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Synapse Formation: Let's Stick Together

Dispatch

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Synapse formation requires the precise alignment and attachment of presynaptic and postsynaptic cells. Homophilic cell adhesion molecules have now been found to have a role in these processes on both sides of the synaptic cleft.

The formation of synapses — the specialized junctions between nerve cells through which they communicate — is a complex process requiring the coordinated assembly of components on either side of the synaptic cleft. Synapse assembly begins when the immature presynaptic terminal contacts the postsynaptic cell, leading to the formation of an 'active zone' where neurotransmitters are released into the synaptic cleft. On the postsynaptic side, receptors and signalling molecules are induced and localised, conferring the capacity to transduce the given signal into a postsynaptic response (Figure 1).

These sites of cell contact are precisely aligned and are therefore likely to be physically linked. Several molecules have been implicated in this linkage, for example the cadherins, the neuroligin–β-neurexin cell adhesion complex and the ephrins/Eph receptors [1–3]. But although each of these molecules has been shown to play a role in the various aspects of synapse formation, no single molecule has been found to be essential for all the stages, from initial synapse specification to the formation of mature, functional connections.

Furthermore, levels of complexity exist beyond that of synapse specification *per se*. Specific connectivity between the peripheral and central nervous systems is the culmination of numerous processes, including the targeted outgrowth of axons to specific regions, and the formation of laminae – stratified areas each containing a unique complement of neuronal types [4]. Axons growing towards laminated areas often confine their projections to individual laminae, displaying 'laminar restriction'. Such restriction is thought to be a major component of synaptic specificity [1].

The developing visual system is a convenient model for studying synaptic laminar restriction. In the chick, the retinal ganglion cells receive visual input from external stimuli and then project predominantly to the optic tectum, where each axon synapses in an individual lamina; the retinal ganglion cells projecting to a given lamina are known to have characteristic and distinct neurochemical identities [5]. In addition to this stratification, regions of lamination exist within the retina itself. The dendrites of the retinal ganglion cells arborize in the adjacent inner plexiform layer (IPL), which itself receives input from the amacrine and bipolar cells in the overlying inner nuclear layer (INL) [6]. The IPL is further subdivided into laminar regions within which the amacrine and bipolar cells communicate with the retinal ganglion cells, giving rise to laminar-specific patterns of information transduction *en route* to the brain.

Recent work has brought to light three proteins which are involved in synapse formation. SynCAM [7] appears to act in synapse formation, whereas the others, Sidekick (Sdk)-1 and Sdk-2 [8], have been shown to be involved in laminar-specific synapse formation. Both SynCAM and the Sdks are thought to act via homophilic interactions — the binding of like molecules with like. This is a relatively novel concept in the process of vertebrate synapse formation, which has been hitherto thought of as a fundamentally asymmetric process.

Invertebrates have several immunoglobulin domain-containing proteins known to function as homophilic cell adhesion molecules at the synapse, for example *Drosophila* Fascilin II and *Aplysia* apCAM [9,10]. Based on this information, Biederer *et al.* [7] used a database-search approach to look for similar proteins in vertebrates. They were successful in the identification of SynCAM, a single-pass transmembrane protein with three extracellular immunoglobulin domains. On the

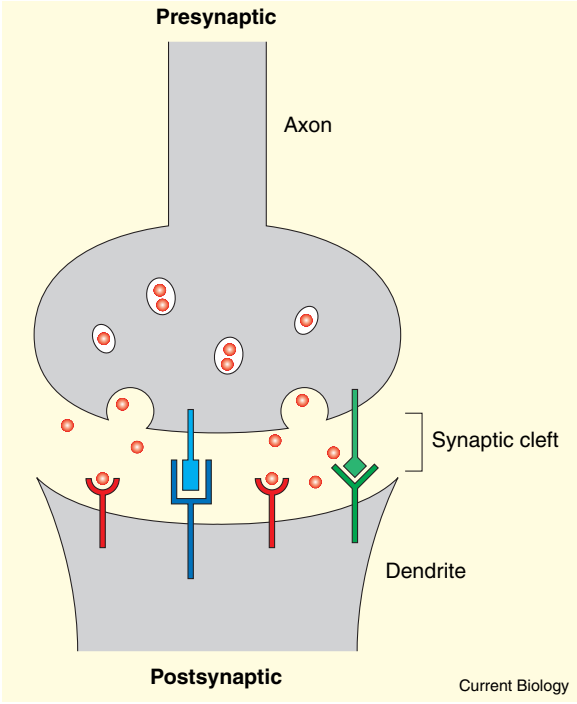

Figure 1. Diagram of a mature synapse.

Vesicles of neurotransmitter (red) fuse with the plasma membrane of the presynaptic cell. Neurotransmitter molecules travel across the synaptic cleft and bind to receptors (red) on the postsynaptic cell. The integrity of the synapse is maintained by tight adhesion of ligand–receptor pairs (blue, green) across the cleft.

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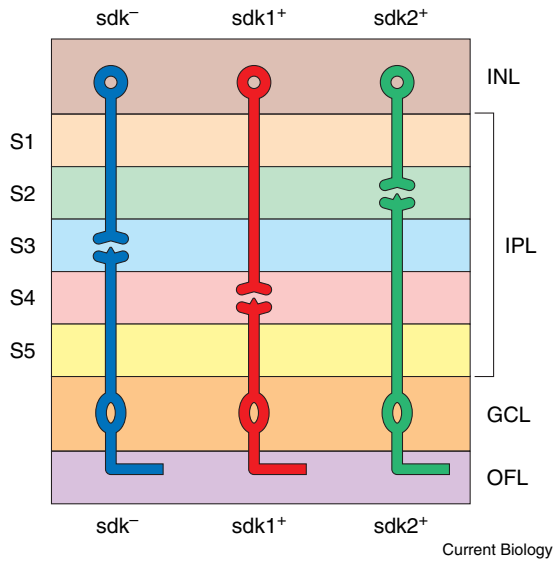

Figure 2. Sublamina-specific functions of Sdk-1 and Sdk-2.

Cells in the INL synapse with retinal ganglion cells in different sublaminae of the IPL. This precise targeting may depend on the expression of molecules such as the Sdks. INL, inner nuclear layer; IPL, inner plexiform layers; GCL, ganglion cell layer; OFL, optic fibre layer; S1–S5, sublaminae of the IPL.

intracellular side, SynCAM has a PDZ protein–protein interaction domain, which is involved in recruiting other proteins to the cell membrane. SynCAM is expressed in the brain of the young rat and the protein levels increase in the first few weeks after birth, corresponding with the main period of synaptogenesis.

Biochemical assays showed that SynCAM is localised to the plasma membrane and is capable of binding to itself homophilically via its immunoglobulin domains, in a Ca^{2+}-independent manner. Further investigation determined that SynCAM is particularly enriched in synaptic plasma membranes, where it colocalises with synaptic proteins such as synaptophysin, neuroligin-1 and CASK. SynCAM interacts specifically and directly with CASK via its PDZ domain.

So far, these findings suggested a correlation between the presence of SynCAM and synapse formation, but little about SynCAM's physiological importance. On a functional level, however, Biederer *et al*. [7] were able to show that the overproduction of SynCAM in hippocampal neurons leads to an increased level of spontaneous synaptic activity. Moreover, perhaps a little surprisingly, SynCAM was found to have the capacity to induce functional synapse formation in non-neuronal 293 cells, when co-cultured with hippocampal neurons. Biederer *et al.* [7] propose that SynCAM alters synaptic inputs, either by enhancing neurotransmitter release or by inducing the formation of new synapses. Hence, SynCAM may prove to be one of the elusive molecules with a function at every stage in synaptogenesis, from the initial induction of localised cell adhesion to the regulation of neurotransmitter release.

The vertebrate Sidekick (Sdk) molecules were identified by Yamagata *et al*. [8] in the course of their screen for proteins expressed by subsets of chick retinal ganglion cells. The two proteins are in some ways similar to SynCAM, but are considerably larger in size – Sdk-1 and Sdk-2 both contain six immunoglobulin domains, a single transmembrane domain and a PDZ interaction motif, but in addition they have thirteen fibronectin-type III repeats. *In vivo* assays showed that the Sdks are also capable of homophilic adhesion and also localised to the plasma membrane at synapses.

It is perhaps the expression profile of the Sdks which is initially most striking. Sdk-1 and Sdk-2 proteins are found in non-overlapping subsets of post-synaptic cells in the retinal ganglion cell layer, with each expressed in approximately a quarter of the cells there. In the inner plexiform layer, however, a complementary expression pattern is found in the synaptic regions. Dividing the IPL into five sublaminae (S1–S5), each occupying roughly 20% of the layer's depth, showed that Sdk-1 is concentrated in S4 with lower levels in S2, whereas the converse is the case for Sdk-2. Surprisingly though, ectopic expression of Sdk-1 by *in ovo* electroporation of early embryos diverted projections of presynaptic amacrine cells from their normal position in S3 to Sdk-1^+ areas in S4, implying that the Sdks may modulate laminar specificity.

Of course, the Sdks can only account for a small proportion of laminar targeting in the IPL, since there are several other layers which are not affected by their activity. But the hypothesis can be made that these molecules induce synapse formation between Sdk$^+$ cells and that they are sufficient to do so (Figure 2).

In summary, the primary function of both SynCAM and the Sdks is thought to be cell adhesion, via homophilic interactions between the immunoglobulin domains across the synapse. In both cases, the PDZ interaction domains are thought to bind to intracellular 'scaffold' proteins, thus helping the tight anchorage between the pre-synaptic and post-synaptic cells. Additionally, the Sdks have motifs which are also found in many axon guidance molecules, for example L1/NgCAM and Robo [11,12], which may be of relevance in the determination of synaptic laminar specificity. It is becoming clear that synaptogenesis is not as asymmetric in vertebrates as it was once thought to be.

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