Orphan GPCR Functional Genomics: trials and tribulations of assay development

Bob Ames
International Human Genome Sequencing Consortium Announces "Working Draft" of Human Genome

June 26, 2000
G-Protein Coupled Receptors

- Superfamily of receptors encoded by 1 to 3% of the genes in our genome
- Transmit signals via heterotrimeric G-proteins
- Contain 7 hydrophobic domains (20-30 a.a.)
  - 7 transmembrane (7TM) domain receptors
- 160+ different subtypes with known ligand
- Respond to a wide range of agents
- 200+ ‘orphan’ receptors with unknown ligand
### GPCRs are “Good” Drug Targets:

**Targets of top 200 selling prescription drugs (1997)**

<table>
<thead>
<tr>
<th>Protein Family</th>
<th># of drugs</th>
<th># distinct targets</th>
<th>Worldwide sales ($US billions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 TMRs</td>
<td>38</td>
<td>25</td>
<td>21.3</td>
</tr>
<tr>
<td>Enzymes (non-proteases)</td>
<td>28</td>
<td>15</td>
<td>16.8</td>
</tr>
<tr>
<td>Ion Channels</td>
<td>28</td>
<td>5</td>
<td>12.0</td>
</tr>
<tr>
<td>NHRs</td>
<td>20</td>
<td>8</td>
<td>7.6</td>
</tr>
<tr>
<td>Bio-therapeutics</td>
<td>20</td>
<td>--</td>
<td>9.02</td>
</tr>
<tr>
<td>Proteases</td>
<td>10</td>
<td>2</td>
<td>7.11</td>
</tr>
<tr>
<td>Symporters</td>
<td>6</td>
<td>3</td>
<td>6.36</td>
</tr>
<tr>
<td>Pumps</td>
<td>4</td>
<td>2</td>
<td>6.02</td>
</tr>
<tr>
<td>Structural</td>
<td>1</td>
<td>1</td>
<td>0.94</td>
</tr>
<tr>
<td>Unknown</td>
<td>11</td>
<td>--</td>
<td>3.71</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>2</td>
<td>1.03</td>
</tr>
<tr>
<td>Total</td>
<td>169</td>
<td>59</td>
<td>91.94</td>
</tr>
</tbody>
</table>

M. Birkeland (IM) & P. Agarwal (Blx): Feb-99
GPCR Superfamily Diversity:
Structurally similar GPCRs bind related ligands

- Catecholamines, neurotransmitters, ATP, LPA, S-1-P
- Neuropeptides (> 50)
- Chemokines
- Glycoprotein hormones
- Glutamate, Ca^{2+}, GABA
- Thrombin

~160 human receptors with characterised ligand

~200 human orphan GPCRs discovered to date
GPCR Functional Genomics Strategy

- In silico
- mRNA expression pattern
- Cloning
- Receptor expression

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Ca²⁺
cAMP

Ligand fishing assays

Extracts / candidate ligands

Ca²⁺
cAMP

HTS

Target Validation

In vitro / in vivo models

Drug discovery effort

Chemistry

Antagonist/agonist

Phase I assays
Recent orphan GPCR ligand pairings
(20 pairings, 43 publications, 15 labs during 1998-2001)

- APJ / apelin
- GPR10 / PrRP
- GPCR97 / H3
- HMTMF81 / CysLT1 2X
- GPR14 / UII 4X
- GPR38 / motilin
- SLC-1 / MCH1 5X
- KIA0001 / UDP-Glu
- HLWAR77 / NPFF2 2X
- OT7T022 / NPFF1 2X
- FM3 / NmU 5X
- FM4 / NmU 3X
- AXOR54 / CysLT2 3X
- FISHBOY / BLT2 3X
- OGR1 / SPC
- AXOR35 / H4 4X
- HHPGS02 / ADP
- CRTH2 / PGD2
- SNORF33 / tyramine
- AXOR21 / MCH2

Excludes EDG receptors and chemokines
Characterization of Orphan GPCRs: *Functional assays*

- **Ca$$^+$$** mobilization (Gq) → (Gs, Gi)
  - FLIPR or apoaequorin
- cAMP flashplates or reporter assay (Gs, Gi)
- Microphysiometer (Gs, Gq, Gi, G?)
- CRE/MRE-reporter (Gi, Gs)
- *Xenopus* oocytes/melanophore (Gs, Gi, Gq)
Primary functional assay used for orphan GPCR ligand pairings

50% of the pairings accomplished on FLIPR or Cytosensor

43 publications describing 20 different receptor / ligand pairings
Characterization of Orphan GPCRs: Functional expression

- Express receptor in a mammalian cell line
  - HEK-293 (*transients*), CHO, COS, others

- Confirm expression
  - Northern blot / TaqMan / epitope tags

- Screen for a functional response
  - Relies on G-protein coupling in the heterologous cell

- Issues: appropriate assay?
  - Endogenous receptors
  - Lack of accessory (RAMPs) or G proteins
Cells used for GPCR functional assay
Neuropeptide FF and Neuropeptide AF “orphan” 7-TM ligands

- Members of the highly conserved cardioexcitatory molluscan peptide FMRF-amide (Phe-Met-Arg-Phe-amide).

- Common feature is the occurrence of Arg-Phe-amide at the C-terminus.

- Implicated as cardiovascular, neuroendocrine, hypothalamic, and opiate functions.

  - Identified as agonists of 2 orphan GPCRs (NPFF1 and NPFF2 / HLWAR77)
Aplysia neurons express a gene encoding multiple FMRFamide neuropeptides

Cell 41: 457-467 (1985)

Preprohormone of Human Neuropeptide FF and Neuropeptide AF
(Human FMRFamide-like Peptide Precursor)

Neuropeptide FF (Human) (hu NPFF)  Neuropeptide AF (Human) (hu NPAF)
SQAFLFQPQRF-amide          AGEGLNSQFWSLAAPQRF-amide

The human HLWAR77 is 420 amino acids and shared 37% identity to the orexin-A receptor.
HLWAR77 (NPFF2) is the Receptor for NPFF and NPAF
Agonist Activity in HEK-293 Gqi5 HLWAR77 Cells

Optical Units

Agonist [M]
Effect of Co-transfection of Individual G-proteins with HLWAR77

- Ga15
- Ga16
- RabGa16
- Gqi5
- Gqo5
- Gqs5
- Gcockt
- No Gprot

Mixture of 5 G-proteins
Agonist Activity in HEK-293 Gqi5 HLWAR77 and NPFF-1R Transfected Cells

**HLWAR77 / NPFF2**
- Human NPFF: EC50 = 21 nM
- Human NPAF: EC50 = 42 nM

**NPFF1**
- Human NPFF: EC50 = 230 nM
- Human NPAF: EC50 = 500 nM
FLIPR$^{384}$

- Tips and pipet head
- Robotics
- Data analysis
  - agonists only
Peptide-based Discovery Services

X-X-R-F-amide Array

Products & Services

- Combinatorial Peptide Arrays Supply
- (Discontinuous) epitope mapping
- Co-development of therapeutic peptides and peptidomimetics
Unique Points

• 20+ years experience in protein-protein interaction studies

• Patented chemistry to produce and use peptides and peptide constructs

• Track record in the (co)-development of diagnostic tests, synthetic vaccines and bio-active peptides

www.pepscan.nl
email: info@pepscan.nl
Screening of PepScan RFamide tetrapeptides
Concentration response curves of select Pepscan peptides vs human NPFFR1 & NPFFR2

HLWAR77/NPFFR2

NPFFR1

Concentration (M) vs Fluorescent Units
GPR10 vs PrRP and Pepscan RF-Amide Array

(assay 3 / plate 1)

PrRP .......... VGRF-NH₂
MCH increases extracellular acidification rates in HEK 293 cells stably expressing MCH$_2$ receptor.
MCH concentration response curve from MCH$_2$ expressing HEK-293 cells

EC$_{50}$ = 1.43 nM ± 0.44 nM
Orphan GPCR functional genomics: challenges ahead

- Fewer new receptors
- For FLIPR based screens will need to continue to explore G-protein chimeras
- Higher throughput Ca$^{2+}$ and cAMP responsive reporter assays are needed
- Accessory proteins (RAMPs, calcyon, etc), receptor dimerization and endogenous receptors
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