51. Evolution of Rhodopsin Supergene Family

Independent Divergence of Visual Pigments in Vertebrates and Insects and Possibly in Mollusks

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(Communicated by Motoo KIMURA, M. J. A., Nov. 13, 1989)

There is a striking similarity between the biochemical pathway of intracellular signalling triggered by light and those by hormones or neurotransmitters and all the signal transduction systems appear to have a similar molecular basis of action.[1],[2] Of the key components involved in the signal transduction systems, the extensively characterized is the receptor that couples with the guanine nucleotide binding protein (G-protein). At present a large variety of the receptor molecules including visual pigments (photoreceptors) as well as hormone and neurotransmitter receptors have been isolated and sequenced. These receptors show remarkable sequence similarities to each other and comprise a large family of genes (rhodopsin supergene family or G-protein-coupled receptor supergene family) derived from an ancestral precursor by gene duplication.[3]-[16] We report here a phylogenetic tree of the members of the rhodopsin supergene family. The phylogenetic tree reveals that 1) the receptors with similar functions appear to be clustered in phylogeny, and 2) different visual pigments expressed in different photoreceptor cells have been diverged independently in each lineage of vertebrates and insects and possibly in mollusks.

Thirty-nine amino acid sequences of the members of the rhodopsin supergene family were compared for highly conserved regions where it is possible to establish unambiguous alignment. On the basis of the alignment, the evolutionary distance k was calculated between each pair of the sequences, excluding amino acid positions where gaps exist in any one of the aligned sequences; the distance k was measured by calculating the amino acid difference K (per site) between sequences compared and correcting multiple substitutions as $k = -ln(1-K)$.[17] Based on the evolutionary distances, a phylogenetic tree was inferred by the neighbor joining method[18]; the evolutionary distances were averaged among different mammalian species. The reliability of the inferred tree was tested by the bootstrap resampling. The bootstrap resampling procedure is repeated 200 times and the probabilities Pr's of occurrence of particular tree topologies (bootstrap probabilities) were estimated.[19]

Fig. 1 shows the phylogenetic tree of the rhodopsin supergene family inferred from the above procedure. The deepest root "a" was determined by

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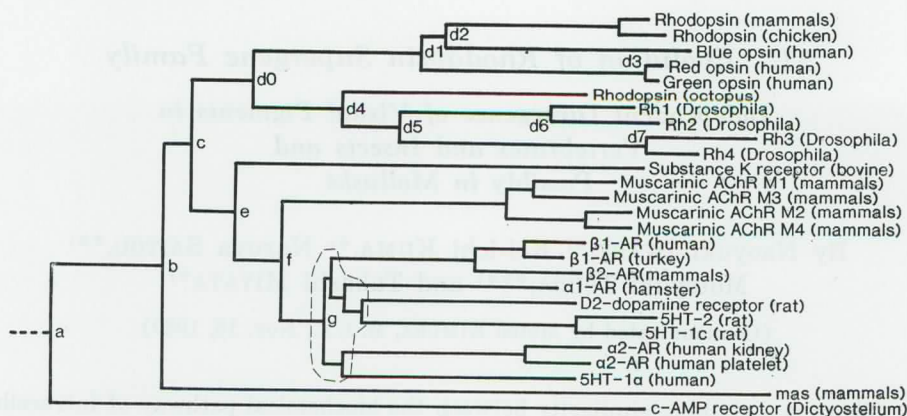***) Department of Physics, Sapporo Medical College, Sapporo 060, Japan.

Fig. 1. Phylogenetic tree of rhodopsin supergene family. This rooted tree was constructed by assuming that the c-AMP receptor is an outgroup. Average evolutionary distances among mammals were used in constructing the tree. The phylogenetic relationships of the a- and β-adrenergic receptors, dopamine receptor and serotonin receptors are somewhat obscure (indicated by a dotted circle); possibly they diverged from each other almost at a same time. ACh, acetylchorine; 5HT-1a, -2, and -1c, serotonin receptors. Sequence data were taken from NBRF database release 20.0 (sequence check was made), except for rhodopsins from mouse,[20] chicken[21] and octopus,[22] muscarinic ACh receptor M1 from human[23] and mouse,[24] M3 from human[23] and porcine,[25] M4 from human[23] and rat,[26] mouse β2-AR,[27] hamster a1-AR[28] human 5HT-1a,[29] rat 5HT-2[30] and *Dictyostelium* c-AMP receptor.[16]

assuming that among all the receptors examined the c-AMP receptor has been diverged from the most ancestral precursor. The reliability of the tree topology was examined based on the bootstrap resampling. Four different tree topologies with non-zero probability were generated (Fig. 2). The tree 1 of Fig. 2, which corresponds to the phylogenetic tree of Fig. 1, is statistically significant with probability of occurrence of 0.87. The phylogenetic tree revealed a strong correlation between the functional similarity and the clustering pattern in phylogeny, except for the case of *mas*/angiotensin receptor[31]; receptors with similar functions appear to be clustered in phylogeny. The node "c" of Fig. 1 separates the rhodopsin family from the hormone and neurotransmitter receptor families; the node "e" separates the peptide hormone receptor (substance K receptor) from the muscarinic and amine neurotransmitter receptors (adrenergic, serotonin and dopamine receptors), and the latter two separate from each other at the node "f". After the functional separation, further divergence by gene duplication has occurred for genes that are expressed tissue-specifically.

There are other supergene families showing such correlation between functions and clustering pattern in phylogenetic tree. These include protein kinase supergene family,[32] G-protein /*ras*/ elongation factor supergene family, steroid/ thyroid hormone receptor supergene family and nicotinic acetylchorine receptor family (Iwabe *et al.*, manuscripts in preparation). These data suggest that this clustering pattern may be a general evolutionary feature characteristic of supergene families, although many supporting data should be accumulated before final conclusion. If this is really the case, it may be possible to infer functions from a phylogenetic tree.
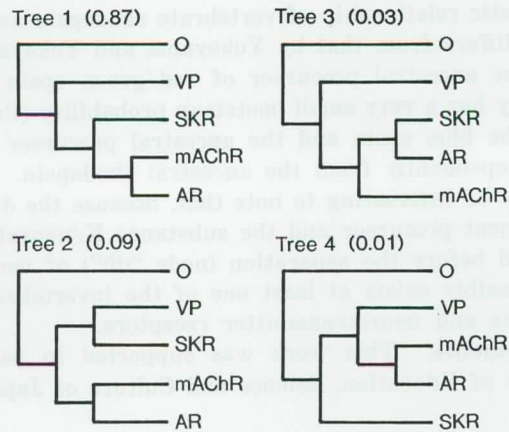
Tree 1 (0.87)   Tree 3 (0.03)

```
        O                                    O
        VP                                   VP
        SKR                                  SKR
        mAChR                                AR
        AR                                   mAChR
```

Tree 2 (0.09)   Tree 4 (0.01)

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        O                                    O
        VP                                   VP
        SKR                                  mAChR
        mAChR                                AR
        AR                                   SKR
```

Fig. 2. Bootstrap probabilities of four possible tree to-
pologies for visual pigments (VP), substance K receptor
(SKR), muscarinic acetylchorine receptor (mAChR) and
adrenergic receptor (AR) subfamilies. The bootstrap
probability of each tree was indicated in parentheses.
O, outgroup.

Tree 1 (0.84)   Tree 2 (0.08)   Tree 3 (0.08)

```
    O                        O                          O
    Mammals                  Mammals                    Mammals
    Octopus                  Rh3/Rh4                    Rh1/Rh2
    Rh1/Rh2                  Octopus                    Octopus
    Rh3/Rh4                  Rh1/Rh2                    Rh3/Rh4
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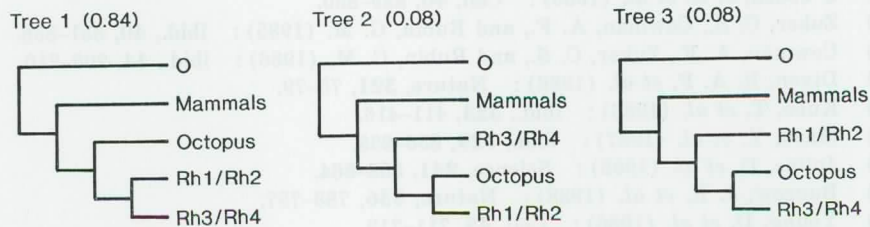
Fig. 3. Bootstrap probabilities of three possible tree topologies of visual pigments
from mammals, octopus, *Drosophila* (Rh1, Rh2, Rh3 and Rh4). O, outgroup. The
bootstrap probability of each tree was indicated in parentheses.

Fig. 1 shows an interesting feature of the evolutionary divergence of visual
pigments. The node "d0" corresponds to the separation of vertebrates and in-
vertebrates. After this separation, three color pigments have diverged in ver-
tebrate lineage. In *Drosophila* the opsin Rh1 is a rhodopsin with absorption
peak of 480 nm and is expressed in the outer six photoreceptor cells (R1–6) of
each ommatidium. Two other opsions Rh3 and Rh4 are expressed in the central
R7 photoreceptor cells that respond maximally to UV light. The remaining opsin
Rh2 absorbs blue light and is expressed in R8 cells.[32] According to the phy-
logenetic tree of Fig. 1, these four visual pigments of *Drosophila* have diverged
in insect lineage after the separation of insects and mollusks (node "d4"), sug-
gesting that the visual pigments of color vision of *Drosophila* evolved independ-
ently from those of vertebrates. The phylogenetic relationship among the visual
pigments of vertebrates, insects and mollusks shown in Fig. 1 is statistically
significant with bootstrap probability of 0.84 (Fig. 3). Although no visual pig-
ments other than rhodopsin is reported from mollusks, it is likely that, also in
mollusks, the visual pigments of color vision may have diverged independently
from those of vertebrates and insects.

The phylogenetic relationship of vertebrate rhodopsin and three color vision opsins in Fig. 1 differs from that by Yokoyama and Yokoyama,[34] in which the blue opsin and the ancestral precursor of red/green opsin are clustered. The latter tree topology has a very small bootstrap probability (Pr=0.04). It is therefore likely that the blue opsin and the ancestral precursor of red/green opsin have diverged independently from the ancestral rhodopsin.

Finally it may be interesting to note that, because the divergence (node "c") of the visual pigment precursor and the substance K/neurotransmitter receptor precursor occurred before the separation (node "d0") of vertebrates and invertebrates, there possibly exists at least one of the invertebrate homologs of the vertebrate hormone and neurotransmitter receptors.

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