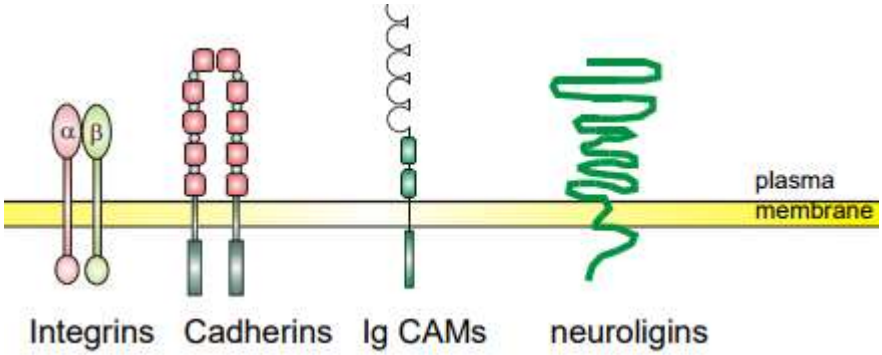
Table of Contents

Cell Adhesion Molecules

Glycoproteins play a major role in recognition between cells and stabilisation of cellular interactions. These molecules are called cell adhesion molecules. They are all bound to the membrane. Accumulates at the cell surface (membrane) i.e. produced intracellularly and delivered to the surface.

E.g.



They bind to other CAMs, or ECM.

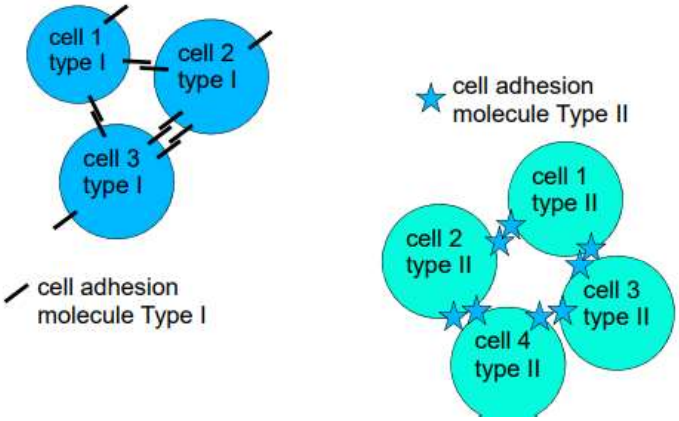
Bead adhesion test to **test binding of these proteins:**

- Recombinant proteins used to coat beads.
- Check the coagulation (clustering) of beads.

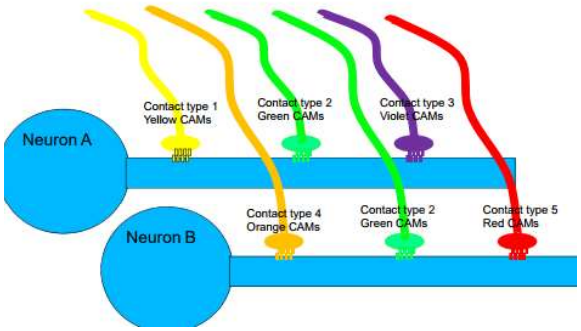
Adhesive interactions are specific i.e. CAMs have a very defined and limited set of binding partner.
Types of adhesion:

- **Homophilic**: same molecules bind to each other
- **Heterophilic**: different molecules bind to each other.

Cells containing different cell adhesion molecules are **segregated into different aggregates**.
Cell adhesion molecules **help cells to recognise other cells of a certain type**.
This could be important for **structuring tissues**



If cells have many contacts, cell adhesion molecules can help cells to recognise distinct “targets”.
Cell adhesion molecules can guide formation of different contacts.

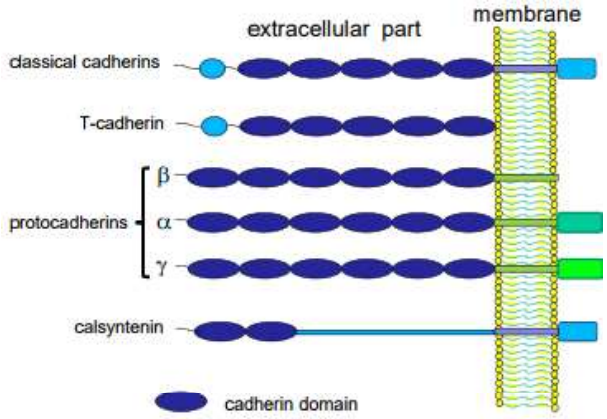


FUNCTIONS OF CELL ADHESION MOLECULES AT THE ORGANSIM LEVEL

- Cellular **attachment**, **clustering** and **segregation**
- Formation of **cellular layers**
- Collection of **fibres into tracts**
- Establishment of **specific connections** between cells (synapses in neurons)

Cadherins

- Characterised by the presence of one or more cadherin domains.
- ~100 cadherin domain containing proteins in the human genome.



- **Diversity generated by alternative splicing.**
- The splicing of proto-cadherins.

Immunoglobulin superfamily (IgSF) of CAMs

- Have modular structure. (divided into smaller components)
- Contain immunoglobulin (Ig)- like domains, which mediate **homophilic adhesion**.
- There are many IgSF members in each organism.
- Can also **contain other domains** such as fibronectin type III domain.
- **Diversity increased by alternative splicing**. Multiple alternative exons responsible for coding regions of the Ig domain.

Integrins

- Function as **dimers, alpha and beta subunits**.
- Structurally complex. Bind to extracellular matrix proteins.
- **Change conformation when binding occurs**. Regulate and **change the affinity of interaction** in intracellular part.
- Diversity created by different combinations of dimers.

- Genomes of vertebrates contain eighteen alpha and eight beta subunits.

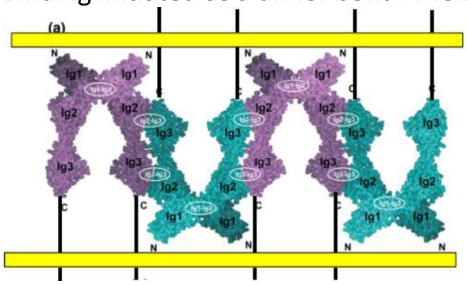
Neurexins and Neuroligins (heterophilic binding partners)

- Diversity increased by alternative splicing.
- >1000 forms of neurexins.

How does binding occur

- In cadherin, homophilic bonding occurs by domain swapping.

- In neural cell adhesion molecule 2 (NCAM2), **an IgSF member**, **bonding by short amino acid** stretches within the IgI and IgII domains.
    - Why so many domains?
        - Interactions can be **enhanced by multiple Ig-like domains**.
        - Binding initiated as a dimer bond. Then dimers start to bind to each other.



        - Multiple bonds strengthen the interaction.
        - This binding is highly cooperative. A 2-fold increase in NCAM levels results in a >30 fold increase in cell adhesion!

Stability of CAMs interactions

- Can be calculated:

dissociation constant

$$Kd = \frac{[A]\,[B]}{[AB]}$$

smaller Kd reflects tighter binding
longer living complexes

Kd of NCAM-to-NCAM interaction is within 10-100 nM range

the half-life of an individual CAM-to-CAM complex is a few seconds

Strength of the bond

- **100pN force** required to break 1 CAM-CAM interaction.
- Although the interactions between individual molecules are transient, adhesion at cell-to-cell contacts can be very strong.
- **Tight interactions** between endothelial cells **form a barrier** between the blood and surrounding tissue.
    - Proteins or toxins cannot pass via cellular junctions.
    - Transport across endothelial cells only by selective uptake and release.

Why CAMs have a multidomain structure

- Allows regulation of the **height of CAMs**. (Shorter CAMs in tighter contacts)
- **Amplify adhesive** interactions.
- Act as **springs** which allow **dynamical** interactions.

Cell adhesion molecule regulation

- By **carbohydrates** i.e. **glycosylation** of the protein.
- E.g. in NCAM, major carrier of the carbohydrate called **polysialic acid (PSA)**
- It is **negatively charged** and occupies a **large hydrated volume** and thereby causes a **direct physical hindrance** of cell–cell contact.
- PSA is highly expressed during development when **cell adhesion remodelling** is required in the brain. PSA expression is dramatically **reduced in mature brain** when stable contacts are important.
- Infusion of an artificial PSA mimetic into the mouse brain enhances performance in certain tasks. PSA mimetics facilitate nervous system repair in mouse models.

- Can also be **regulated by small molecules: Ca^{2+}**
- Ca2+ has to bind to cadherins, to form adhesive bonds. Cadherins mediate **Ca2+-dependent adhesion**.
- Another example is regulation of NCAM adhesion by **extracellular ATP**, which binds to NCAM and **inhibits adhesion**.
    - ATP is a neurotransmitter. Released by neurons.
- Also any other molecules that can bind and inhibit domains: heparin sulphate proteoglycans (NCAMS)

Synapses

Specialised function between two cells

- Transient non-functional contact --> Cells do not change!
- A functional contact is the contact that results:
    - in the **engagement of the molecular machinery** in both cells
    - **changes in the structure** of the contact and/or the whole cell
    - it usually has a certain **function**

“Synapse” - a word coined in 1897, meaning to fasten together.
In 2007: “Synapse: a specialized adhesive junction formed between two closely opposed plasma me mbranes.”
Can form between different cell types.

Neuronal synapses in the brain contacts formed by an axon on one neuron on a dendrite of another neuron.
Neurons form networks in which all **neurons are interconnected via synapses**.

- Easier to **study networks in vitro**: **low density** of neurons and working with **a flat network**.

Synapses have certain **ultrastructure enabling the function**

Molecular composition of synapses also enables the function.

E.g. Neurotransmission: important for neuron to transmit signals across neurons.

1. Action potential
2. Vesicle exocytosis neurotransmitter release.
3. Receptors detect neurotransmitter membrane is depolarised and electrical signal generated

Antibodies against synapse-specific proteins can be used to label synapses.

Why do we study neuronal synapses?

- Importance of synapses (when synapses are not present we die)
- Neurotransmission is required for all **brain functions**.
  - Botulinum toxin is lethal as it blocks neurotransmission.
- Synapses play a central role in **information processing, learning and memory**. They are create d during learning. They also change structurally and functionally during learning.
- Changes in synapses underlie **synaptic plasticity**:
  - E.g. **long-term potentiation** (LTP): a long-lasting enhancement in signal transmission between two neurons. LTP is widely conside red one of the major cellular mechanisms that underlies learning and memory and such pathological conditions as addiction.
- Numbers of synapses and their properties change in **brain disorders**.
  - For example, in Alzheimer's disease, numbers of synapses decline resulting in memory l oss.
  - Mutant mice deficient in adhesion molecules have problem with learning and memory.

Formation of synapses:

- Accompanied by **contact formation**, **recruitment of molecular components, and structure/function acquisition.**
- **CAMs promote** synapse formation because they mechanically **stabilize contacts** and **recruit molecular components**.
  - CAMs knockout show formation of synapse but then disappear over time as not stable.
- Adhesion molecules are among the first molecules accumulating at new contacts.
- **NCAM** travels with synaptic vesicle precursor, when transient contact is made, NCAM stabilises it.
- Other CAMs such as **cadherins** are also delivered to contacts in vesicles, inserted to the surfac e membranes and form adhesive bonds stabilizing contacts.
- Over-expression of NCAM promotes formation of synapses on dendrites of neurons.

When do levels of cell adhesion molecules change?

- During development. In the brain, **CAMs increase during active synaptogenesis**, i.e. the time when synapses are actively formed.
- CAMs **increase during electrical stimulation**, or during learning.
- Change in different disorders.
  - Highly expressed in cancer cells (NCAM or L1): **helps migrate**. Detection of such molecules is used in **diagnostics**.
  - In Down syndrome, triplication of chromosome 21 results in **increased expression of the Down syndrome cell adhesion molecule**, which is required for neuronal development. This **overexpression contributes to malformation of the brain**.
  - **APP** is another cell adhesion molecule encoded by a gene on chromosome 21, which plays a major role in Alzheimer's disease. Down syndrome patients develop Alzheimer's disease.

Cell adhesion molecules recruit other proteins required for the synapse function, E.g. neurotransmitter receptors required for neurotransmission.

- Can be detected through analysis of **protein composition at neuronal synapses**.
- Screening with dyes, western blots, or mass spectrometry.

CAMs recruit synaptic components by **assembling and anchoring macromolecular complexes**.
- A synaptic scaffold around the CAM
- Proteins not bound to the scaffold will diffuse away.
    - E.g. NMDA receptors

Various adhesion molecules accumulating at contacts can play slightly overlapping functions and assemble different complexes.
Differences determine unique synapse function.

What happens to the ultrastructure of the contacts when cell adhesion molecules are removed?
- Ultrastructure can be analysed by high magnification/resolution techniques: **electron microscopy**
    - Can produce 3D reconstructions, of post synaptic density - important for synapse ultrastructure.
- Disruptions in the ultrastructure of synapses reflect a **reduction in contact stability** and protein composition.

Other synapses (non-neural): recruit and activate proteins which bind to CAMs. E.g. Leukocytes to attach and travel between blood barrier.
- Immunologic synapse between T cells and antigen presenting cells.

NCAM and protein life time (hours) is much shorter than the life time of a synapse (years).
- Thus the pool of CAMs refresh in mature synapses.
- Protein distribution visualised by GFP fusion protein.
- CAMs can be delivered to synapses by diffusion in mature neurons.
    - Illustrated by **photobleaching fluorescent CAMs**, and over time they become fluorescent again.
    - Demonstrates CAMs mobility and contacts are continuously refreshed.
    - Important as protein lifetime is short, while contacts should exist much longer.

Cell Adhesion and Signalling

An example:
- CAMs help the growth of neuron cells.

Cell signalling:
- Can be initiated locally and remotely.
- involves a small number of cell surface receptors or adhesion molecules
- results in large effects on cell size and shape
- Is quick

When cell makes contact:
- Intracellular cytosol changes: Intracellular signalling cascade
- changes in: pH, levels of second messengers, Ca2+, cAMP, activities of enzymes (kinases, phosphatases), cytoskeleton structure, gene expression.
- They do not occur simultaneously, but sequentially.