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Hypothesis

# Model of congruency determined molecular coevolution: from homophilic binding to ligand/receptor pairs

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## Abstract

*Peptide hormones depend on reliable recognition by their receptors, based on congruency. If a mutation changes the surface of one of interacting molecules and creates a confined space, a niche, in the otherwise congruent hormone/receptor interface, this would allow further mutations confined to the niche space, until one change in molecular shape fills the entire niche space and stops further mutations. Repetition of this process might lead to a different ligand/receptor pair that shows only remote similarities to the initial pair. This paper is aiming to use this model of congruency determined coevolution on homophilic membrane molecules to describe evolution of endocrine and paracrine ligands and receptors.*

*During evolution, a gene of some ancient homophilic membrane molecule with intracellular enzymatic activity might have been duplicated. A new pair of genes (A & B) evolved toward heterophilic binding. Expression of molecules from both genes on neighboring cells might allow congruency-determined coevolution that resulted in heterophilic recognition (A-B and B-A).*

*Loss of intracellular domains could make one of heterophilic molecules soluble (protoligands) that recognized membrane molecules on other cells (protoreceptors) as a first ligand/receptor pair. New pairs are formed through gene duplication and separate coevolution leading to families of receptors and families of ligands.*

*Survival pressure forces receptors to remain sensitive and specific, while ligands shrink until they are as small as feasible. Occasionally, plant toxins mimic endogenous peptides (morphine versus endorphins), or receptors can become able to interact with nonpeptide endogenous ligands (steroids, catecholamines). Further receptor evolution congruent to the new ligand can make the initial peptide ligand unrecognizable by that receptor (example: membrane receptors for steroids are without known peptide ligands). Finally, lipid soluble ligands can interact with receptors before receptor molecules reach the cell membrane and are still in the cytoplasm. This can make functional membrane receptors less important (aldosterone, estrogen) or abandoned (other steroids, thyroid hormones).*

## INTRODUCTION

Action of peptide hormones depends on functional membrane receptors that can specifically recognize ligand molecules. Since receptors and ligands are products of separate genes, any alteration in their sequence might compromise their recognition. This makes genes

for both molecules conserved during evolution and the everlasting question of explaining the occurrence of ligand/receptor pairs remains to be solved.

Although most of the hormones from one mammalian species are active when injected into another, the incompatibility of human GH receptor (GHR) toward non primate GHs is a notable exception *(1)*. GH species specifity was recently interpreted as a congruency-determined coevolution of GH and GHR molecules that allowed conservation of both molecules to be interrupted by periods of accelerated evolution *(2)*. Coevolution was also noted between the prolactin gene and its receptor in mammals *(3)*.

## THE CONCEPT OF CONGRUENCY-DETERMINED LIGAND/RECEPTOR COEVOLUTION

Peptide hormones that regulate electrolytes, glucose, or any other important regulatory mechanisms depend on reliable recognition by their receptors (as shown in Fig 1A). Specifity and sensitivity are required. Any mutation in one of the involved molecules would endanger survival of the mutation carrier if it compromises recognition and interaction of hormone and receptor molecules (Fig. 1B). The carrier animal of such a trait would not procreate, and the mutation would be lost for evolution. In other words, only mutations that do not compromise recognition based on congruency are allowed. Well-known examples are few amino acid mutations found in insulins of different animals.

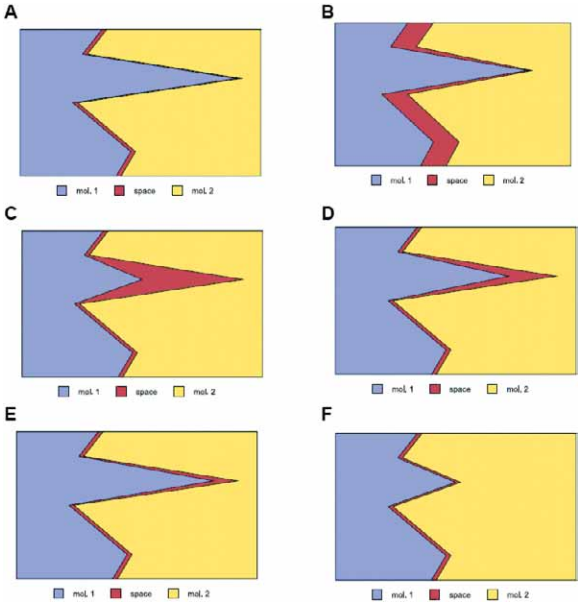


**Figure 1.** *Schematic presentation of the proposed model of two molecules that base their recognition on congruency. A/ initial pair of highly congruent molecules; B/ majority of mutations compromise molecule bonding and are lost in evolution; C/ a mutation that does not compromise molecule binding and leaves space for further mutations of both molecules; D&E/ examples of possible mutations within the allowed space that do not compromise binding; F/ the sealing mutation filled the empty niche and prevented further mutations. Note that both molecules differ from case A.*

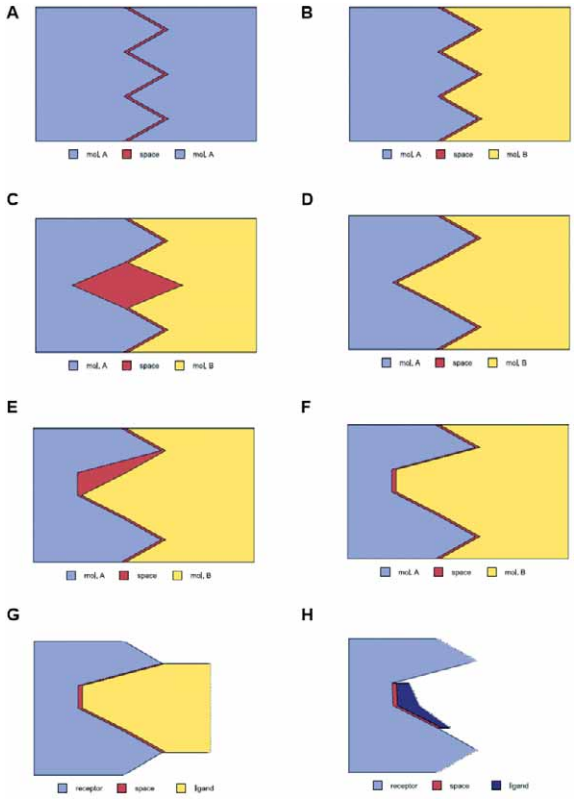


**Figure 2.** *Schematic presentation of the proposed scenario from Table 1. A/ Homophilic binding of extracellular domains of membrane molecules in first multicellular organisms might be the first step toward endocrine/paracrine loops. B to F/ Gene duplication forms a pair of molecules expressed on neighboring cells and allows congruency determined coevolution that leads to heterophilic recognition (A-B and B-A) and reduces homophilic recognition (A-A or B-B). G/ Loss of intracellular domain leads to secretion of soluble heterophilic molecules (protoligands) that recognize membrane molecules on other cells (protoreceptors) H/ Receptors can become sensitive to plant toxins that mimic endogenous peptides, or they can become able to interact with nonpeptide endogenous ligands (steroids, catecholamines).*

A rare situation can be expected when a mutation changes the surface of one of the interacting molecules and creates a confined space, a niche, in the otherwise congruent hormone/receptor interface (Fig. 1C). Although affinity between molecules is probably slightly reduced, recognition and function are not compromised. Formation of this niche allows occurrence of further mutations of both interacting molecules, if they remain confined to the niche space (Fig. 1D&E). This period of accelerated evolution can be abruptly stopped if one consequent mutation fills the entire niche space (Fig. 1F) and stops further mutations conserving a new ligand/receptor pair. The process can be repeated during evolution leading to almost fully different ligand/receptor pairs that show only remote similarities in their structure.

**TABLE 1**

Short description of the presented concept in which homophilic membrane molecules through gene duplication form a pair of molecules that evolve in heterophilic binding and finally in pairs of soluble peptide ligands and membrane-based receptors. Further evolution can introduce nonpeptide ligands and nonmembrane receptors.

| Phases of the ligand/receptor development | Concept of congruency-determined ligand/receptor coevolution | |
|---|---|---|
| Membrane proteins | homophilic interaction of membrane bound proteins | recognition A-A |
| duplication of genes for homophilic proteins | expression of molecules from both genes (A & B) on neighboring cells | allows congruency-determined coevolution that leads to heterophilic recognition (A-B, B-A) and reduces homophilic recognition (A-A or B-B) |
| soluble forms of initially membrane bound molecules | loss of intracellular domain of one of heterophilic molecules | leads to secretion of soluble heterophilic molecules (protoligands) that recognize membrane molecules on other cells (protoreceptors) |
| duplication of genes | new ligand/receptor pairs are formed through gene duplication | separate coevolutions lead to families of receptors and families of ligands |
| congruency-determined ligand/receptor coevolution | receptors remain sensitive and specific, while ligands become as small as feasible | hypothalamic hormones: small enough just to reach adenohypophysis<br>quick action hormones: peptides up to 50 AA<br>slow action hormones: up to 200 AA, or in complex with binding proteins (IGF-I, GH) |
| introduction of nonprotein ligands | External nonpeptide agonists can activate receptors for endogenous peptides | morphine instead of endorphins |
| | Receptor evolution can make membrane receptors sensitive to nonpeptide molecules | steroids, catecholamines etc. |
| | If new ligands become dominant, receptor evolution makes the initial peptide ligand unrecognizable by that receptor | membrane receptors for steroids are without known peptide ligands |
| introduction of nonmembrane receptors | lipid soluble ligands can interact with receptor molecules while they are still in the cytoplasm | membrane receptors become less important (aldosteron, estrogen), or obsolete (steroids without membrane receptors, thyroid hormones) |

The aim of this paper was to use the same model of congruency-determined coevolution of initially homophilic membrane molecules to describe evolution of ligands and receptors.

## BACKGROUND: EMERGING IMPORTANCE OF HOMOPHILIC BINDING IN BIOLOGY

This paper was inspired by the accumulated data on homophilic binding in diverse areas of biology. Reported data show that the same molecule can be homophilic and heterophilic, suggesting frequent evolutionary transition from one type of molecular binding to the other.

Cadherins are homophilic and heterophilic cell adhesion molecules involved in tissue morphogenesis and the maintenance of tissue architecture in adults *(4)*. In the adhesive interaction between E-cadherin and alpha(E)beta7, domain 5 is involved in heterophilic, but not in homophilic adhesion as other domains are *(5)*. Receptor protein tyrosine phosphatases (RPTPs) can mediate either homophilic or heterophilic interactions and suggest a role in cadherin-mediated cell-cell adhesion *(6)*. Nephrin is a signaling cell-cell adhesion protein of the Ig superfamily with extracellular domains that form a network of homophilic and heterophilic interactions building the structural scaffold of the slit diaphragm between the podocyte foot processes *(7)*. SC1 is another immunoglobulin superfamily cell adhesion molecule that shows homophilic binding activity with itself *(8)*. Human NK cells can be activated by the SLAM-related receptors (SRR) with homophilic interaction *(9)*. Erythroblast macrophage protein, expressed on erythroblasts and macrophages, mediates cell-cell attachments via homophilic binding *(10)*. Human monocyte-derived macrophages capture viable and apoptotic human leukocytes through homophilic interactions of CD31 present on both cells *(11)*. The neural cell adhesion molecule, NCAM, is involved in multiple cis- and trans-homophilic interactions (NCAM binding to NCAM), helping cell-cell adhesion through the formation of zipper-like NCAM-complexes *(12)*, and heterophilic (NCAM-fibroblast growth factor receptor) interactions *(13, 14)* with heparan sulfate and chondroitin sulfate glycoconjugates *(15)*. The MEGF1

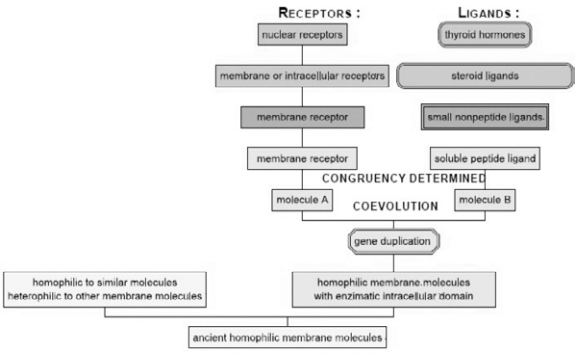


**Figure 3.** *Receptors, paracrine and endocrine peptide ligands might have all originated from homophilic membrane molecules with intracellular enzymatic activities. Through gene duplication and congruency-determined coevolution, new molecular interfaces formed, ligands became soluble and optimally small while receptors remained sensitive and specific. Part of this peptide endocrine and paracrine system was later taken over by nonpeptide agonists making initial peptide ligands less needed or abandoned. Some of the nonpeptide ligands allowed occurrence of intracellular receptors.*

gene encodes a very large protein containing two EGF-like and 34 cadherin motifs *(16)*. Plexins act as receptors of semaphorins, but plexin B3 also shows homophilic interaction in semaphorin-independent signaling mechanisms *(17)*. The homophilic junctional adhesion molecule C (JAM-C) was shown to undergo a heterophilic interaction with the leukocyte beta2 integrin Mac-1 *(18)*.

## THE PROPOSED SCENARIO OF THE OCCURRERNCE OF LIGANDS/RECEPTOR PAIRS

The basic idea, shown in Fig. 2 and described in Table 1., is that the homophilic binding of extracellular domains of membrane molecules could, in early multicellular organisms, be the first step toward development of endocrine/paracrine loops (Fig. 2A).

During evolution a gene of some ancient homophilic membrane molecule capable of intracellular enzymatic activity might have been duplicated, producing a pair of genes whose proteins evolved from homophilic to heterophilic binding. As shown in Figure 2., expression of molecules from both genes (A & B) on neighboring cells (Fig. 2B) allowed congruency-determined coevolution (Fig. 2C to F) that resulted in heterophilic recognition (A-B and B-A) and reduced homophilic recognition (A-A or B-B). Eventual loss of intracellular domain sequence in the gene responsible for one of heterophilic molecules produced soluble molecules (acting as protoligands) that recognized membrane molecules on other cells (acting as protoreceptors) (Fig. 2G). New ligand/receptor pairs were formed through further gene duplication and separate coevolution of these pairs produced families of receptors and families of ligands, as we know them now.

Survival pressure forces receptors to remain sensitive and specific, while ligands shrink until they are as small as feasible. Occasionally, plant toxins mimic endogenous peptides (morphine versus endorphins), or receptors can become able to interact with nonpeptide endogenous ligands (steroids, catecholamines)(Fig. 2H). It is also possible that one ligand interacts with two receptors that differ in structure, as it is known for IGF interacting with type I and type II receptors of different structures *(19)*.

Further receptor evolution congruent to the new ligand can make the initial peptide ligand unrecognizable by that receptor (example: membrane receptors for steroids are without known peptide ligands). Finally, lipid soluble ligands can interact with receptors before receptor molecules reach the cell membrane and are still in the cytoplasm. This can make functional membrane receptors less important (aldosterone, estrogen) or abandoned (other steroids, thyroid hormones).

## CONCLUSIONS

The consequences of the proposed concept are shown in Fig. 3. Receptors, paracrine and endocrine peptide ligands might have all originated from homophilic membrane molecules with intracellular enzymatic activities. Through gene duplication and congruency-determined coevolution, new molecular interfaces formed, ligands became soluble and optimally small while receptors remained sensitive and specific. Part of this peptide endocrine and paracrine system was later taken over by nonpeptide agonists while initial peptide ligands were abandoned. Some of them induced occurrence of intracellular receptors.

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