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Spotlight on vision

Development of retinal layers

Développement de la stratification rétinienne

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ABSTRACT

A noticeable characteristic of nervous systems is the arrangement of synapses into distinct layers. Such laminae are fundamental for the spatial organisation of synaptic connections transmitting different kinds of information. A major example of this is the inner plexiform layer (IPL) of the vertebrate retina, which is subdivided into at least ten sublayers. Another noticeable characteristic of these retina layers is that neurons are displayed in the horizontal plane in a non-random array termed as mosaic patterning. Recent studies of vertebrate and invertebrate systems have identified molecules that mediate these interactions. Here, we review the last mechanisms and molecules mediating retinal layering.

RÉSUMÉ

Une des caractéristiques importantes du système nerveux des vertébrés et des invertébrés est l'organisation des synapses en couches. Ces strates sont essentielles pour l'organisation spatiale des connections synaptiques transmettant l'information neuronale. Un des principaux exemples est la couche plexiforme interne de la rétine de vertébrés, qui est subdivisée en au moins dix sous-couches synaptiques. En plus de cette organisation verticale, les neurones rétiniens sont placés de manière non aléatoire sur le plan horizontal, en une mosaïque régulière. Des études récentes aussi bien chez les vertébrés que chez les invertébrés ont mis en évidence des molécules impliquées dans le contrôle de la stratification. Dans cette revue, nous décrirons les mécanismes et les molécules contrôlant la mise en place de cette organisation en couche.

1. Introduction

One of the most remarkable features of the retina is its extremely ordered structure. As many central nervous system regions, the vertebrate retina is arranged in a multi-layered assembly. The cell bodies of the five types of neurons are distributed among three cellular layers separated by two synaptic strata where connections are constrained. The inner plexiform layer (IPL) is a multi-layered synaptic neuropil constituted of bipolar cell processes, which synapse on retinal ganglion cell (RGC) dendrites, as well as modulatory connections from amacrine cells. The outer plexiform layer (OPL) is formed by ribbon synapses comprising pre-synaptic horizontal and photoreceptor cells and the post-synaptic bipolar cells [1,2]. In addition to this vertical distribution, within the cellular layers, neurons are non-randomly arranged in regular arrays in the horizontal plane. Cell bodies are

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evenly distributed and their dendrites tiled. These patterns are called "retinal mosaic". This very precise organisation is essential in order to establish a functional circuitry of the retina.

Shortly after becoming specified, neurons begin to elaborate neurites and migrate to their final layer. In the mouse, this phase ranges from embryonic day 12 (E12) to post-natal day P7 [3]. All retinal cells derive from a common progenitor, and differentiate following a precise chronological sequence. Ganglion cells, cone photoreceptor, horizontal cells differentiate before birth, while amacrine cells start pre-natally but finish post-natally. Finally, rod photoreceptors, Müller glia and bipolar cells only begin to differentiate after birth [4]. Retinal cells then have to find their way to their final position to cover all the surface of the retina. Then, retinal neurons grow processes and form synaptic contacts within specific layers. Until recently, only a few molecules involved in setting up this complex retinal structure was known. However, genetic tools have led to the discovery of new molecules, which control the different steps that organize the stratification of the retina.

Here, we will review recent data on the mechanisms controlling retinal layering. We will focus on two aspects: the formation of the two plexiform layers and the mosaic patterning.

1.1. Formation of the inner plexiform layer

In mice, the IPL starts to form shortly before birth. In 1893, Ramon y Cajal had already subdivided the IPL into five sublaminae based on his observations of retinal cell processes [5]. In the 1980s, the improvement of neuroanatomical technics allowed for a better description of the IPL and the specificity of each sublamina [6]. Nevertheless, it is only recently that we have begun to understand how bipolar and amacrine cells synapse on the right RGC dendrite to form specific ON/OFF pathways.

Time-lapse studies of the zebrafish retina have shown that RGC dendrites, bipolar cell axons and amacrine neurites seem to grow toward the IPL in a guided manner [7]. Around P14 in mice, the IPL is constituted of more than 10 sublaminae. Structure and function in the IPL are very closely related. For instance, retinal neurons with receptive field centres that are depolarized (ON-cells) by an increase in light intensity have dendrites that project in the innermost sublaminae within the IPL, while hyperpolarized (OFF-cells) will stratified their neurites in the outer sublayers [8]. How do neuronal processes find their appropriate sublamina? In the last five years, numerous molecules have been implicated in the guidance of the IPL processes.

Up to now, five families of molecules were involved in the formation of the IPL, including, several proteins from the immunoglobulin (Ig) superfamily. Sidekick-1 and -2, contain 6 Ig motifs, 13 fibronectin type III (FNIII) repeats and a carboxy-terminal motif (S/TxV) predicted to bind PDZ domains. Sidekicks were discovered through a differential screen of cDNA libraries constructed from single chick RGCs differing by their size and cell surface characteristics. Sidekick-1 and 2 are expressed in the ganglion cell layer just when it starts to be distinguishable from the inner nuclear layer. At E15, Sidekick-1 is expressed in the S4 sublamina of the IPL, while Sidekick-2 is detected in S2 and S4 [9] (Fig. 1). Loss of function experiments has shown that Sidekick-1 and Sidekick-2 are required for lamina specific arborization of processes in the IPL [10]. Overexpression of Sidekick-1 or 2 in the retina redirects neurites to the sublamina in which the corresponding endogenous protein is normally absent [10].

A close relative of Sidekick, Down Syndrome cell adhesion molecule (DSCAM) is also implicated in IPL stratification. This Ig molecule was already known in *Drosophila* for its role in axon tiling and dendrite self-avoidance [11,12]. In the chick, two Dscams were cloned, *Dscam* and *DscamL*, and just like the Sidekicks, they are expressed by distinct subclasses of retinal neurons [10]. More precisely, in the chick IPL, Dscam is expressed in S5 and DscamL in S1, S2 and S4 (Fig. 1). Loss of function of Dscam using interfering RNAs results in the development of ectopic R-cadherin positive processes in S4 instead of S5. Conversely, when Dscam is overexpressed, all the neurites are condensed in S5. Similarly, processes overexpressing DscamL do not project in S3, which is normally DscamL-negative. Thus, at least in the chick, it is likely that these two proteins together with the *sidekicks* regulate the stratification of the IPL. Indeed, in $Dscam^{-/-}$ mice retinal ganglion cells dendrites are fasciculated and cell bodies are clustered. Similarly, bipolar cell processes are also bundled and AII amacrine soma clumped in $Dscaml1^{-/-}$ knockout mice suggesting that these molecules have a role in self-avoidance rather than in lamina specification [13].

As mentioned above, the C-termini of Sideckiks and DSCAM present a motif predicted to bind PDZ domains. Using Sidekick-2, Yamaga and Sanes identified a family of binding partners: the membrane-associated guanylate kinase with inverted orientation (MAGI) [14]. MAGI-2 is

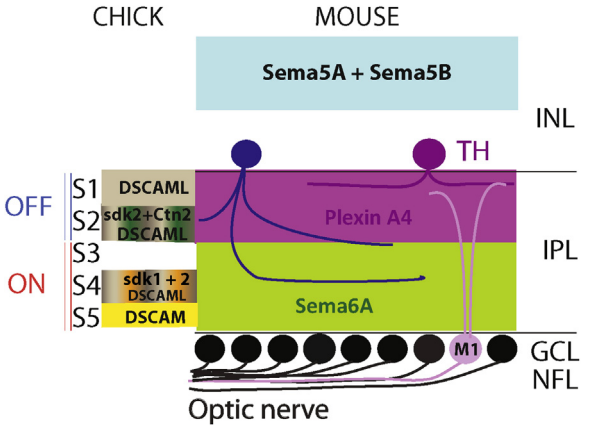

Fig. 1. (Colour online) Molecules regulating inner plexiform lamination. On the left, the expression pattern of the different molecules identified for their role in the IPL stratification in the chick. Sidekick-1 is present in S4 while Sidelick-2 is in S2 and S4 together with DSCAML detected also in S1. DSCAM is found only in S5, and Contactin-2 in S2. In mice, Sema5A and Sema5B are present in the outer retina constrain amacrine and bipolar cells neurites toward the IPL. Sema6A-PlexinA4 signalling is necessary for neurite sublamina targeting of amacrine cells and M1-ipRGC.
Adapted from [17].