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Amyloid beta-protein and lipid rafts: focused on biogenesis and catabolism

Wataru Araki[1], Akira Tamaoka[2]

[1]Department of Demyelinating disease and Aging, National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187-8502, Japan, [2]Department of Neurology, Division of Clinical Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan

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1. ABSTRACT

Cerebral accumulation of amyloid β-protein (Aβ) is thought to play a key role in the molecular pathology of Alzheimer's disease (AD). Three secretases (β-, γ-, and α-secretase) are proteases that control the production of Aβ from amyloid precursor protein. Increasing evidence suggests that cholesterol-rich membrane microdomains termed 'lipid rafts' are involved in the biogenesis and accumulation of Aβ as well as Aβ-mediated neurotoxicity. γ-Secretase is enriched in lipid rafts, which are considered an important site for Aβ generation. Additionally, Aβ-degrading peptidases located in lipid rafts, such as neprilysin, appear to play a role in Aβ catabolism. This mini-review focuses on the roles of lipid rafts in the biogenesis and catabolism of Aβ, covering recent research on the relationship between lipid rafts and the three secretases or Aβ-degrading peptidases. Furthermore, the significance of lipid rafts in Aβ aggregation and neurotoxicity is briefly summarized.

2. INTRODUCTION

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia in the elderly population, characterized pathologically by senile plaques, neurofibrillary tangles and neuron loss. Recent evidence supports the amyloid cascade hypothesis whereby cerebral accumulation of amyloid β-protein (Aβ), a primary constituent of senile plaques, is thought to play a primary role in the molecular pathology of AD (1). Aβ is a hydrophobic peptide of 40-43 amino acids derived from the transmembrane amyloid precursor protein (APP). The peptide is highly prone to aggregation and possesses neurotoxic properties. APP undergoes proteolytic processing to generate Aβ. In this amyloidogenic pathway, APP is initially processed by β-secretase, and the resultant β-C-terminal fragment (β-CTF) subsequently cleaved by γ-secretase, generating Aβ. Alternatively, APP is processed by α-secretase that cleaves within the Aβ region, precluding Aβ production (2). The three secretases have been characterized extensively over recent years (3,4). After Aβ is generated, the peptide is catabolized by Aβ-degrading peptidases or cleared by other mechanisms (5). The balance between synthesis and clearance of Aβ is important to maintain normal Aβ levels in brain. The three secretases and Aβ-degrading peptidases are considered important therapeutic targets for AD. Therefore, inhibition or modulation of β- and γ-secretases and activation of α-secretase and Aβ-degrading peptidases are possible therapeutic strategies (3,5).

Lipid rafts are distinct membrane domains characterized by high concentrations of cholesterol and glycosphingolipids (6,7). These microdomains are insoluble in non-ionic detergents and can be isolated as floating buoyant fractions via sucrose density gradient centrifugation. Accordingly, cellular fractions enriched for lipid rafts are described using acronyms such as 'detergent-resistant membrane' (DRM). Lipid rafts play a central role in various cellular processes, including membrane sorting, trafficking, and signal transduction (6,7). Additionally, these structures are considered important in the pathogenesis of AD, reflecting their involvement in the biogenesis, catabolism, aggregation and accumulation of Aβ, as well as the signaling mechanism of Aβ neurotoxicity (8-11). In this mini-review, we have focused on the roles of lipid rafts in the biogenesis and catabolism of Aβ and reviewed recent studies on the relationship between lipid rafts and β-, γ-, and α-secretases or Aβ-degrading peptidases. Furthermore, the significance of lipid rafts in Aβ aggregation and neurotoxicity is briefly summarized.

3. Aβ BIOGENESIS AND LIPID RAFTS

3.1. APP, Aβ, and lipid rafts

APP is a type I transmembrane protein that undergoes several post-translational modifications, including glycosylation and phosphorylation (4,12). In considering amyloidogenic processing of APP, understanding the mechanisms underlying cellular trafficking of APP and its derivatives, such as β-CTF, is critical. A model of APP trafficking has been delineated as follows: after maturation in the Golgi/*trans*-Golgi network (TGN), APP is transported through the secretory pathway to the plasma membrane. APP is then shed by α-secretase or endocytosed to endosomes where β-site cleavage by β-secretase preferentially occurs. Subsequently, γ-secretase cleaves β-CTF, leading to Aβ production. Recent evidence has suggested that APP also undergoes retrieval from endosomes to the TGN (12,13). Importantly, Aβ is preferentially generated in the endosomal route rather than at the cell surface. Furthermore, Aβ production may depend on membrane composition. In particular, the cholesterol-rich lipid raft domains appear to play a role in Aβ generation, taking into account the presence of Aβ-generating secretases, as described below. Notably, APP is predominantly distributed in nonlipid rafts, with only a minor proportion present in lipid rafts (8,11). In the case of neurons, APP is axonally transported to nerve terminals where it is cleaved by β- and γ-secretases for presynaptic Aβ production (14). Furthermore, Aβ released from synaptic terminals appears to be a principal contributor of extracellular amyloid deposition (15).

3.2. Relationship between lipid rafts and β-secretase

BACE1 is a transmembrane aspartyl protease abundantly expressed in neurons in the brain. BACE1 cleaves APP at the N-terminus of the Aβ region (β-site), and additionally between Tyr10 and Glu11 within the Aβ region (β'-site). The protease undergoes several post-translational modifications, including glycosylation, phosphorylation, and palmitoylation (16,17). Palmitoylation of BACE1 occurs at the four cysteine residues in the transmembrane and C-terminal cytosolic domains, and regulates targeting to lipid rafts (18,19). A number of studies have been performed to delineate the intracellular sorting mechanism of BACE1. Mature BACE1 is transported to the plasma membrane and internalized to early endosomes via the di-leucine motif at the C-terminus. BACE1 is suggested to recycle to the plasma membrane via endosomes or TGN (17,20).

BACE1 processing of APP is likely to occur in endosomal compartments that have a low pH environment favorable for protease activity. β-Cleavage of APP is additionally supported by the Golgi and TGN environments. Interactions of BACE1 with adaptor molecules, such as GGAs (Golgi-localized γ-ear-containing, ADP ribosylation factor-binding proteins), through the di-leucine motif may regulate retrieval of BACE1 from endosomes to the TGN (12,13,17,20). Moreover, BACE1 retrieval or endocytosis may be regulated by BACE1-interacting molecules, such as sorting nexin 6, sorting nexin 12 and sortillins (21-23).

A considerable proportion of endogenous or overexpressed BACE1 is localized to lipid rafts. Disruption of the integrity of lipid rafts via cholesterol depletion inhibits β-cleavage of APP, leading to a BACE1 shift from raft to non-raft fractions (24). These findings, in conjunction with other data, suggest a significant role of raft-associated BACE1 in Aβ generation (7,8,24-28). However, in view of the partial distribution of BACE1 in lipid raft fractions, the issue of whether BACE1 cleavage of APP predominantly occurs in lipid or nonlipid rafts remains controversial. One approach to resolve this issue is to utilize the dependence of lipid raft association of BACE1 on palmitoylation. Our group and that of Dr. Thinakaran

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independently demonstrated that both wild-type and palmitoylation-deficient mutant BACE1 forms produce similar amounts of Aβ in neuroblastoma cells and primary neurons expressing APP (18,19). Furthermore, β-CTF was detected mainly in nonraft domains, both in neurons co-expressing APP and BACE1 (wild-type or mutant) and expressing Swedish mutant APP only (19). These findings indicate that raft association of BACE1 does not influence β-cleavage of APP and Aβ production, and support the view that BACE1 cleaves APP mainly in nonraft domains. Accordingly, we proposed a model of neuronal Aβ generation involving mobilization of β-CTF from nonraft to raft domains (19).

A previous study showed that a mutant form of BACE1 containing the glycosylphosphatidylinositol (GPI) anchor localizes to lipid rafts and exhibits increased β-cleavage activity (26). However, other investigators reported that increased Aβ secretion under conditions of GPI-BACE1 expression is mainly attributed to reduced APP cleavage at the β' site, compared with wild-type BACE1 (29). It is therefore likely that β-site cleavage efficiency of BACE1 is essentially unaltered by its association with lipid rafts.

BACE1 localized at lipid rafts is additionally able to participate in Aβ production. Recent experiments demonstrated that expression of GPI-anchored ADAM10, but not wild-type, is associated with Aβ reduction in neuroblastoma cells, suggesting that GPI-ADAM10 competes with BACE1 for the APP substrate (30). BACE1 in lipid rafts may also function in cleavage of other substrates, including neureglin-1 and β-subunits of voltage-gated sodium channels (17).

A close relationship between cholesterol and BACE1 or β-secretase has been suggested, based on experimental data (31-33). However, it remains to be clarified whether regulation of BACE1 by cholesterol is related to alterations in lipid raft integrity.

3.3. Relationship between lipid rafts and α-secretase

It is generally accepted that the metalloprotease ADAM10, a member of the ADAM (a disintegrin and metalloproteinase) family of proteases, is a major α-secretase, although ADAM17 and ADAM9 display some α-secretase activity towards APP (34,35). ADAM10 is a type I transmembrane protein mainly active at the plasma membrane. During passage through the TGN, the prodomain of ADAM10, which inhibits the enzyme activity, is cleaved off by proprotein convertases. The protease is involved in the ectodomain shedding of numerous type I transmembrane proteins in addition to APP, including tumor necrosis factor-α (34,35).

Since ADAM10 is localized almost exclusively in nonlipid raft membrane domains, α-secretase cleavage of APP occurs mainly in these regions (36). Kojro and co-workers showed that activation of α-secretase cleavage occurs upon inhibition of cholesterol synthesis by statins or zaragozic acid in neuroblastoma cells (37). α-Secretase activation appeared dependent on cholesterol and independent of the isoprenoid pathway. Additionally, the group showed that targeting of ADAM10 to lipid rafts by a GPI anchor has no influence on α-secretase activity (37). These data collectively suggest that the cellular cholesterol concentration influences α-secretase activity, although the underlying mechanism remains unclear.

How are cleavages of APP by ADAM10 and BACE1 controlled? One mechanism is that the cellular compartments where α- and β-cleavages occur are spatially segregated: α-cleavage preferentially occurs at the plasma membrane, while β-cleavage in the endosomal route. In this regard, a recent study showed that constitutive cleavages of APP by ADAM10 and BACE1 are largely uncoupled and that an inverse coupling is observed only partially in neurons (38). An additional mechanism is that phosphorylation of APP at Thr668 is a factor involved in the regulation of APP endocytosis and subsequent amyloidogenic processing (4,39,40).

3.4. Relationship between lipid rafts and γ-secretase

γ-Secretase is a high molecular weight complex composed of presenilin 1 (or presenilin 2), nicastrin, APH-1, and PEN-2. Presenilins 1 and 2 are membrane proteins containing nine transmembrane domains that normally undergo endoproteolysis between transmembrane domains 7 and 8, generating stable N- and C-terminal fragments. These proteins act as the catalytic subunit of γ-secretase. APH-1 and PEN-2 are transmembrane proteins containing seven and two transmembrane domains, respectively. Nicastrin is a type 1 transmembrane protein that appears to function as a γ-secretase substrate receptor (41). Numerous familial AD-associated mutations of the presenilin 1 and presenilin 2 genes are reported to affect

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amyloidogenic processing of APP, resulting in generation of elevated amounts of highly amyloidogenic Aβ42, relative to Aβ40 (41).

γ-Secretase has been shown to be tightly associated with lipid rafts. Fractionation analyses of cultured cells and brain tissues revealed that all components of γ-secretase are primarily localized to the lipid raft fraction (42-47). The four components of the γ-secretase complex are stable in rafts, even at high concentrations of detergents, such as CHAPSO. Moreover, the lipid raft fraction contains high levels of γ-secretase activity, based on assay of APP intracellular domain production (46). Thus, γ-secretase is enriched in lipid rafts, although its substrate levels are relatively low. Subcellularly, γ-secretase mainly localizes to lipid raft microdomains of post-Golgi and endosomes (45). Together, these findings support the theory that Aβ is generated in lipid raft membrane domains.

The group of Dr. Thinakaran also presented evidence that nicastrin is palmitoylated at Cys689, and APH-1 at Cys182 and Cys245 (48). Using palmitoylation-deficient mutants, the authors showed that palmitoylation contributes to raft association of nicastrin and APH-1. This modification appears important for nicastrin and APH-1 stability, but does not directly modulate processing of substrates by γ-secretase.

Earlier studies indicate that membrane lipid composition is important in modulating γ-secretase activity. For instance, cholesterol has stimulatory effects on γ-secretase activity (32,49). Thus, γ-secretase modulation by cholesterol may underlie the reported positive association between cholesterol and Aβ accumulation in animal models (50).

Oxidative stress is an important factor in the pathogenesis of AD (51). Notably, oxidative stress affects BACE1 and γ-secretase activities (51,52). In a previous study, we investigated the relationship between oxidative stress and these secretases in lipid rafts. Treatment of neuroblastoma cells with ethacrynic acid, which induces oxidative stress via glutathione depletion, triggered a significant increase in presenilin 1 mRNA levels as well as protein levels in both cell lysates and the lipid raft fraction without altering BACE1 or other γ-secretase components. Ethacrynic acid treatment additionally promoted Aβ secretion from cells expressing Swedish mutant APP (47). A vicious cycle between Aβ and oxidative stress may thus exist, whereby Aβ triggers oxidative stress, which upregulates PS1 protein in lipid rafts and consequently promotes Aβ production.

4. Aβ CATABOLISM AND LIPID RAFTS

After generation of Aβ, the protein is cleared through multiple pathways. Aβ is subjected to enzymatic degradation by peptidases or cleared via cerebral vessels through special transport mechanisms or drainage along perivascular basement membranes. Aβ clearance is additionally mediated by microglial phagocytosis (5). Several Aβ-degrading proteases are operative, among which neprilysin (NEP), insulin-degrading enzyme (IDE), and endothelin-converting enzyme (ECE) are thought to be most efficient under physiological conditions (53,54).

NEP is a member of the M13 zinc-binding metalloendopeptidase family with a type II transmembrane protein structure (53,54). NEP resides mainly on the plasma membrane, and is suggested to participate in extracellular Aβ degradation. The glycosylated mature form of NEP has been shown to localize in lipid rafts, while both immature and mature forms are present in nonlipid rafts. Delocalization of NEP from lipid to nonlipid rafts was observed upon treatment with the cholesterol-sequestering agent, methyl-β-cyclodextrin. NEP activities in lipid and nonlipid raft fractions were comparable (55). In addition, targeting of NEP to lipid rafts via fusion with the N-terminal domain of growth-associated protein 43 did not affect Aβ clearance activity (56). However, the theory that NEP participates in degradation of Aβ associated with lipid rafts is plausible. Interestingly, previous investigations suggest that NEP controls the Aβ level at presynaptic sites (57). Therefore, NEP may act as an important regulator of Aβ at synapses.

IDE is a thiol zinc-metallopeptidase involved in the hydrolysis of several peptides, including insulin and Aβ (53,54). IDE is primarily cytosolic, but also partly associated with the plasma membrane (58). Bulloj *et al.* further demonstrated that IDE is partly associated with plasma membrane lipid raft fractions in neuroblastoma cells and that lipid raft association of IDE appears to be modulated by brain cholesterol levels (59). IDE may play a complementary role in the catabolism of Aβ generated in lipid rafts.

ECE-1 and ECE-2 are members of the M13 family of zinc-binding metalloproteases with a type II transmembrane structure, which contribute to

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Aβ catabolism (60). Recent studies have shown that ECE-1 regulates both intracellular and extracellular pools of Aβ, while ECE-2 mainly regulates the intracellular peptide pool and co-localizes with markers of the endosomal-lysosomal pathway (61). However, the issue of whether ECEs are associated with lipid rafts remains to be established.

Potent Aβ-degrading activity of BACE2, a homolog of BACE1, has been reported (62). In addition, BACE2 is localized in intracellular compartments relevant to Aβ degradation, including lysosomes (62). Further studies are required to elucidate whether BACE2 is associated with lipid rafts and involved in physiological Aβ degradation.

5. Aβ AGGREGATION, NEUROTOXICITY AND LIPID RAFTS

Aβ is a natively unfolded protein, and under certain conditions, aggregates to form a heterogeneous mixture of soluble oligomers, protofibrils and fibrils. In particular, numerous reports have indicated that soluble Aβ oligomers in the brain are the key pathogenic structures in AD (63,64). Aβ oligomers specifically bind neurons, evoking neurotoxicity and synapse deterioration. Several lines of evidence, including the finding that Aβ accumulates in presynaptic terminals in the AD cortex, co-localizing with the lipid raft markers, cholesterol and ganglioside, GM1, indicate that lipid rafts are pivotal modulators of Aβ aggregation as well as production, resulting in the accumulation of neurotoxic Aβ oligomers in AD brain (9).

Recent studies indicate that lipid rafts play an important role as pathological signaling platforms where receptors for Aβ oligomers, such as PrP^C, glutamate receptors, nerve growth factor receptors and insulin receptors, among others, are assembled (9,10). Thus, multireceptor, pathogenic signaling platforms in lipid rafts are induced by Aβ oligomers. Binding of Aβ oligomers to their receptors is related to aberrant localization of these receptors, with deleterious effects on their physiological long-term synaptic potentiation (LTP: an electrophysiological correlate of learning and memory) and defense against oxidative stress. In this way, lipid rafts appear to be directly responsible for transduction and amplification of Aβ oligomer-mediated neurotoxicity characteristic of AD (9,10).

Lipid rafts play an important role in AD as well as a range of neurodegenerative proteinopathies (65). Interestingly, lipid raft disruption was reported to protect mature neurons against oligomer-induced toxicity (66).

6. CONCLUSIONS

APP is mostly distributed in nonlipid rafts, with only a minor proportion present in lipid rafts. BACE1 is only partially localized in lipid raft fractions. In addition, raft association of BACE1 does not influence β-cleavage of APP and Aβ production. These findings support the view that BACE1 cleaves APP mainly in non-raft domains, and β-site cleavage efficiency is essentially unaltered by its association with lipid rafts. On the other hand, all components of γ-secretase are primarily localized to the lipid raft fraction, suggesting that Aβ is likely to be generated in lipid raft domains. Since ADAM10, a major α-secretase, is localized almost extensively in non-raft membrane domains, α-secretase cleavage of APP occurs mainly in these regions. Notably, the cellular compartments where β- and α-cleavages occur are spatially segregated. Taking the collective findings into consideration, a model of neuronal Aβ generation involving mobilization of β-CTF from non-raft to raft domains has been proposed (19) (Table 1, Figure 1).

The balance between synthesis and clearance of Aβ is proposed to be critical to maintain the Aβ level in brain. After Aβ generation, the protein is cleared through multiple pathways, including degradation by Aβ-degrading enzymes, among which NEP, IDE, and ECE appear to be most physiologically efficient. Both NEP and IDE are partly associated with lipid rafts, whereas the issue of whether ECEs are associated with lipid rafts remains unknown.

In vitro studies to date have highlighted important roles for the lipid microenvironment in modulation of β- and γ-secretase activities. The precise relationship between the lipid microenvironment and secretases *in vivo*, including regulation of BACE1 and γ-secretase activities, control of interactions of BACE1 with APP, and association of APP CTFs and γ-secretase, remains unclear, although lipid rafts are known to be involved in the biogenesis and accumulation of Aβ, playing a key role in the molecular pathology of AD. Thus, resolving these issues should facilitate the development of new therapeutic strategies to manage AD. In addition, since lipid rafts are considered generic platforms for oligomer-mediated neurotoxicity, investigation of the pathophysiology of the downstream effects

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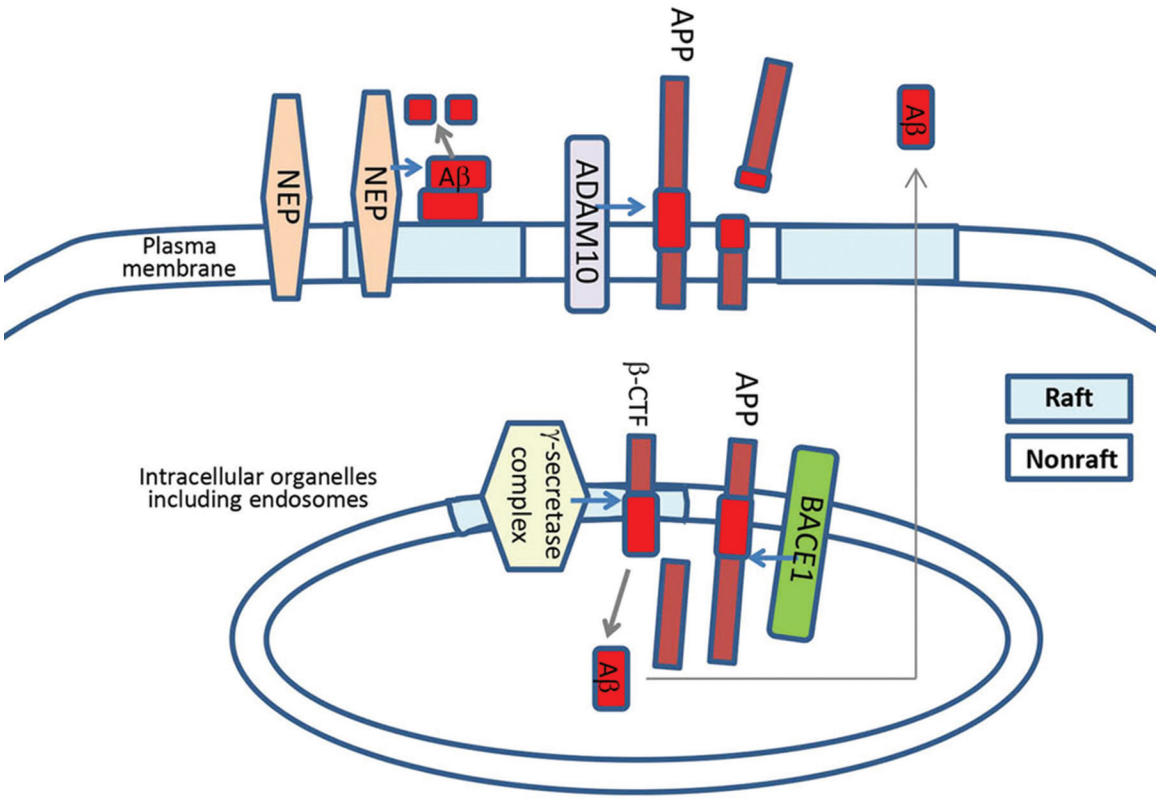

Figure 1. Schema illustrating the relationship between lipid rafts and the proteases involved in Aβ biogenesis and catabolism. APP is processed by BACE1 mainly in nonraft domains to produce β-CTF, which is subsequently cleaved by **γ**-secretase in raft domains, generating Aβ. These cleavages occur predominantly in intracellular organelles including endosomes. APP is alternatively processed by ADAM10 in nonraft domains, precluding Aβ generation. Aβ-degrading proteases such as neprilysin (NEP) degrade Aβ associated with lipid rafts. ADAM10 and NEP are active mainly at the plasma membranes.

Table 1. Relationships of lipid rafts with APP, secretases and Aβ-degrading peptidases

Protein	Relationship with lipid rafts
APP	Only a minor portion is localized in lipid rafts
β-secretase (BACE1)	Partially localized in lipid rafts Raft association does not affect Aβ production
γ-secretase	All γ-secretase components are enriched in lipid rafts
α-secretase (ADAM10)	Almost exclusively localized in nonlipid rafts
NEP	Partially localized in lipid rafts
IDE	Partly associated with lipid rafts
ECE	Unknown

of oligomers binding to lipid raft receptors could lead to the identification of potential therapeutic targets for prevention and treatment of AD and other neurodegenerative disorders.

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Send correspondence to: Wataru Araki, Department of Demyelinating disease and Aging, National Institute of Neuroscience, NCNP, 4-1-1 Ogawahigahi, Kodaira, Tokyo 187-8502, Japan, Tel: 81-42-346-1717, Fax: 81-42-346-1747, E-mail: araki@ncnp.go.jp

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