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VISIONS AND PERSPECTIVES

Evolution game: which came first, the receptor or the ligand?

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Abstract

On the basis of a bioinformatic approach, we suggest that in invertebrates many ligands interact with a single, ancestral and generalized receptor driving ligand evolution. In vertebrates, on the other hand, the occurrence of gene/genome duplications induced the shift to a ligand-directed evolution of receptors.

Key words: cytokines; cytokine receptors; ligand-receptor evolution

Immunocytochemical approaches revealed that a number of invertebrate species contains mammalian cytokine-like molecules (Ottaviani *et al*., 2004; Malagoli *et al*., 2007). Moreover, mammalian cytokines influence some invertebrate immune functions, such as cell motility, chemotaxis, phagocytosis and cytotoxicity (Ottaviani *et al*., 2004) suggesting the presence of cytokine receptors conserved during evolution from invertebrates to vertebrates.

The presence of cytokine receptors has been reported for platelet-derived growth factor (PDGF)-AB and transforming growth factor (TGF)-β1 (Kletsas *et al*., 1998) in molluscan immunocytes, for interleukin (IL)-1-, IL-2-, IL-6- and interferon (IFN)-γ in sea star cells (Legac *et al*., 1996) and for IFN-γ in tobacco hornworm larvae (Parker and Ourth, 1999). Interestingly, studies performed on IL-2 and corticotrophin-releasing hormone (CRH) revealed that the mammalian cytokines IL-1α, IL-1β, IL-2, tumor necrosis factor (TNF)-α, TNF-β and CRH may bind the same receptor in molluscan immunocytes, suggesting the existence of an ancestral common receptor on the invertebrate cell membrane (Ottaviani *et al*., 1994, 1995). This result indicates that different cytokines could interact with a single receptor in invertebrates.

The hypothesis that a single ancestral receptor might bind different ligands is strengthened by data reporting that the mammalian γ chain of the IL-2 receptor is also functionally involved in the IL-4 and IL-7 receptor complex (Kondo *et al*., 1993; Noguchi *et al*., 1993; Russell *et al*., 1993).

The possibility that receptors able to bind different cytokines may be present in invertebrates prompted us to look at the evolutionary interrelationships between cytokines and their receptors from invertebrates to vertebrates.

We took a comparative bioinformatic approach based on the screening of the wholly sequenced *Drosophila melanogaster*, *Anopheles gambiae* and *Caenorhabditis elegans* genomes for the presence of cytokine- and related receptor-coding genes. Standard tools such us BLAST and ClustalW working at both DNA and protein sequence level were used.

The question about the lead role of either receptor or the ligand during evolution could have two different replies. The first consists in a ligand-directed evolution of receptors, where the former acts as selective agent in the evolution of the latter. In this case, a single ligand may have interacted with different receptors, whose structure has been optimized during evolution in order to increase the specificity of the interaction between ligand and receptor (Fig. 1A). If this hypothesis is true, we should find ligands that have been strongly conserved from invertebrates to vertebrates.

The second reply envisages the evolution of ligands depending on receptor structure and the receptor acting as a selective agent in the evolution of ligands. In this view, different ligands can interact with a single receptor that serves as a selective agent driving ligand evolution. This would mean that receptors which have been strongly conserved from invertebrates to vertebrates could be identified (Fig. 1B).

In order to prove these hypotheses, we studied the evolutionary relationship between CRH, TGF-β

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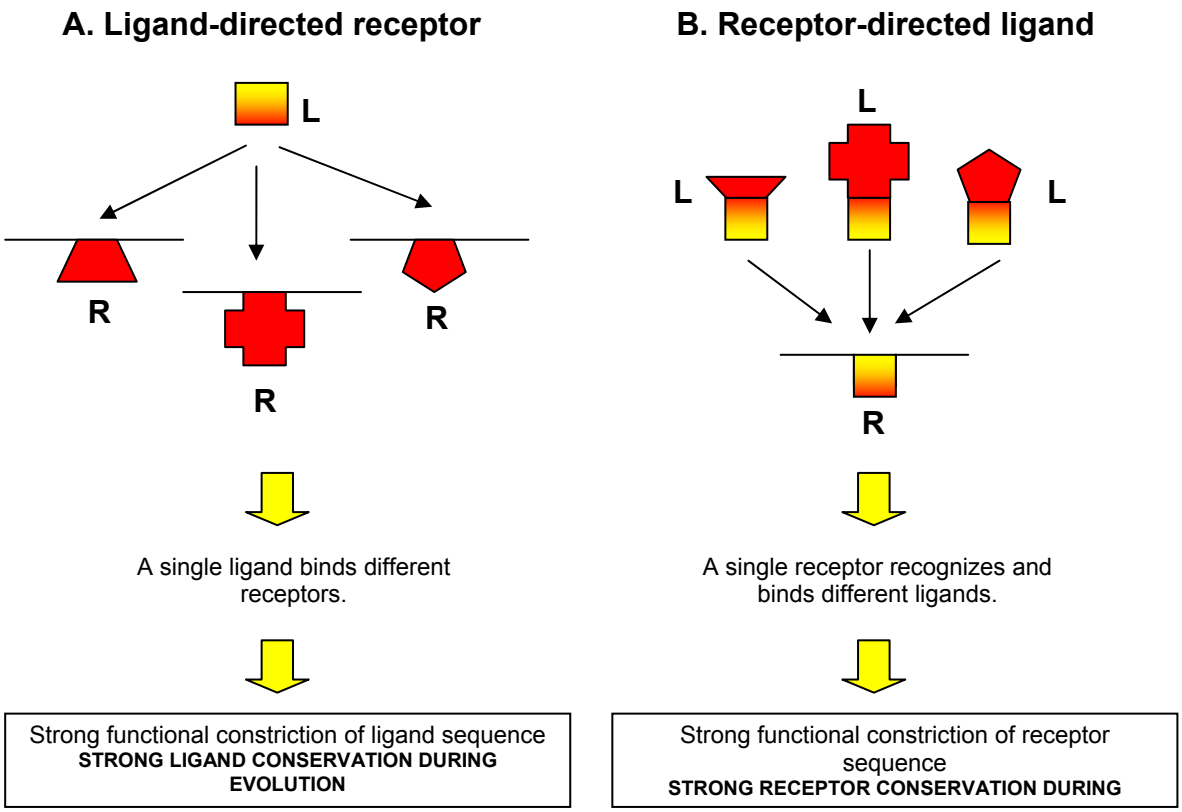

Fig. 1 Comparison of the putative mechanisms involved in the evolution of ligands (L) and receptors (R) in invertebrates.

family ligands and epidermal growth factor (EGF) and their specific receptors.

The analysis of genes coding for CRH and the two related receptors showed that CRH receptors are found in invertebrates with a similarity to mammalian homologues ranging from 43 % to 51 %, whereas the only conserved CRH-coding gene was identified in the lepidopteran *Mamestra brassicae* (Malagoli *et al*., 2002). A similar evolutionary pattern was obtained with the TGF-β receptors, which showed a 49 % to 69 % similarity to mammalian homologues. The present data are in agreement with those reported in Raftery *et al.* (1999), who confirmed that more *Anopheles* and *Drosophila* genes code for putative TGF-β family ligands (Raftery *et al*., 1999). Overall, these findings indicate that there are far fewer receptor-genes than ligand-coding ones (Raftery *et al*., 1999). EGF analysis gives a similar picture: EGF receptor-coding genes are conserved in invertebrates with a sequence similarity ranging from 45 to 67 %.

Our bioinformatic analysis suggests that receptor-coding rather than ligand-coding genes are conserved between vertebrates and invertebrates and that present receptors are more similar to their ancestor than ligands. Moreover, data from invertebrates suggest that more ligands may interact with a single receptor (Ottaviani *et al.*, 1994), supporting the hypothesis that ligand evolution is receptor-dependent. Our assumption founded on cytokine- and cytokine receptor-coding sequences is strengthened by data on the evolution of estrogen receptors (Schwabe and Teichmann, 2004). In particular, Schwabe and Teichmann (2004) suggested that the steroid receptors are much more ancient than previously thought and that the evolution of nuclear receptors is not ligand-directed.

Using a new structure prediction algorithm, developed to find helical cytokines in human databases (Conklin, 2004), we recently have found in *D. melanogaster* a molecule with a structure similar to that of mammalian helical cytokines (Malagoli *et al.*, 2007). This molecule may be involved in the fly immunity, however no information on its receptor is available at present.

We, therefore, suggest that receptor structure undergoes a more tights constraint than ligand origin and that receptors drive ligand evolution in invertebrates. This tendency could also be valid in vertebrates, even if, given the occurrence of gene/genome duplications in vertebrate lineage (Ohno, 1970; Panopoulou *et al*., 2003), a new element has to be added to the previous receptor-ligand scenario. The drastic increase in genome size and gene number occurred in vertebrates as a result of two rounds of whole-genome duplication (2R hypothesis) or one complete genome duplication plus many segmental duplications (Ohno, 1970; Panopoulou *et al*., 2003). This led to the creation of additional gene copies and the evolution of new protein functions (Ohno, 1970; Hughes, 1999).

The gene/genome duplications at the boundary between invertebrates and vertebrates are responsible for the presence of more genes coding

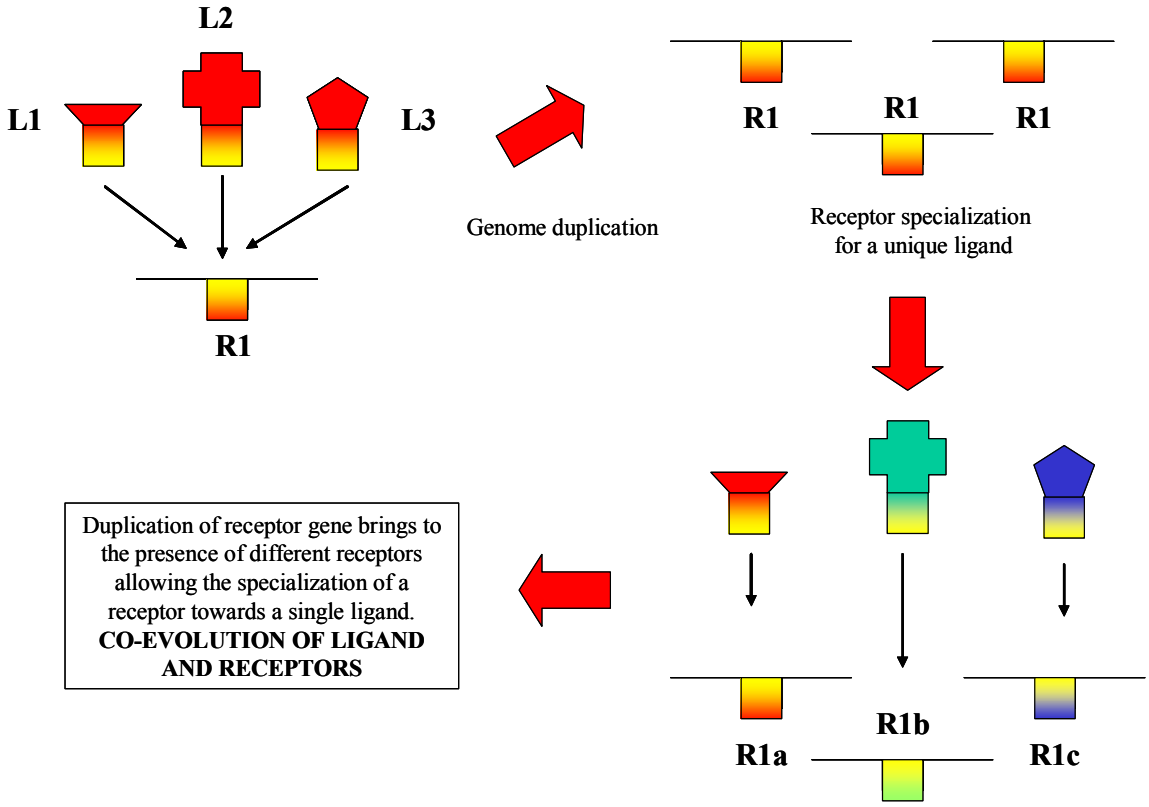

Fig. 2 Transition of the evolution mechanism of ligands (L) and receptors from a receptor(R)-directed mechanism to a ligand-directed one in vertebrates.

for receptors in the vertebrate lineage, so allowing the differentiation of receptors towards different ligands. In vertebrates, therefore, we observe a transition from common receptors for different ligands to a specific receptor for each ligand. This phenomenon modifies the invertebrate relationship between receptors and ligands, and permits ligands to drive the evolution of newly duplicated receptors (Fig. 2). The transition from a protein with a generalized recognition to more specialized proteins is a common model in evolutionary biology, as observed in the evolution of enzymes (Jensen, 1976; O'Brien and Herschlag, 1999).

In conclusion on the basis of the present bioinformatic findings and reports in the literature, we suggest that in invertebrates many ligands interact with a single, ancestral and generalized receptor driving ligand evolution. In vertebrates, on the other hand, the occurrence of gene/genome duplications induced the transition to a ligand-directed evolution of receptors. This transition represented an evolutive advantage because it couples a more refine signalling to a minor sensitivity to receptor gene mutation.

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