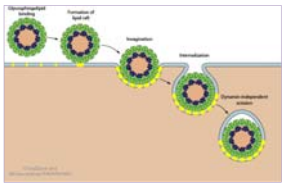
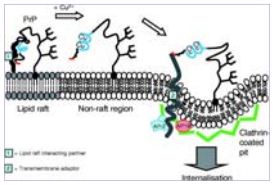




SEMINARIO

RUOLO DEI RAFTS NELL'INFEZIONE PROVOCATA DA TOSSINE, VIRUS, AMILOIDE, E PRIONI

http://viralzone.expasy.org/all_by_protein/5496.html
http://d1dvw62tmnyoft.cloudfront.net/content/joces/118/21/5141/F8.large.jpg

expert reviews

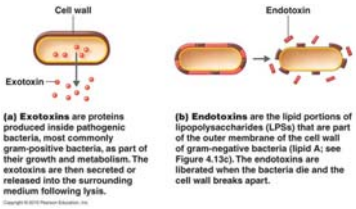
Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases 2002

Jacques Fantini, Nicolas Garmy, Radhia Mahfoud and Nouara Yahi

The fluid mosaic model of the plasma membrane has evolved considerably since its original formulation 30 years ago. **Membrane lipids do not form a homogeneous phase consisting of glycerophospholipids (GPLs) and cholesterol**, but a **mosaic of domains with unique biochemical compositions**. Among these domains, those containing **sphingolipids** and **cholesterol**, referred to as membrane or **lipid rafts**, have received much attention in the past few years. Lipid rafts have unique physicochemical properties that direct their organisation **into liquid-ordered phases floating in a liquid-crystalline ocean of GPLs**. These domains are resistant to detergent solubilisation at 4 °C and are destabilised by cholesterol- and sphingolipid-depleting agents. Lipid rafts have been morphologically characterised as small membrane patches that are tens of nanometres in diameter. **Cellular and/or exogenous proteins that interact with lipid rafts can use them as transport shuttles on the cell surface**. Thus, **rafts act as molecular sorting machines capable of co-ordinating the spatiotemporal organisation of signal transduction pathways within selected areas ('signalosomes') of the plasma membrane**. In addition, rafts serve as a portal of entry for various pathogens and toxins, such as human immunodeficiency virus 1 (**HIV-1**). In the case of HIV-1, raft microdomains mediate the lateral assemblies and the conformational changes required for fusion of HIV-1 with the host cell. Lipid rafts are also **preferential sites of formation for pathological forms of the prion protein (PrPSc) and of the [beta]-amyloid peptide associated with Alzheimer's disease**. The possibility of modulating raft homeostasis, using statins and synthetic sphingolipid analogues, offers new approaches for therapeutic interventions in raft-associated diseases.

Fantini J. **Interaction of proteins with lipid rafts through glycolipid-binding domains: biochemical background and potential therapeutic applications.** Curr Med Chem. 2007;14(27):2911-7**.**

- La ***grande diversità biochimica*** dei ***glicolipidi*** nelle membrane spiega perchè queste molecule sono **spesso selezionate da patogeni** (virus, batteri, protein prioniche) come siti primari di interazioni con la superficie cellulare.
- Inoltre, i glicolipidi si concentrano in microdomini ricchi in colesterolo dove essi possono raggiungere ***elevate concentrazioni locali*** consistenti con il ***legame mulitivalente dei patogeni alla superficie cellulare***.
- Infine, è stato dimostrato che ***i glicolipidi sono in grado di modulare la conformazione delle proteine.***
- Questa **attività tipo chaperonina dei glicolipidi** è stata associata a diversi processi patogenici che includono l'infezione da HIV, la propagazione della proteina prionica, e l'aggregazione amiloide nelle malattie di Alzheimer e Creutzfeldt-Jakob.



Rafts lipidici

TOSSINE BATTERICHE

Rafts come portali d'ingresso per patogeni

- Molti patogeni (virus, parassiti, batterie loro tossine) usano i rafts per invadere le cellule ospite.
- Utilizzano sia **proteine superficiali ancorate a GPI** che i **lipidi dei rafts** (**GSL**, **sfingomielina**, **colesterolo**) come recettori primari o accessori:
  - Tossina del colera: si lega al ganglioside GTM1
  - La tossina Shiga si lega al glicolipide neutro Gb3
  - I miccobatteri si legano al colesterolo
  - Ceppi di *Escherichia coli* che esprimono l'adesina FimH si legano alla proteina CD48 legata a GPI .
  - Tossine del tetano e del botulinium si legano a diversi di- e tri-sialogangliosidi sulla superficie della membrana pre-sinaptica.

Fantini J, Garmy N, Mahfoud R, Yahi N. **Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases**. Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.

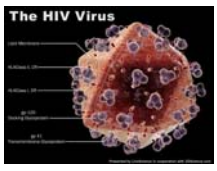
Interazione tossine del tetano e del bottulino con i neuroni

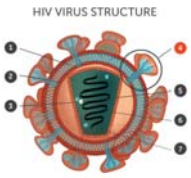
- L'affinità delle tossine per i **recettori di- e tri-sialogangliosidi** è sorprendentemente bassa se si pensa che la tossina è estremamente tossica a concentrazioni dell'ordine del picomolare.
- **Modello del doppio recettore**
  - La tossina si lega alla superficie carica negativamente delle membrane pre-sinaptiche mediante **interazioni a bassa affinità** con un gran numero di recettori gangliosidici
  - In seguito **diffonde lateralmente** per legarsi ad un ipotetico recettore proteico, probabilmente una proteina a 58 kDa (sinaptosomi di cervello di ratto), che si lega a quelle tossine solo in presenza di GT1b e GD1a.
  - L'affinità finale è il prodotto delle due costanti di legame.
- Un'ulteriore ipotesi è che il legame a bassa affinità della tossina con i recettori gangliosidici **induca nella tossina un cambiamente di conformazione** che aumenta l'affinità verso il recettore proteico.

Particolarità dei rafts particolarmente utili ai patogeni e loro tossina (invasori)

1. L'ambiente bidimensionale del raft fornisce **un'elevata concentrazione di recettori a bassa affinità** che stabilizzano l'invasore sulla superficie cellulare.
2. Il raft può **consegnare l'invasore ad adeguati recettori ad alta affinità**.
3. Alcuni lipidi specifici dell'ambiente del raft possono fungere da «**chaperones**» **inducendo alterazioni conformazionali sulla struttura dell'invasore nella vicinanza di recettori ad alta affinità**.

Questo modello è sorprendentemente consistente con la reazione di fusione che ha luogo durante l'infezione provocata dal virus dell'immunodeficienza umano 1 (HIV-1).



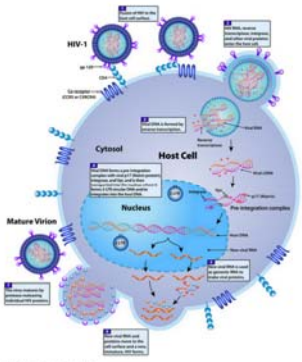


Human immunodeficiency virus (**HIV**) is a lentivirus (slowly replicating retrovirus) that causes *acquired immunodeficiency syndrome* (AIDS),[1][2] a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

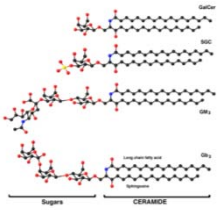
HIV infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells.[3] HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms including: apoptosis of uninfected bystander cells,[4] direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocytes that recognize infected cells.[5] When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections.

http://www.livescience.com/10510-viruses-invade.html
http://en.wikipedia.org/wiki/HIV
http://www.hvtn.org/en/science/hiv-vaccine-basics/_jcr_content/par/imagecomponent/image.img.png/1401393726521.png

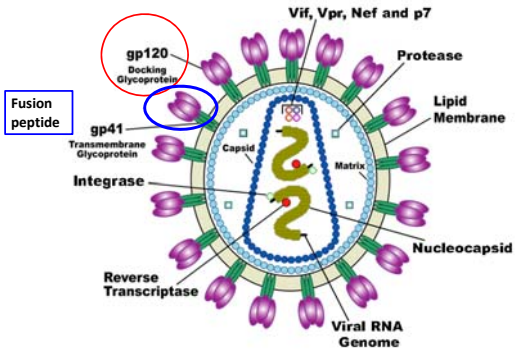
Ciclo di replicazione dell'HIV



Schema degli glicosfingolipidi coinvolti nell'infezione da HIV



http://www.discoverymedicine.com/Clifford-A-Lingwood/2011/04/14/the-role-of-glycosphingolipids-in-hiv-aids/



http://en.wikipedia.org/wiki/HIV

Seminario

Legame e fusione dell'HIV - 1

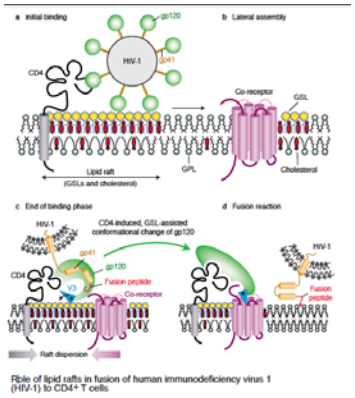
- L'HIV-1 è un virus ad involucro che si fonde con la membrane plasmatica per consegnare il suo RNA genomico alle cellule ospite.
- La fusione dell'HIV-1 con linfociti T $CD4^+$ (linfociti T helper) è un processo altamente controllato, totalmente automatico e irreversibile.
- Per questo evento cruciale, il virus ha il suo proprio "**arpione**", che è dato dalla parte **N-terminale idrofobica della gp41**, una glicoproteina transmembrana dell'involucro virale, che è nota come "**peptide di fusione**".
- **A causa della sua idrofobicità, il peptide di fusione è inizialmente sepolto in una tasca della glicoproteine gp120 della superficie dell'involucro virale, in modo da essere protteto dall'ambiente acquoso**.

Fantini J, Garmy N, Mahfoud R, Yahi N. **Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases**. Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.

Legame e fusione dell'HIV - 2

- In seguito ad un riarrangiamento strutturale dell'involucro virale, il **peptide di fusione viene improvvisamente espulso fuori dagli "spikes" virali dove deve affrontare un ambiente acquoso altamente polare**.
- Per minimizzare la sua energia, il peptide di fusione penetra nella membrana plasmatica della cellula bersaglio dove trova condizioni idrofobiche che lo stabilizzano: «**viral mouse trap model**».

Fantini J, Garmy N, Mahfoud R, Yahi N. **Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases.** Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.



Role of lipid rafts in fusion of human immunodeficiency virus 1 (HIV-1) to CD4+ T cells

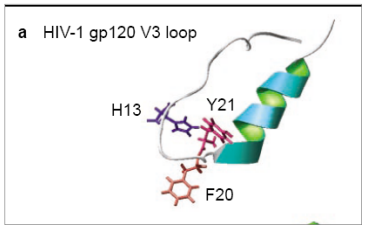
Fantini J, Garmy N, Mahfoud R, Yahi N. **Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases**. Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.

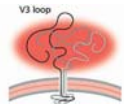
I rafts lipidici giocano un ruolo fondamentale nella fusione dell'HIV (1)

- L'assemblaggio del **machinario di fusione** dell'HIV-1, che funziona essenzialmente per **smascherare il peptide di fusione**, richiede: (1) un **recettore ad alta affinità** (CD4); (2) **GSLs leganti la gp120 a bassa affinità** (il ganglioside GM3 e il globotriosilceramide neutro Gb3); (3) un cofattore di fusione, detto **co-recettore** per l'HIV-1 .
- Gli **co-recettori** per l'HIV-1 identificati includono recettori per le chemochine (sopratutto CXCR4, CCR5, CCR3 e CCR2b) e una serie di recettori orfani, che appartengono tutti alla famiglia di **recettori accoppiati a proteine G con sette domini transmembrana**.
- Il **legame della gp120 (virale)** ai **GSLs (dell'ospite)** è mediato da un **dominio legato da ponti S-S**, detto il **loop V3**, che corrisponde al principale dominio di neutralizzazione della gp120 ed è chiaramente distinto dalla regione di legame con il CD4.

Fantini J, Garmy N, Mahfoud R, Yahi N. Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases. Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.

V3, un dominio di legame con gli sfingolipidi comune all'HIV-1 e alle proteine coinvolte nell'Alzheimer e prioniche - 1





H: HISTIDINE
Y: TYROSINE
F: PHENYLALANINE

Loop V3 della glicoproteina gp120 dell'involucro del virus dell'immunodeficienza acquisita umana di tipo 1 (HIV-1)

Fantini J. **How sphingolipids bind and shape proteins: molecular basis of lipid-protein interactions in lipid shells, rafts and related biomembrane domains**. Cell Mol Life Sci. 2003 Jun;60(6):1027-32.
http://www.uc.edu/news/tsang.htm

I rafts lipidici giocano un ruolo fondamentale nella fusione dell'HIV (2)

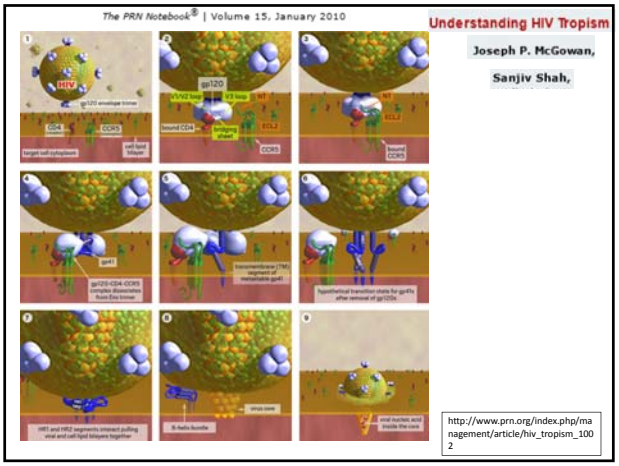
- Nel primo passo della fusione dell'HIV-1 con le cellule T $CD4^+$ si forma un **complesso trimolecolare** fra la gp120, il CD40 e i GSLs in aree di **rafts** della membrana plasmatica.
- I GSLs mediano diversi ruoli in questo processo:
  1. ***Stabilizzano il virus*** sulla superficie cellulare.
  2. ***Facilitano la migrazione dei complessi CD4-gp120 fino ad un co-recettore appropriato***, così mediando gli assemblaggi laterali richiesti per il machinario di fusione dell'HIV-1.
  3. Collaborano alle ***modificazioni conformazionali della gp120*** che portano ***al rilascio del peptide di fusione verso l'esterno dei "virus spikes"*** .

Fantini J, Garmy N, Mahfoud R, Yahi N. Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases. Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.

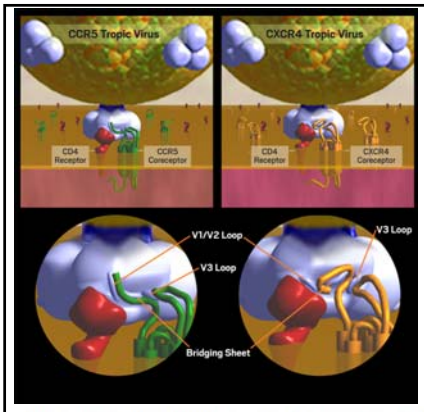
I rafts lipidici giocano un ruolo fondamentale nella fusione dell'HIV (3)

- La stabilizzazione del virus sulla piattaforma che si muove risulta da **molteplici interazioni a bassa affinità fra il dominio V3** della gp120 e la **porzione di carboidrati** del **GM3** e/o **Gb3**.
- Il raft potrebbe allora galleggiare sulla superficie cellulare finchè non trova un co-recettore adeguato, la cui scelta è guidata da un processo di selezione molecolare basata su interazioni del dominio **V3 del virus** con il **co-recettore del linfocito**.

Fantini J, Garmy N, Mahfoud R, Yahi N. Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases. Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.



http://www.prn.org/index.php/management/article/hiv_tropism_1002



HIV tropism (the type of CD4 cell that the virus will be able to infect) is determined by the type of coreceptor recognized by gp120. Binding to CCR5 is known as CCR5 (or R5) tropism, while binding to CXCR4 is known as CXCR4 (or X4) tropism.

http://www.prn.org/index.php/management/article/hiv_tropism_1002

Interazione tra i rafts lipidici e la pp120 dell'HIV-1

In the intestinal mucosa, lipid rafts have been shown to be involved in the transfer of infectious HIV-1 virions through intact intestinal epithelial cells (Ref. 56) and in the pathogenesis of HIV-1 enteropathy (Refs 57, 58). In both cases, the interactions of HIV-1 with intestinal lipid rafts are mediated by the GSL GalCer, a high-affinity receptor for gp120 that was initially discovered in neural cells (Ref. 59) and in the intestinal epithelium (Ref. 60). GalCer is recognised by the V3 loop of gp120, as demonstrated by various biochemical and physicochemical techniques

Overall, it is now clearly established that the V3 loop of gp120 is a sphingolipid-binding domain that mediates the attachment of HIV-1 to lipid rafts from various cell types. Indeed, V3-derived synthetic peptides bind to GSLs and inhibit HIV-1 infection in $CD4^-$ and $CD4^+$ cells (Ref. 63). Thus, both low/moderate-affinity (Gb3 and GM3, with K_d values ranging from 10^{-7} to 10^{-6} M) and high-affinity (GalCer, with a K_d of 10^{-9} M) GSL receptors are recognised by the gp120 V3 loop. The affinity between two ligands depends on the number of structured water molecules that are released to bulk solution as a result of the binding reaction (Ref. 64). The terminal galactose residue of GalCer, GM3 and Gb3 is involved in gp120 binding. In the case of GalCer, this sugar is in close interaction with the membrane, so that gp120 binding might result in the release of numerous water molecules ordered around the lipid–aqueous interface. In GM3 and Gb3, the galactose residue is distant from the membrane, so that fewer water molecules might be displaced by gp120. This

Fantini J, Garmy N, Mahfoud R, Yahi N. Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases. Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.

Coinvolgimento del GalCer nell'enteropatia da HIV-1 - 1

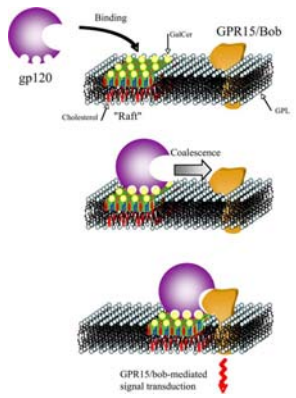
- Il malassorbimento e la diarrea sono problemi comuni e pericolosi nei pazienti con AIDS in parte dovuti ad enteropatie dovute all'HIV non ancora ben capite.
- **La glicoproteina gp120 dell'involucro del HIV-1 è in grado di legarsi al GalCer sulla superficie degli enterociti**.
- In seguito, il complesso è consegnato ad una proteina di membrana chiamata GPR-14/Bob.
- I rafts lipidici contenenti GalCer permettono la migrazione dell'HIV-1 alla superficie della cellula invasa finchè non raggiunge GPR15/Bob.

http://www.chm.bris.ac.uk/motm/galcer/6receptor.htm
Maresca M, Mahfoud R, Garmy N, Kotler DP, Fantini J, Clayton F. **The virotoxin model of HIV-1 enteropathy: involvement of GPR15/Bob and galactosylceramide in the cytopathic effects induced by HIV-1 gp120 in the HT-29-D4 intestinal cell** line. J Biomed Sci. 2003 Jan-Feb;10(1):156-66.

Coinvolgimento del GalCer nell'enteropatia da HIV-1 - 2

- Il GPR/Bob è un recettore con sette domini che attraversano la membrana associato a proteine G.
- Perciò, la stimolazione indotta da gp120 di questo recettore provoca una cascata di attivazione che porta ad un grande aumento del Ca^{2+} intracellulare, estesa depolimerizzazione dei microtubuli, una diminuzione del 80% della barriera intestinale e una diminuzione del 70% dell'assorbimento del glucosio.
- Anticorpi contro il GalCer (e anticorpi contro GPR15/Bob) bloccano questa serie di eventi e proteggono efficacemente le cellule da infezione da HIV-1.

http://www.chm.bris.ac.uk/motm/galcer/6receptor.htm
Maresca M, Mahfoud R, Garmy N, Kotler DP, Fantini J, Clayton F. **The virotoxin model of HIV-1 enteropathy: involvement of GPR15/Bob and galactosylceramide in the cytopathic effects induced by HIV-1 gp120 in the HT-29-D4 intestinal cell** line. J Biomed Sci. 2003 Jan-Feb;10(1):156-66.

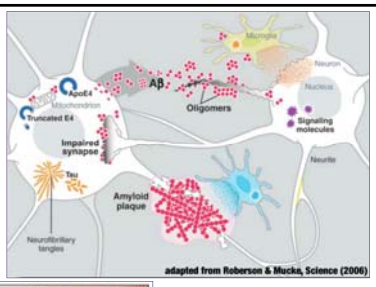


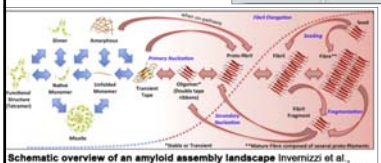
Coinvogimento del GalCer nell'entropatia da HIV-1

http://www.chm.bris.ac.uk/motm/galcer/6receptor.htm
http://www.chm.bris.ac.uk/motm/galcer/6receptor_fichiers/image003.jpg

http://www.cmu.edu/biolphys/smsl/pictures/AD%20basics%20shadow.jpg

Malattie neurodegenerative





Schematic overview of an amyloid assembly landscape Invernizzi et al., InternationalJournal of Biochemistry & Cell Biology, 2012, Vol 44, pp. 1541-1554

http://www.rpi.edu/dept/chem-eng/WWW/faculty/belfort/Figures/Amyloids/Figure2.png

Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases

Jacques Fantini* and Nouara Yahi

Alzheimer, Parkinson and other neurodegenerative diseases involve a series of brain proteins, referred to as 'amyloidogenic proteins', with exceptional conformational plasticity and a high propensity for self-aggregation. Although the mechanisms by which amyloidogenic proteins kill neural cells are not fully understood, a common feature is the concentration of unstructured amyloidogenic monomers on bidimensional membrane lattices. Membrane-bound monomers undergo a series of lipid-dependent conformational changes, leading to the formation of oligomers of varying toxicity rich in β-sheet structures (annular pores, amyloid fibrils) or in α-helix structures (transmembrane channels). Condensed membrane nano- or microdomains formed by sphingolipids and cholesterol are privileged sites for the binding and oligomerisation of amyloidogenic proteins. By controlling the balance between unstructured monomers and α or β conformers (the chaperone effect), sphingolipids can either inhibit or stimulate the oligomerisation of amyloidogenic proteins. Cholesterol has a dual role: regulation of protein-sphingolipid interactions through a fine tuning of sphingolipid conformation (indirect effect), and facilitation of pore (or channel) formation through direct binding to amyloidogenic proteins. Deciphering this complex network of molecular interactions in the context of age- and disease-related evolution of brain lipid expression will help understanding of how amyloidogenic proteins induce neural toxicity and will stimulate the development of innovative therapies for neurodegenerative diseases.

Fantini J, Yahi N. **Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases**. Expert Rev Mol Med. 2010 Sep 1;12:e27.

1

- L'**Alzheimer**, il **Parkinson** e altre malattie degenerative coinvolgono una serie di proteine del cervello («**proteine amiloidogeniche**»), che hanno una **plasticità conformazionale** eccezionale ed elevata **propensione all'auto-assemblaggio**.
- Nonostante i meccanismi tramite i quali le proteine amiloidogeniche uccidono i neuroni non siano ancora interamente noti, un aspetto comune è la **concentrazione di monomeri amilogenici non-strutturati per formare reti bidimensionali di membrane**.
- **I monomeri legati alle membrane subiscono una serie di alterazioni conformazionali, dipendenti dai lipidi, che portano alla formazione di oligomeri con tossicità variabile**, ricchi in strutture a **β-foglietto** (pori annulari, fibrille di amiloide) o **ad α-elica** (canali transmembrana)

Fantini J, Yahi N. **Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases**. Expert Rev Mol Med. 2010 Sep 1;12:e27.

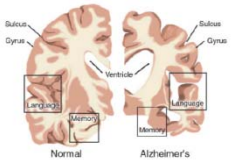
2

- **Nano- e microdomini condensati di membrana formati da sfingolipidi e colesterolo** sono siti privilegiati per il **legame** e **l'oligomerizzazione delle proteine amiloidogeiche**.
- Mediante il controllo dell'equilibrio tra monomeri non-strutturati e i conformeri α e β (**effetto chaperone**) gli sfingolipidi possono sia inibire che stimolare l'oligomerizzazione delle proteine amiloidogeniche.
- Il **colesterolo** gioca un doppio ruolo: **regola le interazioni proteina-lipide** mediante una regolazione fine della conformazione del sfingolipide (effetto indiretto) e **facilita la formazione di pori** (o canali) mediante legame diretto con le proteine amiloigeniche.

Fantini J, Yahi N. **Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases**. Expert Rev Mol Med. 2010 Sep 1;12:e27.

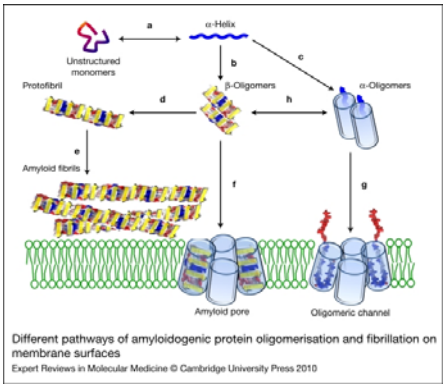
ALZHEIMER

Amyloid fibrils formation is one of the pathological hallmarks of Alzheimer's disease (Dumery, L. et al., 2001)



Fibrils are enriched of β-amyloid peptides (Aβ), a 39–42 residue fragment that is processed from a larger transmembrane protein known as the amyloid precursor protein (APP)

Lipid rafts bind to Aβ and might promote fibrils formation by stabilizing a β-sheet or a β-helix



Different pathways of amyloidogenic protein oligomerisation and fibrillation on membrane surfaces

Expert Reviews in Molecular Medicine © Cambridge University Press 2010

Fantini J, Yahi N. **Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases**. Expert Rev Mol Med. 2010 Sep 1;12:e27.

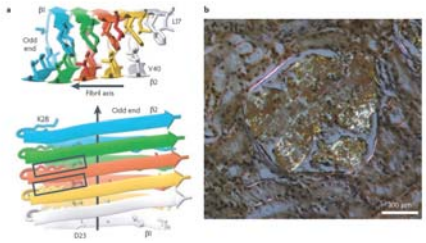
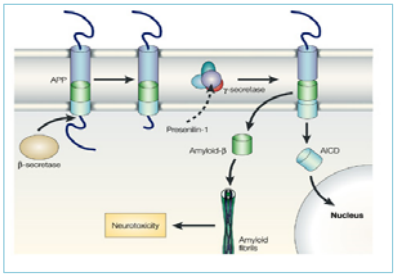


Figure 1 | **Common features of protein aggregation and amyloids. a** | Ribbon diagrams of the three-dimensional structure of amyloid-β (Aβ42) (residues 17–40). Amyloid-forming proteins such as Aβ are all thought to form similar tertiary structures when aggregated, known as a cross-β spine or amyloid[182]. The cross-β spine consists of an ordered arrangement of β-sheets (thick coloured arrows). **b** | Congo red-stained sections of human kidney affected by amyloidosis. The cross-β spine structure is able to intercalate with molecules of the azo-dye Congo red and cause them to emit a characteristic apple-green birefringence upon exposure to polarized light[183]. This unique feature of amyloids has historically been used to identify them histologically.

Aguzzi A, O'Connor T. **Protein aggregation diseases: pathogenicity and therapeutic perspectives**. Nat Rev Drug Discov. 2010 Mar;9(3):237-48.

Roles of lipid rafts in Alzheimer's desease



Amyloid Precursor Protein (APP), β-secretase and presenilin 1 all reside in lipid rafts → Rafts coalescence → Activation of two sequential cleavages which release the β-amyloid peptide (Aβ) → Aβ binding to cholesterol and GM1 promotes fibrils formation by α-helix to β-sheet transition

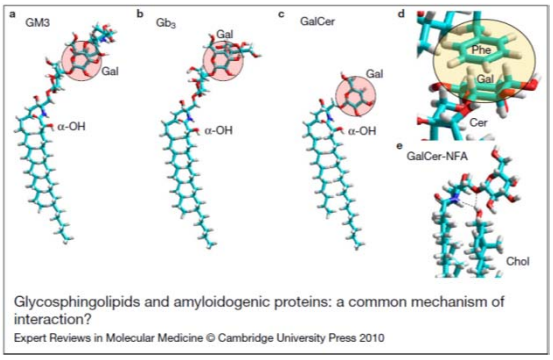
Stassi, Dottorato 2013

Lipid rafts and Alzheimer's disease

Amyloid fibril formation is one of the pathological hallmarks of Alzheimer's disease (Ref. 79). In this case, the fibrils form cerebrovascular senile plaques composed of the β-amyloid peptide (Aβ), a 39–42 residue fragment that is processed from a larger transmembrane protein known as the amyloid precursor protein (APP). Aβ plays a key role in the development of Alzheimer's disease, since all known inherited forms of the disease are associated with changes in Aβ processing and production. Aβ is produced from APP as a result of two sequential proteolytic cleavages involving: (1) a membrane-bound aspartyl protease (referred to as β-secretase); and (2) two homologous membrane proteases (presenilins 1 and 2, which probably correspond to the formerly described γ-secretase activities)

APP, β-secretase and presenilin 1 all reside in lipid rafts (Refs 80, 81). Thus, the production and accumulation of Aβ might occur primarily in these microdomains. Two raft lipids (cholesterol and GM1) bind to Aβ and might promote fibril formation (Refs 28, 80, 82). The molecular mechanism of amyloid fibril formation involves a major conformational change of Aβ, transforming an α-helix to a β-sheet or β-helix

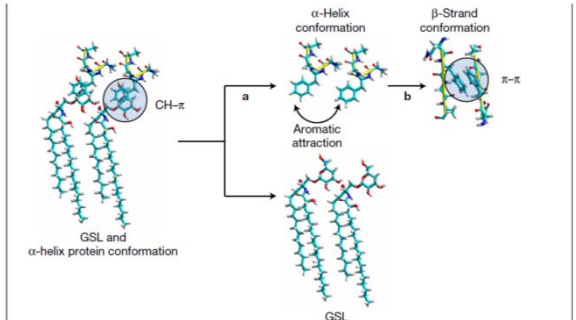
A sphingolipid-binding domain similar to the V3-like domain of PrP has been identified in Aβ (Fig. 6), suggesting a common way by which HIV-1, prion and Alzheimer proteins interact with lipid rafts (Ref. 74). The molecular model proposed above to explain the role of raft lipids in the PrP^{C} to PrP^{Sc} conversion might also apply for Aβ. Amyloid formation proceeds by hydrophobic interactions among conformationally altered Aβ amyloidogenic intermediates. Short synthetic



Glycosphingolipids and amyloidogenic proteins: a common mechanism of interaction?

Expert Reviews in Molecular Medicine © Cambridge University Press 2010

Fantini J, Yahi N. **Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases**. Expert Rev Mol Med. 2010 Sep 1;12:e27.

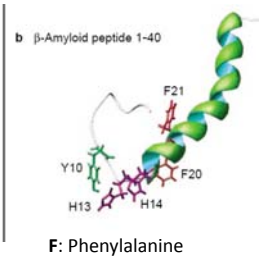


How glycosphingolipids could promote α-helix structures in amyloidogenic proteins

Expert Reviews in Molecular Medicine © Cambridge University Press 2010

Fantini J, Yahi N. **Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases**. Expert Rev Mol Med. 2010 Sep 1;12:e27.

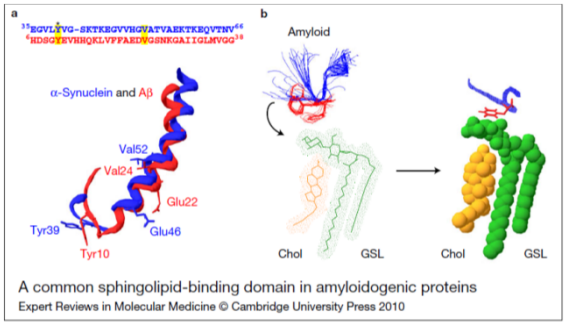
V3: dominio di legame con gli sfingolipidi comune a HIV-1 e proteine coinvolte nell'Alzheimer e prioniche - 2



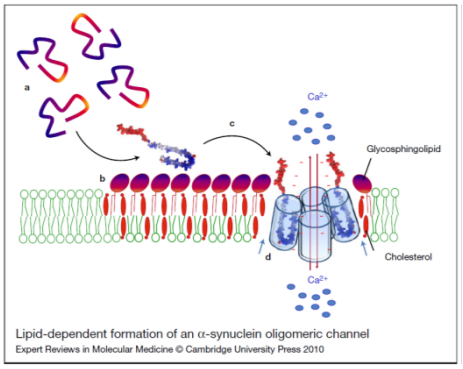
Peptide β-amiloide 1-40, coinvolto nella malattia di Alzheimer

F: Phenylalanine
H: Histidine
Y: Tyrosine

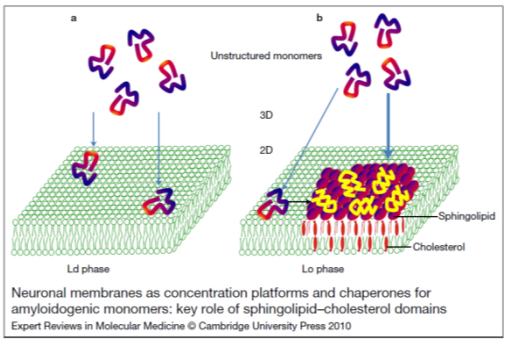
Fantini J. **How sphingolipids bind and shape proteins: molecular basis of lipid-protein interactions in lipid shells, rafts and related biomembrane domains**. Cell Mol Life Sci. 2003 Jun;60(6):1027-32.



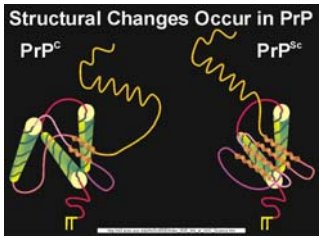
Fantini J, Yahi N. **Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases**. Expert Rev Mol Med. 2010 Sep 1;12:e27.

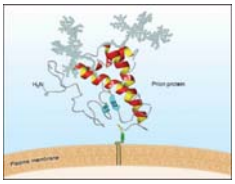


Fantini J, Yahi N. **Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases**. Expert Rev Mol Med. 2010 Sep 1;12:e27.



Fantini J, Yahi N. **Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases**. Expert Rev Mol Med. 2010 Sep 1;12:e27.



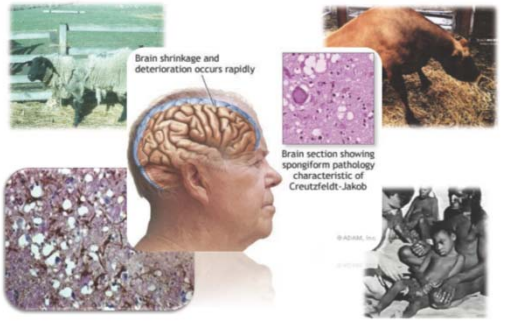


Rafts lipidici

PROPAGAZIONE PRIONICA

http://www.felloweducators.com/brick/bio/images/bioi_prion_isoforms.jpg
https://prionsfactorfiction.files.wordpress.com/2013/10/priolafig1.gif

Rafts are excellent candidate sites for the generation of the scrapie PrP (PrP^{sc})

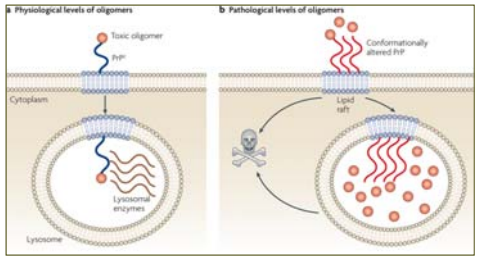


Marco Falchetto, Dottorato 2013

Prioni

- Un **prione** ("**P**roteinaceous **I**nfectious **ON**ly") è un agente infettivo che si ritiene sia la causa delle encefalopatie spongiformi trasmissibili (TSEs).
- E' composto interamente da material proteico, detto "**prion protein**, **PrP**", che si può ripiegare in modi molteplici molto diversi tra di loro, almeno uno dei quali è trasmissibile ad altre proteine prioniche, portando alla malattia che è simile ad un'infezione virale.
- La proteina PrP si trova in tutto il corpo, anche in persone e animali Tuttavia la PrP che si trova nel material infettivo ha una struttura differente ed è resistente alle proteasi.
- La forma **normale** della proteina viene chiamata **PrPC (C: cellulare),** mentre la forma **infettiva** è chiamata **PrPSc** (Sc: "scrapie", il prototipo di malattia prionica delle pecore).
- Mentre la struttura della PrPC è ben definita, la PrPSc è sicuramente polidispersa e poco definita.

https://en.wikipedia.org/wiki/Prion



a | La proteina prionica ripiegata normalmente (**PrPC**) è una **proteina** della superficie cellulare **ancorata a GPI** e **residente permanente nei rafts lipidici**. La funzione fisiologica di PrPC è sconosciuta; tuttavia, una possibilità intrigante è che la PrPC della superfice cellulare possa legare specie extracellulari oligomeriche potenzialmente tossiche e marcarle per la degradazione nei lisosomi. **b** | Ad elevate concentrazioni patologiche, gli oligomeri tossici potrebbero indurre l'aggregazione e/o un cambiamento conformazionale sulla PrPC della superficie cellulare. Ciò può portare all'induzione diretta di percorsi di morte cellulare. In alternative (o in combinazione), l'accumulo mediato da PrPC degli oligomeri potrebbe indurre la tossicità cellulare.

Aguzzi A, O'Connor T. **Protein aggregation diseases: pathogenicity and therapeutic perspectives**. Nat Rev Drug Discov. 2010 Mar;9(3):237-48.

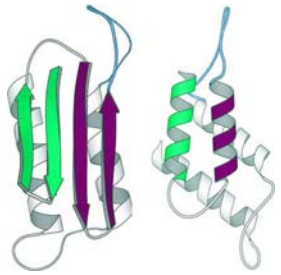
Fantini J, Garmy N, Mahfoud R, Yahi N. **Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases**. Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.

Lipid rafts and prion propagation

Evidence for prion conversion in lipid rafts

Spongiform encephalopathies are an intriguing group of neurodegenerative diseases caused by an agent consisting exclusively of a protein usually referred to as a prion (from 'proteinaceous infectious only'). One of the hallmarks of prion diseases is the cerebral accumulation of a protease-resistant, misfolded isoform of the prion protein (PrP), the so-called PrP^{Sc} (for scrapie PrP), which is derived from the normal cell-surface glycoprotein PrP^{C} (for cellular PrP) (Ref. 66). (Scrapie is one of the major degenerative diseases caused by infectious prions in sheep.) PrP^{Sc} and PrP^{C} have the same amino acid sequence but differ in their conformation. Upon physical interaction with PrP^{Sc}, PrP^{C} is converted into PrP^{Sc}, inducing an endless chain reaction. The conformational changes associated with the PrP^{C} to PrP^{Sc} conversion consist of an α-helix to β-helix transformation (see discussion below).

Non conversione da α-elica in β-foglietto, bensì da α-elica in β-elica



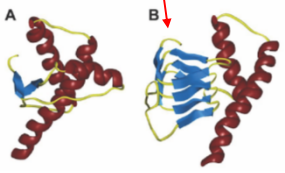
Due possibili conformazioni di una proteina prionica. A sinistra sotto forma di **β-sheet**; a destra come **α-elica**

http://www.learner.org/courses/physics/unit/text.html?unit=9&secNum=4
http://www.learner.org/courses/physics/visual/visual.html?shortname=protein_folding

I **prioni**, noti dal loro ruolo nella malatia della mucca pazza, possono assumere due conformazioni molto diverse. **Il prione a destra, in conformazione elicoidale, si dissolve facilmente in acqua ed è relativamente benigno**. **Il prione a sinistra,** nella conformazione **"beta-sheet"** [N.B. PRECEDENTEMENTE RITENUTA QUELLA DELLA FORMA PATOLOGICA]. **Le forme infettive tendono ad incollarsi ad altri prioni simili formando placche.** Queste placche alterano la struttura del tessuto sano, provocando la tessitura spongiforme che si trova nei cervelli degli animali infetti.

TUTTAVIA

Recent electron-crystallography results suggest that PrP^{sc} contains parallel β-helix and not an antiparallel β-sheet like previous modeling studies



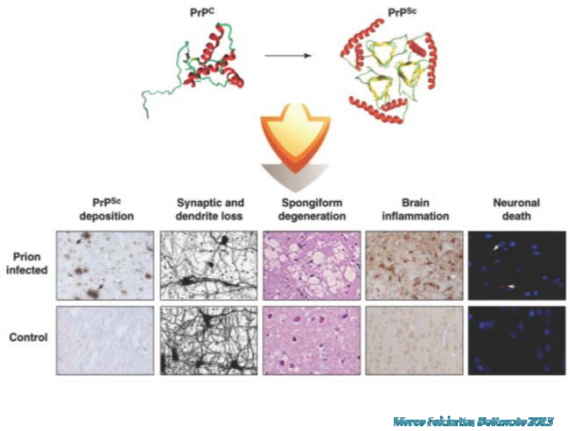
β-helix are stable and suitable for polymerization

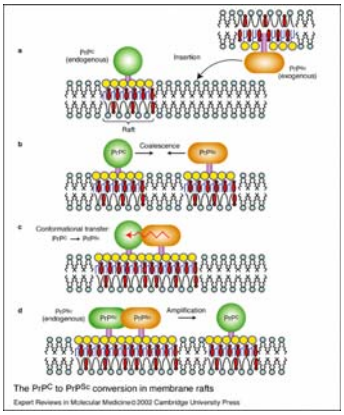
Falchetto, Dottorato 2013

Beta-eliche

- Struttura proteica formata ***dall'associazione di foglietti beta paralleli formando un pattern elicoidale*** con due o tre facce.
- E' un tipo di dominio proteico solenoidale.
- La struttura è stabilizzata da legami di idrogeno intra-catenari, da interazioni proteina-proteina e talvolta da ioni metallici legati.
- Sono state identificate beta-eliche sia destrorse che sinistrorse.

http://en.wikipedia.org/wiki/Beta_helix





The PrP^{C} to PrP^{Sc} conversion in membrane rafts

Expert Reviews in Molecular Medicine © 2002 Cambridge University Press

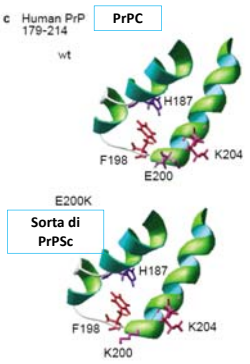
Fantini J, Garmy N, Mahfoud R, Yahi N. **Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases**. Expert Rev Mol Med. 2002 Dec 20;4(27):1-22.

Figure 5. The PrP^{C} to PrP^{Sc} conversion in membrane rafts. A possible model for transmission of PrP^{Sc} from an infected cell to an uninfected cell. This model is based on recent data from the Caughey laboratory showing that the conversion of raft-associated prion proteins requires insertion of PrP^{Sc} into contiguous membranes (Ref. 27). (a) Infectious prions (either individual PrP^{Sc} molecules or small membrane vesicles enriched in PrP^{Sc}) are shed from the surface of an infected cell, and PrP^{Sc} is inserted into the plasma membrane of an uninfected cell. (b) At this stage, endogenous PrP^{C} and infectious PrP^{Sc} proteins are probably localised into distinct rafts of the recipient cell. The coalescence of these rafts will allow a close contact between PrP^{C} and PrP^{Sc}. (c) The PrP^{C} to PrP^{Sc} conversion occurs in membrane rafts. (d) The infection is propagated on the surface of the host cell **(fig005jfm)**.

sensitive; PrP^{sen}) and PrP^{Sc} (proteinase-K-resistant, PrP^{res}) (Ref. 66). Several lines of evidence suggest that rafts are a candidate site for the generation of PrP^{Sc} in infected cells: (1) like other GPI-anchored proteins, PrP is naturally enriched in lipid rafts (Ref. 67); (2) both PrP^{C} and PrP^{Sc} are recovered within DRMs (Ref. 68); (3) cholesterol depletion decreases the formation of PrP^{Sc} whereas sphingolipid depletion increases PrP^{Sc} (Refs 69, 70); and (4) infectious prion rods were found to contain the two sphingolipids GalCer and sphingomyelin (Ref. 71), suggesting that selected raft lipids might interact with normal and/or pathogenic prion proteins. Recently, a

The importance of the membrane environment in the conversion reaction has been underscored by several studies. In particular, PrP^{C} can bind to raft-like membranes enriched in cholesterol and sphingomyelin (Ref. 72). This interaction appears to induce folding of the unstructured N-terminal domain of PrP^{C}, resulting in a protein with a higher content of α-helix compared with the structure of the protein in solution. These data suggest that the interaction of PrP^{C} with lipid rafts might stabilise the 'normal' conformation of PrP^{C}. These protective lipid–PrP^{C} interactions should be destabilised when exogenous PrP^{Sc} is inserted in the vicinity of PrP^{C} in the raft environment (Fig. 5). Since a chaperone activity appears essential to assist the conformational change of PrP (Ref. 73), it is likely that the conversion reaction involves a co-factor that might be either a raft-associated protein ('protein X', according to Prusiner; Ref. 66) or selected raft lipids. In this respect, there is a striking similarity between HIV-1 gp120 and PrP, since both proteins undergo major conformational changes in rafts. Indeed, a sphingolipid-binding domain that is structurally related to the V3 loop of gp120 has been characterised in PrP^{C} (Ref. 74). The V3-like domain of PrP consists of a helix-turn-helix motif formed by 33 of the 36 amino acid residues of a disulphide-linked loop (Cys179–Cys214). This loop includes the α2 and α3 helix of PrP^{C} (Fig. 6). In the V3 loop of HIV-1 gp120, the motif is a hairpin structure with only one α-helix corresponding to α3 in PrP. Interestingly, the V3-like motif of PrP contains His, Tyr and/or Phe residues that mediate binding to individual sugar rings of complex carbohydrates (Ref. 75).

Synthetic peptides derived from the predicted V3-like domain of PrP^C were found to interact with GalCer and sphingomyelin. Moreover, the V3-like domain of PrP^C includes the E200K mutation site associated with familial Creutzfeldt–Jakob disease (Fig. 6). This mutation abrogated sphingomyelin recognition, probably because of an electrostatic repulsion between the positive charges of the Lys residue and of the phosphorylcholine group of sphingomyelin. Taken together, these data strongly suggest that sphingolipids such as GalCer and/or sphingomyelin stabilise the non-pathological conformation of PrP^C in the lipid raft through specific interactions with the V3-like domain of PrP^C. When exogenous PrP^{Sc} is inserted in the target cell membrane, these low-affinity interactions are destabilised, allowing the formation of the PrP^C–PrP^{Sc}–co-factor complex (Refs 72, 73). The consequence of this autocatalytic process is the pathological formation of amyloid fibrils (prion rods), which accumulate in brain tissues.



H: Histidine
F: Phenylalanine
E: Glutamic acid
K: Lysine

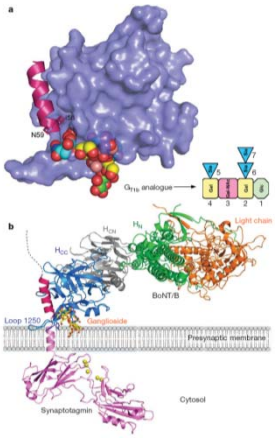
V3, un dominio di legame con gli sfingolipidi comune a HIV-1 e proteine coinvolte nell'Alzheimer e prioniche - 3

- Paragone fra la forma wild-type (wt) della proteina prionica umana (PrP) con la **mutazione E200K** che è associata alla forma familiare della **malattia di Creutzfledt-Jacob**.
- Questa mutazione potrebbe ostacolare l'associazione della proteina mutata PrP^c con lipidi dei rafts quali la sfingomielina mediante repulsione elettrostatica tra le cariche positive della testa di colina della sfingomielina e quella del residuo di **lisina (K)**.

Fantini J. **How sphingolipids bind and shape proteins: molecular basis of lipid-protein interactions in lipid shells, rafts and related biomembrane domains.** Cell Mol Life Sci. 2003 Jun;60(6):1027-32.

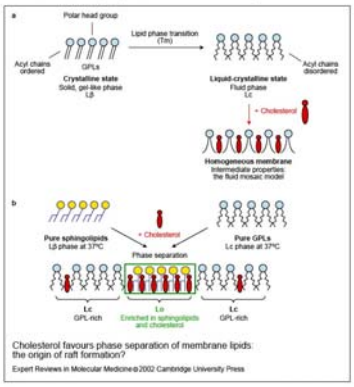
Seminario infezione

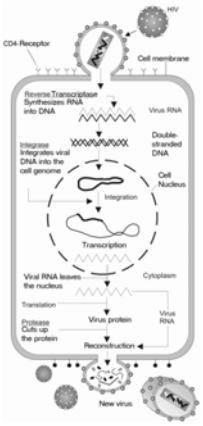
DIAPOSITIVE AGGIUNTIVE



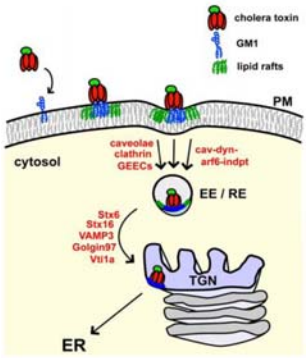
ure 4 | Model of BoNT/B binding to its receptors on a neuron. a, Surface resentation of H_{CC} domain of BoNT/B (light blue) in a complex with Syt-recognition domain (magenta) with a modelled G_{T1b} heptasaccharide heres) docked to the BoNT/B ganglioside binding site, modelled by steins deposited in the PDB under accession numbers 1F31 (ref. 9) and V3 (ref. 21). A cartoon model is shown to indicate the orientation of the ptasaccharide G_{T1b}. **b**, Model of BoNT/B binding using both Syt-II and sglioside receptors at presynaptic membrane. The cytoplasmic domain of aptotagmin is presented as two C2 domains (light pink) bound with cium ions (yellow spheres), modelled using the coordinates of proteins JX[28] and 1DQV[18], in the PDB.

Fantini et al., Expert Rev Mol Med. Dec 20;2002:1-22.



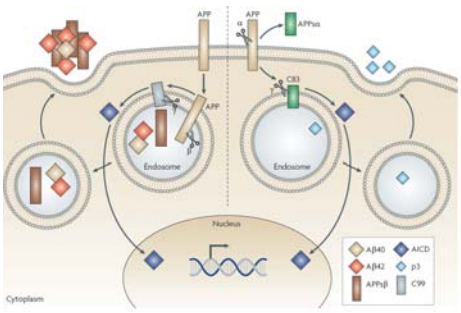


- Il primo passo della fusione coinvolge il **legame al CD4** dei domini leganti il CD4 della **gp120**.
- Una volta che il gp120 è legato alla proteina CD4, il complesso dell'involucro subisce un **cambiamento strutturale che espone i domini leganti le chemochine del gp120** e permettendo ad essi di interagire con il recettore per le chemochine bersaglio.
- Ciò permette un **legame bivalente** più stabile, che permette al peptide di fusion del N-terminale pg41 di penetrare nella membrane cellulare
- Sequenze ripetute nella gp41, HR1, e HR2 interagiscono allora, provocando il collassamento della porzione extracellulare della gp41 formando una sorta di forcina.
- **Questa struttura a loop avvicina molto il virus e la membrane cellulare permettendo la fusion delle membrane e la successive entrata del capside virale**.



Wernick NL, Chinnapen DJ, Cho JA, Lencer WI. **Cholera toxin: an intracellular journey into the cytosol by way of the endoplasmic reticulum.** Toxins (Basel). 2010 Mar;2(3):310-25.

Formazione di β-amiloide



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Protein aggregation diseases: pathogenicity and therapeutic perspectives

Adriano Aguzzi and Tracy O'Connor

Abstract | A growing number of diseases seem to be associated with inappropriate deposition of protein aggregates. Some of these diseases — such as Alzheimer's disease and systemic amyloidoses — have been recognized for a long time. However, it is now clear that ordered aggregation of pathogenic proteins does not only occur in the extracellular space, but in the cytoplasm and nucleus as well, indicating that many other diseases may also qualify as amyloidoses. The common structural and pathogenic features of these diverse protein aggregation diseases is only now being fully understood, and may provide novel opportunities for overarching therapeutic approaches such as depleting the monomeric precursor protein, inhibiting aggregation, enhancing aggregate clearance or blocking common aggregation-induced cellular toxicity pathways.

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Interaction of Proteins with Lipid Rafts Through Glycolipid-Binding Domains: Biochemical Background and Potential Therapeutic Applications

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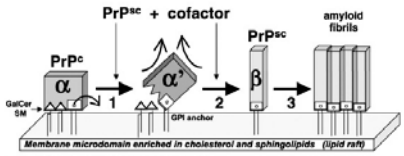
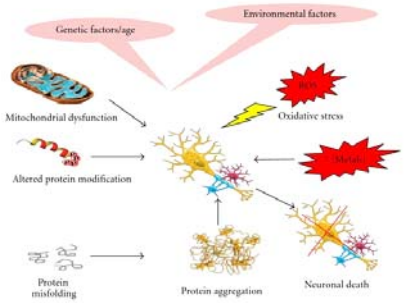


Fig. (1). Protective role of glycolipid for amyloid proteins : a working hypothesis.
The non-pathological cellular isoform of the prion protein (PrP) is located in a lipid raft where it interacts with sphingolipids (GalCer and sphingomyelin SM). These sphingolipids stabilize the α-helix conformation of the protein (1). PrP is inserted in the plasma membrane by a glycosyl-phosphatidyl-inositol (GPI) anchor. In presence of the pathological form of the protein (PrPsc) and probably other co-factors, PrP is separated from its protective lipids, which let the protein free to adopt the more stable β-conformation (2). Amyloid aggregation then results from the π–π driven assembly of aromatic side chains, forming amyloid fibrils which in turn associate (again with their exposed aromatic residues) to form amyloid fibrils (3).

Malattie neurodegenerative



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