The cellular prion protein (PrP^C) is a glycosylphosphatidylinositol (GPI)-anchored protein. We investigated whether PrP^C can move from one cell to another cell in a cell model. Little PrP^C transfer was detected when a PrP^C expressing human neuroblastoma cell line was cultured with the human erythroleukemia cells IA lacking PrP^C. Efficient transfer of PrP^C was detected with the presence of phorbol 12-myristate 13-acetate, an activator of protein kinase C. Maximum PrP^C transfer was observed when both donor and recipient cells were activated. Furthermore, PrP^C transfer required the GPI anchor and direct cell to cell contact. However, intercellular protein transfer is not limited to PrP^C, another GPI-anchored protein, CD90, also transfers from the donor cells to acceptor cells after cellular activation. Therefore, this transfer process is GPI-anchor and cellular activation dependent. These findings suggest that the intercellular transfer of GPI-anchored proteins is a regulated process, and may have implications for the pathogenesis of prion disease.

The normal cellular prion protein (PrP^C) is a highly conserved glycoprotein bound to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor (1, 2). Although the general function is not understood, recent studies have established that PrP^C has several activities. There is strong evidence that PrP^C is a metal-binding protein that has antioxidation property (3–6). Cell surface PrP^C has also been reported to bind laminin and glycosaminoglycans, and to participate in signal transduction (7–10).

PrP^C also plays a central role in a group of fatal neurodegenerative disorders, commonly known as transmissible spongiform encephalopathies or prion diseases (11, 12), which occur in many non-central nervous system tissues (28–30), the pathogenic mechanism shared by all the prion diseases occurs exclusively in the central nervous system (12, 31). It is believed that in prion diseases acquired by infection such as variant Creutzfeldt-Jakob disease, the PrP^C to PrP^Sc conversion happens through a series of intermediates in different tissues until PrP^Sc reaches the central nervous system.

GPI-anchored proteins are diverse and mediate various functions such as: cell to cell adhesion, nutrient uptake, signal transduction, and regulation of complement activity (32, 33). Purified GPI-anchored proteins can incorporate spontaneously onto the target cell membrane in vitro, a phenomenon known as “cell painting” (34, 35). Accumulated evidence also suggests that GPI-anchored proteins may detach from the cell surface and re-attach to another cell. For example, spontaneous cell to cell transfer of CD4-GPI has been demonstrated between co-cultured HeLa cells (36). In a transgenic mouse model, human CD59 and CD55, which are expressed only on red blood cells, were observed to transfer to vascular endothelial cells (37). In both cases, the transferred molecules retained their normal functions. However, any intercellular transfer of GPI-anchored proteins in vivo must be tightly regulated, to assure that GPI-anchored proteins do not lose their cell type specificity. The in vivo significance and the mechanism by which GPI-anchored protein moves between cells remain unclear (38).

In the current study, we established a co-culture system using a PrP^C expressing (PrP^C+) cell line M17-PrP and the cell line IA lacking PrP^C (PrP^C−) to study whether PrP^C can move into a pathogenic and infectious conformer, called scrapie PrP (PrP^Sc) (13, 14).

The mechanism and the cellular locale of PrP^C to PrP^Sc conversion remain unclear. It has been suggested that the conversion is either through the direct binding of PrP^Sc to PrP^C or mediated by a chaperone protein (8, 15–17). Horiuchi and Caughey (18) found that direct interaction between PrP^C and PrP^Sc through the region around amino acid residues 219 to 232 is required for the subsequent conversion in the cell-free system (18). Plasma membrane and subcellular compartments of the protein recycling pathway have been suggested to be sites of conversion (19–22), because in the cell model, the generation of PrP^Sc is inhibited by brefeldin A, an agent, which blocks the delivery of membrane proteins from the cytosol to the membrane (23). Furthermore, treatment of cells with phospholipase C (PI-PLC), an enzyme that hydrolyzes the GPI anchor, or with proteases, which degrade PrP^C also inhibits PrP^Sc formation (24). In addition, PrP^C is not converted to PrP^Sc when it is expressed as a transmembrane protein rather than a GPI-anchored protein (25, 26). The GPI anchor may be needed because GPI-anchored proteins occupy microdomains on the cell membrane, known as detergent insoluble complex or lipid rafts, in a concentrated and multimeric form (27). An additional relevant issue is that although PrP^C is expressed in many non-central nervous system tissues (28–30), the pathology of prion diseases occurs exclusively in the central nervous system (12, 31). It is believed that in prion diseases acquired by infection such as variant Creutzfeldt-Jakob disease, the PrP^C to PrP^Sc conversion happens through a series of intermediates in different tissues until PrP^Sc reaches the central nervous system.

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+ The abbreviations used are: PrP^C, cellular prion protein; GPI, glycosylphosphatidylinositol; PrP^Sc, scrapie prion protein; PI-PLC, phosphatidylinositol-phospholipase C; mAb, monoclonal antibody; PMA, phorbol 12-myristate 13-acetate; ConA, concanavalin A; PKC, protein kinase C.
from cell to cell. We found that PrP$^{C}$ could be transferred from M17-PrP cells to IA cells by a GPI-dependent process in vitro. However, this process does not efficiently occur spontaneously but requires cell activation and direct cell to cell contact.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Animals**—The human neuroblastoma cell line M17 and the human PrP-transfectant M17-PrP have been described in detail (39). The PrP-CDD4-PCEP4 expression construct was generated by replacing the GPI-anchor signaling sequence with the transmembrane and cytoplasmic domain of human CD44. The PrP-CDD4-PCEP4 plasmid was transfected into M17 cells using the DOTAP L脂膜转

**Co-culturing and Separation of Cells**—Co-culture was carried out in 6-or 12-well tissue culture plates (Corning, Corning, NY). M17 or M17-PrP cells were first plated and allowed to grow to 90% confluence. IA cells were then added onto the plate and incubated overnight. Except in the experiments to determine the kinetics of co-culture, a 1:1 of donor cell (1 × 10$^7$) to recipient cell was used for all the experiments. Phorbol 12-myristate 13-acetate (PMA, Sigma) was added at 20 ng/ml at the beginning of the culture. After co-culture, the IA cells, which grow in suspension, were carefully collected. In some experiments, an anti-CD44 mAb was used with a secondary reagent conjugated to magnetic beads to isolate the CD44$^+$ IA cells according to the protocol provided by the manufacturer (Matenyi Biotec, Auburn, CA).

For treatment with PI-PLC, cells were incubated with 60 ng/ml phosphatidylinositol-specific phospholipase C for 30 min in a 37°C incubator (39). After treatment, cells were washed extensively and stained with the anti-PrP monoclonal antibody (mAb) and flow cytometry as described below. The human erythroleukemia cell line IA, derived from K562, was kindly provided by Dr. M. E. Medof of our institution. IA cells failed to express any GPI-anchored protein because of a defect in the assembly of the GPI-anchor core structure (40). The original breeding pairs of FVB PrP$^-$$^-$/H11032 mice were kindly provided by Dr. S. Prusiner, University of California, San Francisco, CA, and Dr. G. Carlson, McLaughlin Institute, Great Falls, MT.

**Stimulation of Spleen Cells from PrP$^-$$^-$/H11032 Mice and Co-culture with M17-PrP Cells**—Single cell suspensions from the spleen of PrP$^-$$^-$/H11032 mice were prepared as described (41). Cells were cultured in a 12-well plate at 3 × 10$^6$ per well with complete medium: RPMI, 1% antibiotics, and 10% pre-selected fetal calf serum. Either PMA (20 ng/ml) or ConA (15 μg/ml) were used as activators of the cells. Plates were incubated at 37°C in a CO$_2$ incubator as described (39). After treatment, cells were washed extensively and stained with the anti-PrP mAb as described.

In some experiments, the donor and recipient cells were cultured in transwells that were separated by a membrane with a 0.4-μm pore size (Costar, Corning). IA cells were placed in the top chamber and the M17-PrP cells in the lower chamber.

**Immunofluorescent Staining and Fluorescence-activated Cell Sorter Analysis**—The IA cells or spleen cells were collected and washed with washing buffer (phosphate-buffered saline supplemented with 0.5% newborn calf serum, 0.1% Na$_2$HPO$_4$, pH 7.4), and blocked with Fc-block$^\text{TM}$ (Pharmingen) on ice for 25–30 min. Cells were then incubated with purified anti-PrP mAb 8H4 or anti-human CD90 mAb SE10 (Phar-
mingen) or an isotype matched, control mAb on ice for 45 min. Cells were washed twice and then incubated with a fluorescein isocitrate-conjugated F(ab)$_2$, goat anti-mouse IgG Fc-specific antibody (Chemicon, CA), for 25 min on ice. Finally, the samples were washed and immediately analyzed using a FACScan$^\text{TM}$ (BD Biosciences). At least 10,000 viable cells were analyzed in each experiment, and all experiments were repeated at least three times for consistency.

**Confocal Microscopy**—Following co-culture, IA cells were collected for two-color immunofluorescent staining. The cells were first blocked with 5% human serum for 30 min on ice, then incubated with antibodies in the following sequence, anti-human CD44 mAb 4D1, Alexa Fluor 488 (green) goat anti-mouse IgG, F(ab)$_2$ (Molecular Probes), biotinylated anti-PrP mAb 8H4 and Alexa Fluor 594 (red) streptavidin conjugates (Molecular Probes). Samples were fixed with 3.7% formaldehyde after staining. A cytospin was then used at 500 rpm for 10 min to allow cells to adhere onto glass slides and mounted immediately with Permount (Sigma). The slides were analyzed using a dual scanning confocal microscopy system (LSM 510, Zeiss, Oberkochen, Germany). Adhesion Assay—IA cells were covalently labeled with the amine-reactive fluorescein dye, 5-carboxyfluorescein, succinimidyl ester (Molecular Probes). The reaction was carried out at 37°C for 15 min according to the protocol provided by the manufacturer. Labeled cells were washed extensively and loaded into the 96-well plate with M17-PrP monolayers, then co-cultured with or without PMA for 6–8 h. The plate was gently washed and the remaining fluorescent cells were quantified using a CytoFluor multiplate reader (PerSeptive Biosys-
tems, Series 4000, MA).

**Isolation of Microvesicles**—M17-PrP cells were first cultured with or without PMA for 24 h, the supernatants were then collected and spun at 10,000 × g to remove the cell debris. The supernatant was further subjected to a 100,000 × g ultraspase centrifugation for 1 h at 4°C (42). In some experiments the pellets were re-suspended in lysis buffer to determine the PrP$^C$ content on immunoblots. In other experiments, the pellets were re-suspended in culture medium and various amounts of the microvesicles were cultured with recipient cells.

**Western Blotting**—Cells or microvesicle fractions were incubated with lysis buffer (100 mM NaCl, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 10 mM Tris, pH 7.4, 2 mM phenylmethylsulfonyl fluoride). Predetermined amounts of total protein from each lysate were loaded and separated in 12% polyacrylamide gels, and then transferred to Immobilon P (Bio-Rad) for 2 h at 90 V. Membranes were incubated overnight at 4°C with the anti-PrP mAb 8H4. Bound mAbs were detected with an horseradish peroxidase-conjugated F(ab)$_2$, goat anti-mouse IgG Fc region specific antibody (Chemicon, CA). The blots were developed on an enhanced chemiluminescence system (Fierce) as described by the manufacturer. Prestained molecular weight markers (Bio-Rad) were used as standards.

**RESULTS**

**PrP$^C$ Does Not Spontaneously Transfer from M17-PrP Cells to IA Cells**—M17-PrP, a stably transfected human neuroblas-
toma cell line that expresses high levels of human PrP$^C$ (Fig. 1A) and IA a human erythroleukemia cell line, which does not express PrP$^C$ because it has a defect in the assembly of the GPI anchor (Fig. 1A), were used as donor and recipient cells, respect-
ively. Furthermore, the M17-PrP cells adhere to the substrate and are CD44 negative (CD44$^-$), whereas the IA cells grow in suspension and are CD44 positive (CD44$^+$) so that the two cell lines can be easily separated to examine PrP$^C$ transfer from one cell to the other.

Following co-culture of M17-PrP cells and IA cells for 12 h, little or no PrP$^C$ was detected on the cell surface of IA cells with mAb 8H4 to PrP (Fig. 1B) (43, 44). However, we detected a significant amount of PrP$^C$ in the IA cells when we co-cultured M17-PrP cells and IA cells in the presence of PMA, an activator of protein kinase C, indicating that PMA had triggered the efficient transfer of PrP$^C$ (Fig. 1C). We next used an anti-CD44 mAb and magnetic beads to isolate IA cells after co-culture. All the preparations enriched in CD44 positive cells (>98% CD44$^+$) were PrP$^C$ positive (Fig. 1D). This finding rules out the possibility that the presence of PrP$^C$ in IA cell preparations results from contaminating M17-PrP cells because the latter are CD44$^-$.

**INTERFACIAL TRANSFER OF PRP C**

**Isoelectric Focusing—** PrP$^C$ transfer and the kinetics of the transfer. Activation of either the donor or acceptor cells separately prior to co-culture was sufficient for PrP$^C$ transfer. However, the activation of both cell types consistently resulted in the highest level of transfer (Fig. 3, A and B). PrP$^C$ transfer could be detected as early as 3 h after co-culture, increased subsequently, and became stable at about 12 h (Fig. 3C). Approximately 25 ng/ml PMA was the optimal
concentration for triggering intercellular transfer (Fig. 3D). The transfer was cell-dose dependent because more PrPC was detected on IA cells when IA cells were co-cultured with increased numbers of M17-PrP cells (Fig. 3E).

**Direct Cell to Cell Contact Is Required for PrPC Transfer**—Significant levels of free PrPC were detected in the culture supernatant of PMA-treated M17-PrP cells as determined by capture enzyme-linked immunosorbent assay (not shown). We investigated whether PrPC is transferred as soluble and free PrPC present in the culture supernatant. No PrPC was detected on the surface of IA cells after culture with supernatant from PMA-treated or nontreated M17-PrP cells, even when the IA cells were also treated with PMA (not shown).

We next investigated whether purified microvesicles released by activated M17-PrP cells were the source of the transferred PrPC. The microvesicle PrPC content determined by immunoblotting showed that microvesicles from PMA-treated M17-PrP cells contained more PrPC than those from the nontreated M17-PrP cells (Fig. 4A). Therefore, microvesicles purified from activated M17-PrP cells were added directly into IA cell cultures, which had been preactivated with PMA. Only a very low level of PrPC was detected on IA cells exposed to microvesicles isolated from a number of M17-PrP cell 10 times higher than that used in common co-culture experiments (Fig.
Therefore, neither the soluble PrPc in the supernatant nor the PrPc in the isolated microvesicles is the major source of the transferred PrPc.

A transwell co-culture system was then used to investigate whether cell to cell contact is required for PrPc transfer. After culture for 12 h in two chambers separated by a membrane, little or no PrPc was detected on IA cells (Fig. 4C).

Finally, an adhesion assay showed that after co-culture with nonlabeled M17-PrP cells for 6 h, about 30–40% more IA cells adhered to M17-PrP cell monolayers following PMA than without PMA (Fig. 4D). Therefore, direct contact between the M17-PrP donor and IA acceptor cells appears to be required for efficient PrPc transfer and is enhanced by activation with PMA.

The GPI Anchor Is Important for Transfer of PrPc—Treatment with PI-PLC that specifically cleaves GPI anchors carrying one or two acyl substituents, drastically reduced transferred PrPc on the surface of IA recipient cells (Fig. 5A) indicating that transferred PrPc is linked to the surface of IA cell by the GPI anchor, and is sensitive to PI-PLC cleavage. We further investigated the importance of the GPI anchor in PrPc transfer by replacing the GPI anchor of PrPc with the transmembrane and cytoplasmic domains of human CD44 to generate a transmembrane form of PrPc. In immunoblots, PrP-CD44 chimeric protein migrated as a broad band at ~55 kDa and was expressed at a level similar to that of GPI-anchored PrPc in the M17-PrP cells (Fig. 5, B and C). Co-culture of the M17-PrP-CD44 cells with IA cells under conditions identical to those used with the M17-PrP cells did not result in PrPc transfer (Fig. 5D) suggesting that the GPI anchor is required for PrPc transfer.

Intercellular Protein Transfer Is Not Only Limited to PrPc—We investigated whether CD90 (homologue of rodent Thy-1), another GPI-anchored protein, is transferred from M17-PrP cells to IA cells. We chose CD90 because CD90 is a small cell surface molecule, is highly expressed on neurons, and is likely to be present of M17-PrP cells.

We found that a moderate level of CD90 is present on the surface of M17-PrP cells (Fig. 6A). No CD90 immunoreactivity is detected on the surface of IA cells with or without PMA (shaded peak). We then co-cultured M17-PrP cells with IA cells. We chose CD90 because CD90 is a small cell surface molecule, is highly expressed on neurons, and is likely to be present of M17-PrP cells.

We found that a moderate level of CD90 is present on the surface of M17-PrP cells (Fig. 6A). No CD90 immunoreactivity is detected on the surface of IA cells with or without PMA (shaded peak). We then co-cultured M17-PrP cells with IA cells either with or without PMA as described earlier. After coculture, we found that CD90 immunoreactivity was detected on activated IA cells (Fig. 6B, dark line), but not on nonactivated IA cells (gray line). These results provide direct evidence that the phenomenon of intercellular protein transfer is applicable at least to another GPI-anchored protein.

Transfer of PrPc from M17-PrP Cells to Splenocytes from PrPc−/− Mice—We also examined whether PrPc could be transferred from M17-PrP cells to normal cells using spleen cells
from PrPC<sup>C</sup>−/− mice. Co-culture of PrPC<sup>C</sup>−/− spleen cells with M17-PrP cells for 16 h without PMA did not result in PrPC<sup>C</sup> transfer (Fig. 7A). Following activation by PMA or ConA, a T lymphocyte mitogen, PrPC<sup>C</sup> was detected on the surface of spleen cells (Fig. 7, B and C). These results indicate that PrPC transfer also occurs in normal cells and is not species specific.

**DISCUSSION**

The present study shows that after cellular activation PrPC<sup>C</sup>, a GPI-anchored protein, can be transferred from neuronal cells to other cell types. Based on the levels of immunofluorescent intensity on M17-PrP cells and IA cells, we estimate that −1 to 5% of the PrPC<sup>C</sup> present at the surface of the donor cells is transferred to the acceptor cell (n > 10). This transfer requires cellular activation by PMA, membrane docking by the GPI anchor, and direct cell to cell contact. These requirements imply that the GPI-anchored protein transfer is tightly regulated and depends on the physiologic state of cells as well as the microenvironment. Whereas these conclusions are based mainly on studies carried out with PrPC<sup>C</sup>, it is likely that they are applicable to other GPI-anchored proteins, at least for CD90 as demonstrated here.

PMA increases PrPC expression in donor M17-PrP cells, but up-regulation of PrPC<sup>C</sup> expression is not required for PrPC<sup>C</sup> transfer, because activation of recipient IA cells or PrPC<sup>C</sup>−/− spleen cells, which lack PrPC<sup>C</sup> also resulted in PrPC<sup>C</sup> transfer, albeit at a lower level. PMA activates protein kinase C (PKC), which is known to induce changes in plasma membrane properties, such as fluidity (45), ruffling (46, 47), and lipid domain reorganization (48–51). In addition to PMA, other PKC activators such as ingenol and thymeleatoxin also induce PrPC transfer. Accordingly, inhibitors of PKC such as bisindolylmaleimide I and Go<sup>6976</sup>, which is specific for PKC α and βI isoforms, abolish PrPC<sup>C</sup> transfer (not shown). Moreover, inhibitors of intracellular calcium release such as 8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride also completely inhibited PrPC<sup>C</sup> transfer (not shown). Collectively, these results suggest that it is the calcium-dependent PKC isozymes (α or βI or both) that are important in PrPC<sup>C</sup> transfer. We also demonstrated that activation of PrPC<sup>C</sup>−/− spleen cells with ConA, a T cell mitogen also resulted in PrPC<sup>C</sup> transfer. Recently, it has been shown that PrPC<sup>C</sup> has signaling activity that plays an important role in neuronal differentiation (7). This activity

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**Fig. 4.** PrPC<sup>C</sup> transfer requires direct cell to cell contact and cellular adhesion. A, more PrPC<sup>C</sup> is present in microvesicles from PMA-activated than nonactivated M17-PrP cells. Microvesicles purified from the culture supernatants (see "Experimental Procedures") were blotted in equal protein amounts with mAb 8H4. B, culture of PMA-activated IA cells with microvesicles obtained from M17-PrP cells and containing PrPC<sup>C</sup> did not show any evidence of PrPC<sup>C</sup> transfer (light and dark gray lines). IA cells (1 × 10<sup>6</sup>) were co-cultured for 16 h with microvesicles corresponding either to 2 × 10<sup>6</sup> M17-PrP cells (light gray line) or to 10 × 10<sup>6</sup> cells (dark gray line) and immunostained with mAb 8H4. Nonactivated IA cells co-cultured with microvesicles from nonactivated M17-PrP cells were used as control (shaded area). C, PrPC<sup>C</sup> transfer did not occur when direct cell-cell contact between IA and M17-PrP cells was prevented (thick line, PMA treated; shaded area, no PMA treatment). Cells were co-cultured in a top and lower chamber separated by a membrane with 0.4-µm pores. D, an adhesion assay (see "Experimental Procedures") showed 30–40% more IA cells adherent to M17-PrP cells in the co-cultures pretreated with PMA (dark gray bar) than in untreated co-cultures (light gray bar). *p < 0.05 compared with the non-PMA-treated sample.
depends on PrP\(^C\) coupling to the tyrosine kinase Fyn. It will be important to determine whether transferred PrP\(^C\) contributes to, or generates, this activity.

In agreement with our finding that direct cell to cell contact is essential for PrP\(^C\) transfer, we consistently observed a small increase in cellular adhesion between activated donor cells and acceptor cells. Increase in cell adhesion may be because of activation of adhesion molecules, or a general effect on the membrane, such as the distribution and organization of membrane microdomains (52). GPI-anchored proteins associate with lipid rafts, which are membrane microdomains rich in cholesterol and glycosphingolipids (53, 54). Lipid rafts play an important role in intracellular trafficking and sorting (27, 55). Although we have established that PrP\(^C\) is associated with lipid rafts in M17-PrP cells,\(^2\) it remains to be determined whether the transferred PrP\(^C\) is also associated with lipid rafts on IA cells, and whether lipid rafts are important in intercellular transfer of GPI-anchored proteins. On confocal microscopy both CD44 and transferred PrP\(^C\) formed a punctuate pattern, and partially co-distributed on the IA cell surface. This suggests that PrP\(^C\) may transfer to IA cells as individual molecules, which are subsequently re-organized into membrane domains. Alternatively, membrane fragments or microdomains that contain multiple PrP\(^C\) molecules might selectively be transferred.

It has been reported that intercellular transfer of CD4-GPI occurred spontaneously from transfected HeLa cell to recipient cells, and direct cell-cell contact was not required. Transfer of CD4-GPI was mediated by microvesicles released from donor cells (36, 56). This discrepancy may be because of the nature of donor cells; M17 is a neuronal tumor cell line, HeLa is an epithelial tumor cell line. The cellular activation and cell-cell contact-dependent mechanism described here may be cell type-dependent. This interpretation is in good accordance with the observation that the efficiency of CD4-GPI transfer is cell type-dependent. Alternatively, the expression levels of the protein may determine the efficiency of transfer. GPI-anchored proteins express at very high levels, such as in the CD4-GPI-transfected HeLa cell may permit the protein to relocate to the recipient cells without activation and adhesion.

Another important issue we also addressed is whether in-
crease in adhesion alone is sufficient for the transfer to occur. We first “force” the IA cells to adhere to the M17 cell monolayer by centrifuging the co-culture plate. We found that simply physically forcing the two cells to adhere without cellular activation does not result in PrPC transfer (not shown). This result provides additional evidence that cellular activation is critical in triggering efficient PrPC transfer.

We also show that the GPI anchor is required for the transfer, because the trans-membrane protein PrP-CD44 is not transferred from M17-PrP cells to IA cells. This finding also indicates that transfer is unlikely to result from the fusion of M17-PrP cells and IA cells as a consequence of cellular activation. The importance of the GPI anchor is also supported by our findings, which showed that free PrPC in the culture supernatant of activated M17-PrP cells was not the source of transferred PrPC on IA cells. Recent studies revealed that free PrPC in the supernatants of neuronal cells lacked GPI anchors (57). Intercellular protein transfer is not limited to PrP C because CD90, another GPI-anchored protein, is transferred from M17-PrP cells to IA cells. Recent studies suggested that PrP C and Thy-1 are organized in different domains on the surface of rat neuronal cell lines (58). Whether PrP C and CD90 (Thy-1) are also present on different membrane domains on M17-PrP cells and whether cellular activation alters the distribution of these domains are not known.

In contrast to intracellular trafficking, the mechanisms of activation-induced intercellular transfer of GPI-anchored proteins are not known. Lipid transfer has been reported to occur between parasites and human epithelial cells as well as neutrophils (59, 60). There is also evidence indicating that hemifusion of the outer leaflet of the plasma membrane can occur between two cells under in vitro conditions (61, 62). Based on these observations, we hypothesize that after cellular activation, there is a transient and focal fusion of the outer leaflets of the donor cell and the acceptor cell membrane. This focal fusion allows the exchange of lipids, which include GPI-anchored proteins. Experiments are now in progress to determine whether cellular activation results in the exchange of lipids between M17-PrP cells and IA cells.

In addition to providing new insights into the biology of GPI-anchored protein transfer our findings may also have implications for the pathogenesis of prion diseases. In prion diseases acquired by infection, such as Kuru and variant Creutzfeldt-Jakob disease in humans, scrapie in sheep, and bovine spongiform encephalopathy in cattle, the infectious prion most likely enters the host through the gastrointestinal tract, subsequently migrates to the spleen, and causes pathology in the central nervous system. Therefore, from the portal of entry to the target organ, the infectious prion must be transferred through different cell types. Infectious PrPSc has first been detected in the spleen even following PrPSc intracerebral injection indicating that PrPSc can also travel from central nervous system to the peripheral tissues (63, 64). Furthermore, it has been known since the 1970s that activation of the immune system enhances susceptibility as well as shortens the incubation time in experimentally infected mice (65, 66).
PrPC and PrPSc are GPI anchored on the cell surface (20, 26, 67). Whether PrPSc also transfers between cells in a similar manner to PrPC is not known. It has been reported that PrPC to PrPSc conversion required PrPSc and PrPC to be present in the same continuous membrane (68). This result suggests that PrPC has to be transferred from infected cell to the uninfected cell surface in order for the conversion to occur. The GPI anchor-mediated intercellular transfer of PrPC might provide a mechanism for this transfer. Our results indicate that activation-induced PrPC transfer may promote or enhance the conversion by providing PrPSc negative cells or cells with low levels of PrPC with more substrate. On the other hand, the transfer of PrPSc would implant the seed of the infectious agent. Experiments are now in progress to determine whether transfer of PrPSc also occur under similar culture conditions.

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