A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade


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Signal Amplification or Organization?

In the past, the biochemistry and in vitro systems emphasized enzymatic activities. …Amplification.

Today, in vivo studies demonstrate the importance of organization in signaling.

• Different signaling cascades can use the same set of proteins if organized into functionally distinct signaling units.
• Organized complexes lead to enhanced response time, specificity, and selectivity.
• Signal complexes prevent crosstalk between different signaling pathways.

⇒ “transducisome:” multivalent, highly organized unit of signaling.

…inaD.
In this paper…

• Experiments done *in vivo*.

• Generated new mutant alleles of inaD.

• Identified InaD domains involved in interacting with signaling components.

• Genetic & physiological analyses of InaD function:
  - localization of signaling components.
  - stability of signaling components.
  - physiology (signaling capacity, kinetics) of photoreceptors.
PHOTOTRANSDUCTION
(DIVERSITY IN)
• Light induced photoisomerization of cis- to trans retinal.
• Activation of Rhodopsin (heterotrimeric G-protein coupled receptor).
• Transducin (Gt) signaling leads to phosphodiesterase activation.
• cGMP is converted to GMP and CNG ion channels close.
• Cells become hyperpolarized.
Phototransduction is different in invertebrates.

Rhodopsin activation signals different channels…

Channel activation in ~20 ms. Channel closing in <100 ms. …fastest known GPCR cascade!

(Montell, 1999)

Channel opening, depolarization
Fly eye →

(~800 ommatidium/eye)

Structure of Ommatidium

R cells = Photoreceptor Cells

Cross-sections show rhabdomeres (black circles)

(Montell, 1999)
Structure of Photoreceptor cell in Drosophila

Ranganathan & Ross, 1997
The importance of PDZ domains in organizing signal transduction: Scaffolds in the post-synaptic density

Zhang & Wang, 2003
The importance of PDZ domains in signal organization:
Scaffolds in the post-synaptic density

"scaffold of scaffolds"
The importance of PDZ domains in signal organization: Scaffolds in the post-synaptic density

“scaffold of scaffolds”

Crosstalk between receptors

Zhang & Wang, 2003
inaD:
A PDZ domain containing protein.
inaD
(inactivation no-after potential)

• discovered genetically: abnormal electrical responses in the retina. (Pak, 1970s)

• inaD first cloned in 1995. (Shieh & Niemeyer, 1995)

• inaD associated with TRP channel in photoreceptors. (Shieh & Zhu, 1996)

• InaD associated with other signaling components: PLCβ, eye-PKC. (Huber et al, 1996)

• inaD215 causes mislocalization of TRP in vivo. (Chevesich et al, 1997)
inaD is a PDZ domain-containing protein

a: Structural components of inaD
b: Amino acid alignment of PDZ domains
The PDZ domain

PSD-95, DLG, ZO-1, coined in 1992 by Cho et al. for associated proteins.

100+ PDZ domain sequences.

Conserved in all metazoans; similar domains in yeast, plants, and bacteria.

Highly modular domain (~90 amino acids).

Recognition of specific C-terminal motifs (often cytoplasmic tails of TM receptors or channels) just 3-5 amino acids long. (C-term. carboxylate recognition) … can recognize internal motifs as well.

PDZ-peptide affinities nM to µM? (probably low µM range of SH2, SH3 interactions).

Present in proteins that typically contain multiple protein-protein interacting domains… scaffolding proteins that organize signaling complexes (often membranous).
PDZ domain structure & peptide recognition

Fig. 1. Structure of the PDZ domain and mechanism of peptide recognition. (A) Ribbon diagram of PSD-95 PDZ domain 3 (residues 306-394, shown in red) with a bound peptide (NH2-KQTSV-COOH, shown in blue). Names of β-strands and α-helices are indicated. The side chains of the peptide P0 residue (valine) and P_2 residue (threonine) are shown in stick form, as is the terminal carboxylate. (B) Diagram of the peptide-binding pocket. Residues in the PDZ-domain-binding pocket are shown in black; the peptide is shown in blue. Hydrogen bonds are drawn as red dotted lines, and hydrophobic packing is indicated by green arcs. (C) Solvent-accessible surface representation of the structure shown in (A) (probe radius=1.4 Å). The peptide is drawn as in A, and key binding pockets are indicated by circles (Doyle et al., 1996).

Harris & Lim, 2001
Generation of new inaD mutants
Why new mutants? What kind of mutants?

How to get inaD mutants?

EMS as a mutagen?

Immunoblot assay for nulls?

…Polyclonal vs. Monoclonal Antibodies?
• Loss of InaD protein in inaD1 mutant flies.

• confirm mutants by sequence analysis.
  
  - inaD1: “null,” amber mutation, truncated at 270
  
  - inaD2: “hypomorph,” missense in PDZ5.
  
  - inaD215: dominant negative, missense in PDZ3.

**Why is inaD215 a dominant negative?**
Co-immunoprecipitation

IP: InaD
IB: TRP, PLC, PKC, Ga, Rh1

What are they trying to show?

norpA = PLC-null
inaC = PKC-null
• InaD does not associate with Ga or Rhodopsin in vivo.

• Different PDZ domains have specific targets:
  PDZ3 ⇔ TRP
  PDZ4 ⇔ PKC
  PDZ5 ⇔ PLC

• Complex formation is independent of other components.

⇒ InaD functions as a “modular multivalent PDZ protein.”
InaD mutants lack transduction complexes
Previous work had shown that TRP is mislocalized in inaD215 mutants.
Localization of complexed proteins in inaD1 mutants.
Localization of complexed proteins in inaD1 mutants.

- InaD1 mutants have mislocalized TRP, PKC, & PLC
- Rhodopsin, Gq, TRPL are properly localized without InaD

Questions? Concerns with figure?
Figure 4

Physiological effect with loss of InaD protein?... Phenotype?

Electroretinograms?
Defective signaling in inaD mutants:
- weaker depolarization.
- lag in resetting proper polarization.
• Further characterize the inaD phenotype.

• Find source of inaD mutant’s reduced signaling strength.

• Confirm specificities of inaD PDZ domains.

⇒ Immunoblot analysis.
Reduced levels of InaD target proteins with null allele.

InaD mutants disrupt stability of target proteins:

No effect on upstream proteins (rhodopsin, Gq, Trpl).
Figure 5

**inaD mutants disrupt stability of target proteins:**

Reduced levels of InaD target proteins with null allele.

No effect on upstream proteins (rhodopsin, Gq, Trpl).

**Effects of specific PDZ domain mutations...**
InaD mutants disrupt stability of target proteins:

Reduced levels of InaD target proteins with null allele.

No effect on upstream proteins (rhodopsin, Gq, Trpl).

InaD215 is defective in PDZ3… TRP unstable.

InaD2 is defective in PDZ5… PLC unstable.

Specific PDZ domain mutants lead to instability of specific target proteins.
Protein localization in specific PDZ domain mutants
Effects on localization of specific PDZ domain mutations in inaD.

Figure 6

Immunofluorescence:

<table>
<thead>
<tr>
<th>Protein</th>
<th>inaD^{215}</th>
<th>inaD^{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRP</td>
<td></td>
<td></td>
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<tr>
<td>PKC</td>
<td></td>
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<tr>
<td>PLC</td>
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Immuno-EM:

wt

inaD

[Diagram showing localization differences]
- Immunofluorescence shows mislocalization of specific target proteins.

- Immuno-EM shows decreased levels of TRP in inaD215 mutants as well as mislocalization of TRP throughout the plasma membrane.
inaD mutants have trp phenotype in aged flies.

Why do inaD215 and trp301 have similar phenotypes?

(20 days post-eclosion)
Figure 7

⇒ Whole-cell Patch Clamping.

Physiological phenotype of inaD215.

What are quantum bumps?
Figure 7

Slow deactivation kinetics in inaD whole-cell assay.
- single vs. sum of two exponentials.
- defect in deactivation?

Normal termination kinetics in inaD quantum bumps.
- bump activation defect?
- increased latency in channel activation.
- inaD215 is DN, sequesters signaling components away from TRP, or simply less TRP.
A Model
InaD is a PDZ domain-mediated organizing complex

Figure 3  Organization of the phototransduction complex by INAD in Drosophila photoreceptors. A schematic of actin-filled microvilli of rhabdomeres is shown at bottom. INAD is depicted beneath the plasma membrane multimerized via its PDZ3/PDZ4 domains. The major interacting proteins are shown binding to specific PDZ domains of INAD. G protein is depicted in dark gray, associated with the membrane in its GDP-bound form. Activated (GTP-bound) Gaq subunit interacts with phospholipase C (PLC). PDZ domains (numbered) are represented by red ovals, F-actin by purple lines.
InaD is a PDZ domain-mediated organizing complex

Harris & Lim, 2001
The importance of PDZ domains in organizing signal transduction: Scaffolds in the post-synaptic density

Zhang & Wang, 2003

“TRANSDUCISOMES”