are all essential for viral replication, but despite considerable functional analysis relatively little is known about their structure. PA and PB2 both bind PB1, but not each other. Here, we have solved crystal structures of the two subunit interaction surfaces, PA-PB1 and PB2-PB1. We have found highly conserved residues which are essential for these interactions, and demonstrated that the interruption of these interfaces dramatically reduces viral replication. These interfaces have considerable potential as a drug target sites, which are entirely independent of surface antigen type.

1SA3-02 インフルエンザ RNA ポリメラーゼ PB2 サブユニット病原性関与ドメインの構造と機能の解析

Structural basis of the influenza A virus RNA polymerase PB2 RNA-binding domain containing the pathogenicity-determinant lysine 627 residue

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Since the influenza A virus has an RNA genome, its RNA-dependent RNA polymerase, comprising three subunits, the PA, PB1 and PB2 subunits, is

essential for viral transcription and replication. The binding of RNA primers/promoters to the polymerases is an initiation step in viral transcription. In our current study, we reveal the 2.7 Å tertiary structure of the C-terminal RNA-binding domain of PB2 by X-ray crystallography. This domain incorporates lysine 627, and this residue is associated with the high pathogenicity and host range restriction of influenza A virus. We found from our current analyses that this lysine is located in a unique "Φ"-shaped structure consisting of a helix and an encircled loop within the PB2 domain. By electrostatic analysis we identified a highly basic groove along with this loop and found that lysine 627 is located in the loop. A PB2 domain mutant in which glutamic acid is substituted at position 627 shows significantly lower RNA-binding activity. This is the first report to show a relationship between RNA-binding activity and the pathogenicity-determinant lysine 627. Using the Matras program for protein 3D structure structural comparisons, we further found that the helix bundles in the PB2 domain are similar to that of activator 1, the 40 kDa subunit of DNA replication clamp loader (replication factor C), which is also an RNA-binding protein. This suggests a functional and structural relationship between the RNA-binding mechanisms in underlying both influenza A viral transcription and cellular DNA replication. Our present results thus provide important new information for developing novel drugs that target the primer/promoter RNA-binding of viral RNA polymerases.

1SA3-03 光ピンセットを用いたインフルエンザウイルス感染による細胞変位の解析

Analysis of the changing of cell by influenza virus infection using the optical tweezers

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Influenza virus RNA dependent-RNA polymerase is composed of three proteins, PB2, PB1 and PA. The genome of influenza virus was negative sense RNA. This polymerase functions both transcriptase and replicase. It is required the capped RNA as a primer for transcriptase activity, while when the RNA polymerase functions as replicase, RNA synthesis initiates primer independently. Using the purified RNA polymerase that formed different complexes, PB2-PB1-PA, PB2-PB1 and PB1-PA, it was found that both PB2-PB1-PA and PB1-PB2 functioned as transcriptase, while PB1-PA complex functioned as replicase. We expected host protein(s) involved in the conversion of influenza virus RNA dependent RNA polymerase from transcriptase to replicase. To identify the host protein(s) we attempted to screen the host protein using yeast two-hybrid screening system. We identified nine candidates. Ebp1 was interacted with PB1 subunit at C-terminal region. Ebp1 was also interacted with several proteins, ErbB3, P53, Rb, HDAC at C-terminal region (reported by Humberger's group). Ebp1 inhibited in vitro RNA synthesis, but not endonuclease and cap binding activity of influenza virus RNA polymerase. Ebp1 did not inhibit the reverse transcriptase activity of AMV. The assay using Ebp1 over-expression plasmid and siRNA indicated that Ebp1 inhibited the virus multiplication. Interestingly western blotting assay for Ebp1 indicated that Ebp1 expression induced by influenza virus infection. I would like to discuss the inhibitory mechanism of Ebp1 on influenza virus RNA polymerase.

1SA3-04 インフルエンザウイルス RNA ゲノム-タンパク質複合体輸送の生細胞観察

Live cell imaging of the trafficking of influenza viral ribonucleoprotein complexes

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An individual segment of the influenza virus RNA genome forms a ribonucleoprotein complex (vRNP) with viral RNA polymerase and nucleoprotein (NP), and is replicated in the nucleus. At the late phase of infection, progeny vRNPs are exported to the cytoplasm, and finally a set of 8 vRNP segments is incorporated into a virion, which occurs at the plasma membrane. Because of lack of a specific antibody that only recognizes vRNPs, it has been unsuccessful to trace the intracellular trafficking pathway of progeny vRNPs.

We produced an anti-NP monoclonal antibody (mAb61A5) that preferentially recognizes the NP forming RNP complex rather than free NP. We initially performed confocal microscopy using mAb61A5, and found that cytoplasmic vRNPs were present as punctate signals that were accumulated around the microtubule organizing center (MTOC) soon after the nuclear export and were distributed along microtubules later on. To visualize individual vRNPs, we introduced fluorescently labeled mAb61A5 into infected cells using protein transfection reagents and carried out live-cell imaging. The vRNP signals moved along microtubules rapidly (the average velocity of 0.7-1.5 μm/s) but intermittently in both directions. These data suggest that the trafficking of progeny vRNPs from the MTOC to the plasma membrane is likely mediated by the microtubule network and motor proteins.

1SA3-05 インフルエンザウイルスゲノム機能発現のウイルスおよび宿主基盤