DIP/Dpr interactions direct wiring in the fly visual system

(or an informal title: "DIPping into the fly visual system"?)

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The nervous system relies on a combinatorial code of protein interactions that determine wiring specificity. In this issue of Neuron, two complementary studies by Cosmanescu et al. and Xu et al. present the most comprehensive biophysical and structural analysis of the cell-recognition proteins DIP and Dpr to date, and the discovery of key functions of DIP-α and Dpr6/10 in the wiring of the *Drosophila* visual system (Figure 1).

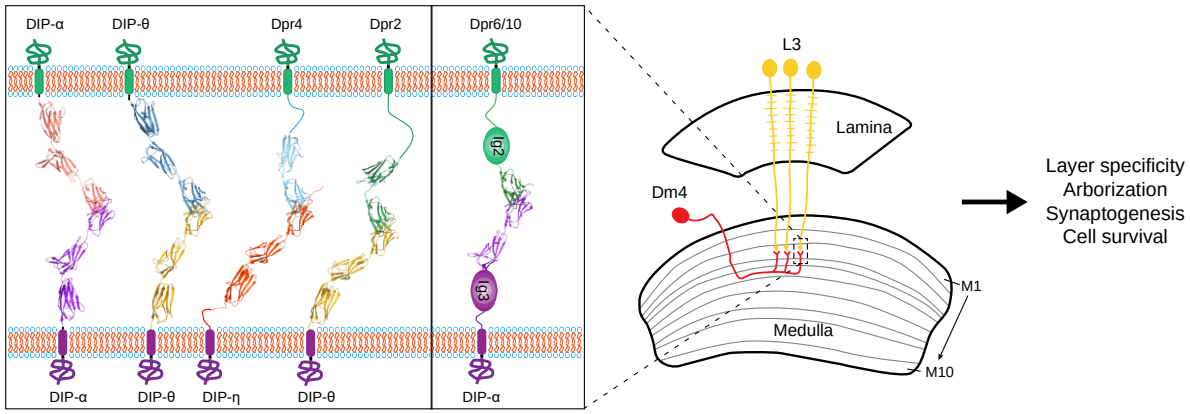

Figure 1: DIP and Dpr proteins form paralogue-specific homophilic and heterophilic interactions. DIP-α and Drp6/10 interact '*in trans*' to regulate layer-specific synaptic circuitry in the fly visual system. For simplicity, the figure shows all DIP- and Drp-mediated interactions *'in trans'*, however it is possible that certain interactions occur *'in cis'* (i.e. on the same cell). The mechanism by which Dpr proteins are attached to the membrane is also not fully understood.

Inter-cellular communication through cell surface receptors is of critical importance in the assembly of complex tissues such as the brain. In organisms with complex nervous system architectures, families of such 'cell-recognition' proteins have typically diversified to include multiple homologues. Subtle structural differences between these homologues lead to specific homophilic and/or heterophilic interaction affinities, which vary in strength depending on the family members involved. This is exemplified by a number of cell surface receptors (Seiradake et al., 2016) and classical cell adhesion molecules (a recent example in (Brasch et al., 2018)). The signals elicited by a specific cell recognition event will reflect the nature and combination of the interacting molecules present, and are therefore highly context-dependent. Neurons can up- and down-regulate cell surface proteins during development, meaning that the context changes as a function of time and space (Zhang et al., 2016). Understanding how these interactions lead to patterning of the brain is one of the biggest open questions in molecular neurobiology.

In this issue of Neuron, Cosmanescu and colleagues investigate the structure-function properties of two groups of Ig superfamily proteins, the 21-member Dpr (Defective proboscis extension response) and the 11-member DIP (Dpr Interacting Proteins) families. Localization of DIPs and Dprs in layer-specific patterns, and with family members that bind one another expressed in synaptic partners, led to the idea that DIP/Dpr interactions might play roles in layer-specificity in the *Drosophila* visual system (Tan et al., 2015). In a comprehensive series of biophysical experiments, the authors analyse the extracellular interactions between all DIP and Dpr members in 231 surface plasmon resonance (SPR) experiments. In addition to previously described interactions identified using an ELISA-based method (Carrillo et al., 2015; Özkan et al., 2013), they uncover 21 new interactions involving 8 of the DIPs and 13 of the Dprs. A revised binding network focused on interactions with K_Ds of 1-200μM revealed four distinct DIP specificity groups. All Dprs interact with one or more DIPs from within a single group, except for Dpr6 and Dpr9, which interact across DIP groups. DIPs and Dprs within a specificity group tend to also be related by phylogeny. Using sedimentation equilibrium analytical ultracentrifugation (AUC) and size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS), the authors reveal that Dpr8, 12, 21 and DIP-α, -θ, -ζ, -η also form homodimers with K_Ds ranging from ~22 μM to 71 μM.

Crystal structures of whole ectodomain homodimers of DIP-α and DIP-θ reveal that interactions between distal Ig1 domains underlie the homophilic binding properties of these proteins. Conserved hydrophobic contacts form the centre of the interaction, and an extensive network of hydrogen bonds complete the binding interface. Notably, the positions of the interacting Ig1 domains of the DIP homodimers is overall similar (RMSD ~0.8 Å of C_α atoms) to the Ig1 domains of the previously solved DIP-α/Dpr6 structure (Carrillo et al.,

2015). Two new DIP/Dpr complex structures from a different specificity group, DIP-θ/Dpr2 and DIP-η/Dpr4, also have similar Ig1-Ig1 interaction topology, suggesting that this is a highly conserved interaction mode. Most of the core hydrophobic and several of the hydrogen-bonding residues are conserved across DIPs and Dprs, with specificity encoded in peripheral regions. The authors integrated the results derived from the structural data, biophysical binding data and sequence alignments to discover the specificity-conferring residues in Dprs and DIPs. Indeed they demonstrate the function of these residues in mutagenesis and SPR experiments designed to convert the specificity of Dpr6 to that of Dpr4, and *vice versa*. Using structure-based protein engineering, Dpr6 was mutated to resemble Dpr4 by introduction of the N102D, H110K and V164K mutations. These three point mutations abolished binding of Dpr6 to DIP-α and enhanced binding to DIP-θ and DIP-η, albeit with a 2-fold weaker affinity compared to Dpr4. In analogy, the Dpr4 mutant (D74N, A76T, K82H and K136V) does not bind to DIP-θ and DIP-η, but binds DIP-α with a K_D of 16.0 μM.

Cosmanescu et al. also assessed the expression profiles of eight DIP family members in 60 medulla neurons using Minos-mediated integration cassette (MiMIC) (Venken et al., 2011) insertions. They found 26 neurons expressing a single DIP, 12 that expressed two DIPs, and one that expressed four DIPs. Based on the data, and given that three paralogs were not assessed, the authors estimate that around 90% of the neurons will express one or, less frequently, two different DIPs. By contrast, multiple Dprs are expressed in each lamina neuron, and their expression appears to be dynamic over time (Tan et al, 2015). The complementary paper by Xu et al., also in this issue of Neuron, makes use of these molecular advances to dissect the functions of DIPs and Dprs in neural circuit assembly *in vivo*.

Understanding the molecular rules that underlie cellular recognition is a challenging task and requires powerful molecular, imaging and genetic tools. Xu et al utilize some of the elegant structure-based mutants presented by Comanescu and colleagues: a DIP-α mutant that specifically lacks the ability to homodimerise (DIP-α^{homo}), and a DIP-α mutant that lacks both homo- and heterodimerisation with Dprs (DIP-$\alpha^{het\text{-}homo}$). DIP-α is expressed in Dm4 and Dm12 neurons in the fly medulla after the onset of neuronal differentiation (Tan et al., 2015). It binds the ligands Dpr6 and Dpr10 with K_Ds of 1-2 μM (Cosmanescu et al.). Dpr6/10 are expressed in L3 neurons, which are presynaptic to Dm4 neurons and pre- and postsynaptic to Dm12 neurons. Xu et al. generate antibodies against DIP-α, Dpr6 and Dpr10 proteins to show that these proteins are expressed in a layer specific fashion from 24 hours after pupa

formation, and are especially prominent in the M3 layer where L3 neurons synapse with Dm4 and Dm12 neurons. The authors use Mosaic Analysis with a Repressible Cell Marker system (MARCM, (Luo et al., 1999)) to show that Dm12 *DIP-α*^{null} mutant neurons display a profound targeting defect. 60% of the mutant neurons mistarget to the M8 layer. Targeting of Dm4 *DIP-α*^{null} mutant neurons was not affected, but these neurons showed a decrease in arbor coverage. Interestingly, *DIP-α*$^{het\text{-}homo}$ mutant neurons displayed similar defects compared to *DIP-α*^{null} mutant neurons, but *DIP-α*^{homo} neurons had no obvious targeting defect. This suggests that heterophilic DIP-α–Dpr6/10 interaction is specifically required for targeting. A gain of function approach also supported these findings: in flies that misexpress Dpr10 in T4 neurons, which arborize within the M10 layer, both Dm4 and Dm12 neurons partially mistargeted to M10. Complete Dm4 mistargeting to M10 was achieved by Dpr10 misexpression in Tm4 neurons in a *dpr6/dpr10* double mutant background. These exciting results suggest that targeting of Dm4 and Dm12 neurons can be directed by DIP-α/Dpr10 interactions, and that perturbing their expression allows specific retargeting of these neurons. The authors also find that *DIP-α*^{null} and *DIP-α*$^{het\text{-}homo}$ mutant Dm4 and Dm12 neurons had reduced synapse numbers. In contrast, *DIP-α*^{homo} mutant Dm4 neurons had increased synapse numbers. This suggests that homophilic and heterophilic interactions impact on synapse formation itself, possibly in an antagonistic fashion.

Loss of DIP-*γ* has been shown to lead to loss of Dm8 neurons (Carrillo et al., 2015). Here, Xu and colleagues show that the number of Dm4 and Dm12 cells is reduced in both *DIP-α* and *Dpr6/10* double mutant animals, suggesting they promote cell survival. The reduction of neurons in the mutants was indeed rescued by the expression of apoptosis inhibitors (p35 and Diap1). Also, the pro-apoptotic protein Hid was increased in Dm4 cells of *DIP-α* mutants. Chimeric DIP/Dpr knock-ins, where the Ig1 domains of DIP-α and Dpr10 are replaced with two exogenous binding domains (those of the vertebrate cell adhesion proteins Nectin1 and 3, respectively) confirmed that DIP-α/Dpr10 binding per se is sufficient to promote cell survival. Nectin 1 and Nectin 3 were chosen because the K_Ds of their heterophilic and homophilic binding are close to those of DIP-α and Dpr10, respectively. It remains unclear whether DIP/Dpr interactions directly stimulate cell survival or whether they bridge other signalling machineries/interactions to activate anti-apoptotic pathways. Given that cell number reduction is also observed in other DIP-expressing neurons, it is tempting to speculate that heterophilic DIP/Dpr interactions provide a general mechanism to regulate cell survival in different layers. Expression of *DIP-α* in the outer medulla neurons of *Dpr6/10*

mutant flies rescued the Dm4 cell loss phenotype, suggesting that DIP-α/DIP-α homophilic interactions also stimulate cell survival. However, *DIP-α* misexpression in T4 neurons did not alter Dm4 or Dm12 targeting, suggesting that the homophilic *DIP-α* interaction can rescue cell survival, but does not re-route neuronal targeting.

Deciphering how the cell surface proteome of neuronal populations dictates their functional behaviour is perhaps the biggest next frontier on the way towards a mechanistic understanding of brain development. The complexity and technical challenges involved are massive, and require a cutting edge and multidisciplinary approach. The work by the Shapiro/Honig and Zipursky labs and colleagues combine some of the most powerful tools available today to assess binding affinities, expression patterns, signalling properties and gain-of-function/loss-of-function paradigms. While this exemplary combination of studies yields important new insights on DIP/Dpr function in the developing nervous system, a central remaining question has not been answered: How does the network of DIP/Dpr interactions relate to the structures of the neural networks they help to pattern? While this remains unclear, the in-depth biophysical characterization of DIP/Dpr interactions presented here provide a firm basis to address this question in future studies.

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