Receptors for intercellular messenger molecules in microbes: Similarities to vertebrate receptors and possible implications for diseases in man

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Outline and aim

Our focus is on the evolutionary origins of receptors for vertebrate hormones, neuroactive peptides, and related messengers. The first part will survey the possible evolutionary origins and phylogenetic distribution of the vertebrate-type messenger peptides providing a possible clue or guide to the same speculation for the receptor. Also, we will explain current data which suggest why receptors might need to be at least as old or as widely distributed as the messengers. In the latter part we will survey examples of materials in microbes that resemble vertebrate-type receptors and also highlight some possible applications to an understanding of human disease problems.

Introduction

A widespread pattern of intercellular communication involves release from one (secretory) cell a soluble messenger molecule which travels through an extracellular fluid compartment to another (target) cell (fig. 1).

The target cell has specific receptor molecules that recognize the messenger by binding it and the combination of messenger with receptor activates the target cell to yield a characteristic biological response.

This pattern of communication is well known for hormones, neuroactive substances, paracrine agents and pheromones in multicellular organisms e.g. vertebrates, invertebrates, and higher plants. Unicellular organisms including both eukaryotes (e.g. yeast) and prokaryotes (e.g. bacteria) utilize similar communication systems with soluble messengers designated pheromones to solve problems related to reproduction or nutrition[32, 40].

While communication systems with this overall design are very widespread, we may now ask how widespread are the molecules that mediate the communication? The peptide messenger molecules that are broadly represented among the vertebrates, e.g. hormones and neuroactive peptides, appear in many cases to be quite similar to substances found in a wide range of non-vertebrate animals[41, 18]. In some cases, the non-vertebrate forms appear to have essential biological functions in that organism.

In insects, insulin-related substances are present especially in neural elements[7, 21]. Injections of insulin accelerate glucose disposal, while surgical removal of the insulin-rich cells of the brain of the blowfly produces a hyperglycemic state, which can be ameliorated by insulin or extracts from insects that are rich in their own insulin-related materials[8]. Receptors for insulin are present in *Drosophila* which are similar to their vertebrate counterparts in binding properties, overall structure, and insulin-stimulated enzyme activity[34]. In molluscs, investigators have suggested a gastrointestinal tract site for the insulin-rich cells and have used anti-insulin antibodies to produce a catabolic ('diabetes-like') state[35].

We and others have found in microbes as well as in flowering plants, substances that closely resemble vertebrate messengers which suggest that these materials have early evolutionary origins and are distributed widely among different forms of life (tables 1 and 2)[1–3, 6, 10, 15, 20, 22–25, 29–31, 33, 42–44, 53]. Outside of the multicellular animals, no function of these vertebrate-type materials is known, although extreme conservation of the surface structure of these molecules including the receptor-reactive regions, suggests possibly (a) function and (b) binding to receptors. Thus the presence of the ligand raises the possibility of the presence of the homologous receptor, or at least that portion of the receptor that contains the specific ligand binding site.

Support for that suggestion is heightened by our current ideas of how hormone and related messenger and their

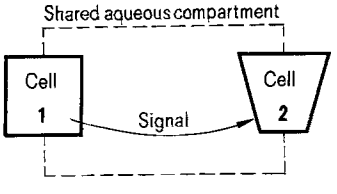


Figure 1. Basic features of intercellular systems. Cell 1 represents a secretory cell which is capable of synthesizing and releasing a signal messenger molecule into the shared fluid compartment. Cell 2, the target cell, contains a receptor which reacts to the signal messenger. In the endocrine system, cell 1 is a glandular cell and the messenger is a hormone; whereas in the nervous system, cell 1 is a neuron and the messenger is a neurotransmitter released into the synaptic space.

Table 1. Materials in unicellular organisms that resemble messenger peptides of vertebrates[1–3, 6, 20, 22, 24, 25, 29, 30, 33, 42, 43]

Hormone-related materials	Microbe
TSH	*Clostridium perfringens*
hCG	Many bacteria
Neurotensin	*E. coli, Caulobacter crescentus, Rhodopseudomonas palustrus*
Insulin	*Tetrahymena, E. coli, Neurospora crassa, Aspergillus fumigatus, Halobacterium solinarium, Bordetella pertussis*
Somatostatin	*Tetrahymena, E. coli, B. subtilis*
ACTH, β-endorphin	*Tetrahymena*
Relaxin	*Tetrahymena*
Arginine vasotocin	*Tetrahymena**
Calcitonin	*Tetrahymena, Candida, E. coli*

* Diabetes Branch unpublished observations.

Table 2. Materials in higher plants that resemble messenger peptides of vertebrates [10, 15, 23, 44, 52, 53]

Hormone-related material	Plant
LHRH	Oak leaves
TRH	Alfalfa
Opioid	Wheat
Interferon	Tobacco
Insulin	Spinach, Lemna, Rye*
Somatostatin − 14 and − 28	Spinach, Lemna, Tobacco

*Diabetes Branch unpublished data.

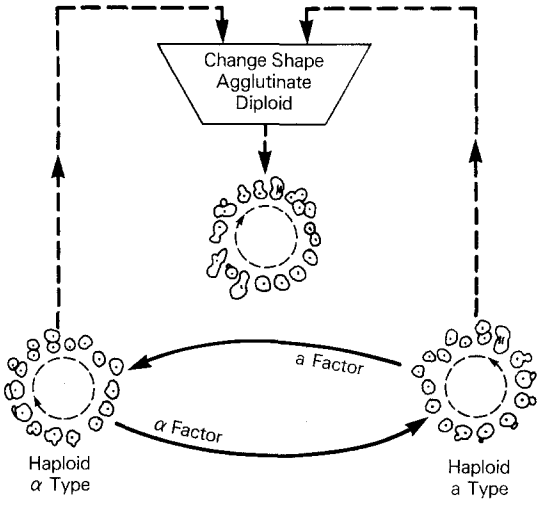
Table 3. Intrinsic biological properties of receptors for soluble messenger molecules

Function	Ligand
DNA-binding proteins	Steroid hormones; vitamin D-related sterols; thyroid hormones
Activator of G-proteins	Hormones and other messengers that activate (or inhibit) directly adenylate cyclase
Tyrosine-specific protein kinases	Epidermal growth factor (EGF), insulin, insulin-like growth factor-I, (IGF-I), platelet derived growth factor (PDGF)
Ion channels	GABA-diazepam; acetylcholine (nicotinic)

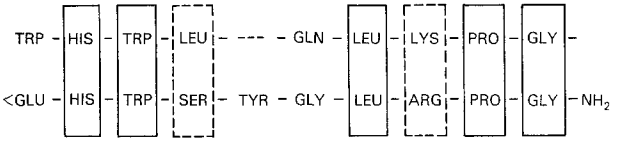
respective receptors function in vertebrate systems. First, these messengers have no known function except as messengers. Secondly at the level of mechanism, the messenger peptides act solely to activate the receptor; the receptor is the proximate mediator of action at the target cell. Indeed in many systems we now know the particular kind of activity in the receptor that is turned on by the messenger (table 3).

Unicellular eukaryotes

Receptors for the mating factors in Saccharomyces cerevisiae. Saccharomyces cerevisiae is a budding yeast which exists in diploid form as well as two haploid forms, designated α and a (fig. 2). Union of the two haploids of opposite type is a process which requires two peptide pheromones or mating factors, α factor and a factor, each produced by its namesake cell type to act on cells of the opposite type, via specific cell surface receptors[46]. (Interestingly, α factor bears a striking structural resemblance to the mammalian hypothalamic peptide known as GnRH or gonadotropin releasing hormone, can bind to the specific hormone binding site on the GnRH receptors of rat pituitary cells, and at high concentrations causes release of gonadotropins from the pituicytes)[14, 28].
More direct evidence for the existence of a receptor for the α mating factor has been reported by Jenness et al.[16]. These authors have demonstrated specific binding of α mating factor to a cells and estimated that each cell has about 8000 binding sites. More recently, the nucleotide sequence of the gene coding for the α mating factor receptor has been determined and from it the amino acid sequence of the gene product (A. C. Burkholder, personal communication). A hydropathy analysis of the primary structure of the peptide reveals several adjacent, evenly spaced, hydrophobic regions in the N-terminal 70% of



YEAST α FACTOR:



MAMMALIAN LHRH:

Figure 2. Sex pheromones in *Saccharomyces cerevisiae*. The life cycle of *S. cerevisiae* involves two mating type haploids (α and a) as well as sporulating diploids. Each haploid produces a pheromone (mating factor) that induces specific changes in the other haploid cell. This results in sexual conjugation with formation of diploid zygotes. Amino acid sequences of the N-terminus of α mating factor demonstrate significant homology with gonadotropin-releasing hormone of mammals (GnRH).

the molecules, while the remainder of the molecule, i.e. the C-terminal portion, is largely hydrophilic. The hydrophobic regions probably represent the transmembrane domains. Multiple transmembrane domains in receptors have also been demonstrated with rhodopsin[11] whereas typical vertebrate hormone receptors such as that for epidermal growth factor (EGF) and insulin have only one or two transmembrane domains per receptor complex[9, 49, 50]. In parallel studies, Sprague and colleagues have deduced the structure of the gene which is thought to encode the a factor receptor and determined that the hydrophobic structure of this receptor is indeed similar to that described for the α factor receptor (G. Sprague, personal communication). Thus the mating system of *Saccharomyces cerevisiae* has several features in common with classic vertebrate hormone receptor systems, in that communication between the two haploid cells is brought about by soluble peptide messenger molecules and the receptors for these mating factors resemble vertebrate transmembrane proteins; in addition the α mating factor is structurally related to mammalian GnRH.
Another similarity between hormone-related systems in

yeast and higher organisms involves the yeast adenylate cyclase system. The RAS1 and RAS2 genes of yeast are homologous to vertebrate ras oncogenes[47] and encode similar proteins. Genetic studies have shown that these RAS gene products are functional components of the yeast adenylate cyclase complex[36]. It has also been demonstrated that the yeast RAS proteins are membrane-bound and exhibit GTP-binding and hydrolyzing activities[48] involved in adenylate cyclase stimulation. Thus, the yeast RAS gene products are unicellular examples of the transducing proteins associated with vertebrate hormone-sensitive adenylate cyclase systems.

Estrogen binding in Saccharomyces. In the cytosol of *Saccharomyces cerevisiae* is a protein which selectively binds with high affinity a vertebrate estrogenic hormone, 17β-estradiol[4]. Unlabeled 17β-estradiol competes for the binding with tritiated estradiol while 17α-estradiol has only about 5% of the activity (fig. 3). Diethylstilbesterol, demoxaphin, nephoxidine and zearalenones, other natural and synthetic substances with estrogenic agonist or antagonist activity in mammals which bind strongly to the mammalian estrogen receptor are inactive in competing for a binding of tritiated estradiol by the *Saccharomyces* cytosolic protein. Despite this difference in the specificity of ligand binding, the yeast cytosolic protein shares physico-chemical properties with the estrogen receptor and other steroid hormone receptors of mammals. Feldman and co-workers also demonstrated that *Saccharomyces* contain lipid soluble materials that are capable of binding to the steroid binding site of estrogen receptors of mammals[4]. In addition, the yeast material can substitute for estrogen in stimulating uteri of ovariectomized mice. Thus, both ligand and receptor of yeast resemble similar components in mammals.

Corticosterone binding in Candida. Another yeast, *Candida albicans,* contains an intracellular protein that binds corticosterone (table 4). The specificity and affinity of binding of corticosterone to this yeast protein lies intermediate between those of two mammalian proteins, CBG, the cortisol binding globulin of plasma, and the glucocorticoid receptor, a cytosolic protein[26]. These investigators further suggest that the yeast probably produces an alternative ligand, possibly a lipid soluble substance which accumulates in the culture medium and has the ability to compete with labeled corticosterone for binding sites on the yeast protein.

Paracoccidioides brasiliensis. Paracoccidioides brasiliensis, a yeast that is pathogenic for humans, contains specific binding sites for labeled estradiol. Labeled steroid hormones including testosterone and corticosterone do

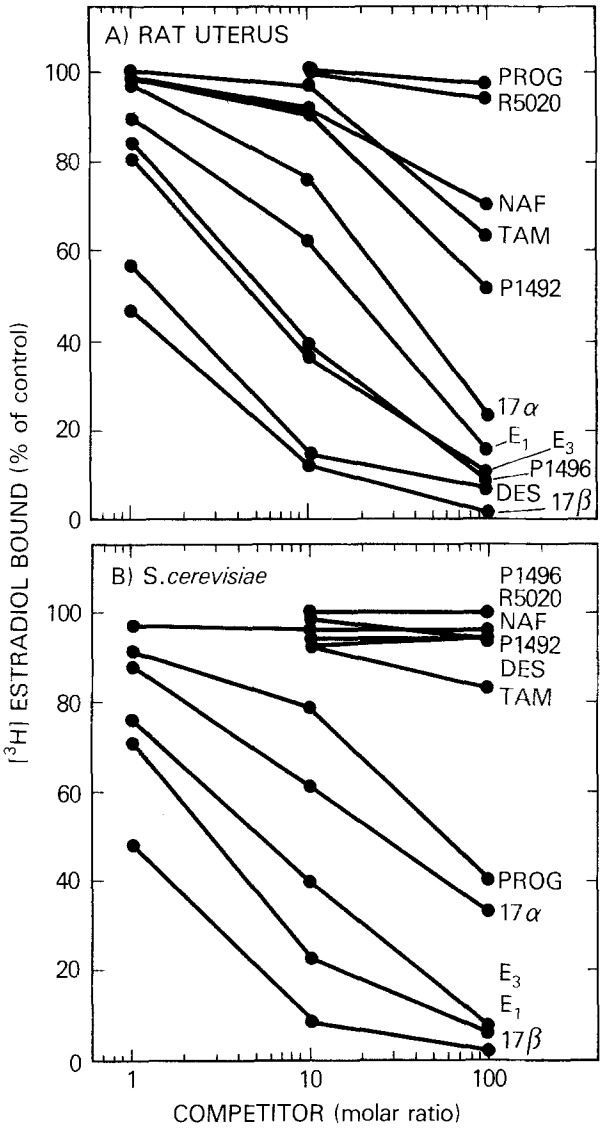


Figure 3. Competitive binding analysis of yeast estrogen binding protein and rat uterine estrogen receptor. A) Binding profile of [^{3}H] estradiol in rat uterine cytosol. B) Binding profile in yeast. Competitors used; 17β-estradiol (17β); 17α-estradiol (17α); estrone (E_1); estriol (E_3); progesterone (Prog); tamoxifen (TAM); nafoxidine (NAF); diethylstilbestrol (DES); zearalanol (P1496); zearalenone (P1492); promegestone (R5020). (Reproduced from ref. 4).

Table 4. Comparison of glucocorticoid binding proteins of *Candida albicans* and mammals

Property	*C. albicans* binding protein	Corticosterone binding globulin	Glucocorticoid receptor
Ligand	[^{3}H]corticosterone	[^{3}H]corticosterone	[^{3}H]dexamethasone
Binds synthetic glucocorticoids	No	No	Yes
Molecular weight	43,000	53,000	102,000
Hypertonic buffer	NEC4	3.4–4.1	4
Hypotonic buffer	NEC4	3.4–4.1	7–8
K_d(nM) (4 °C)	7	1–7	3–31
K_{off} 4 °C (min^{-1})	0.04	0.027	0.003
Stable at 37 °C/1h	Yes	Yes	No

The properties of the *C. albicans* binding protein is closer to corticosterone binding globulin (CBG) of mammals than to the glucocorticoid receptor, but clearly not identical to either.

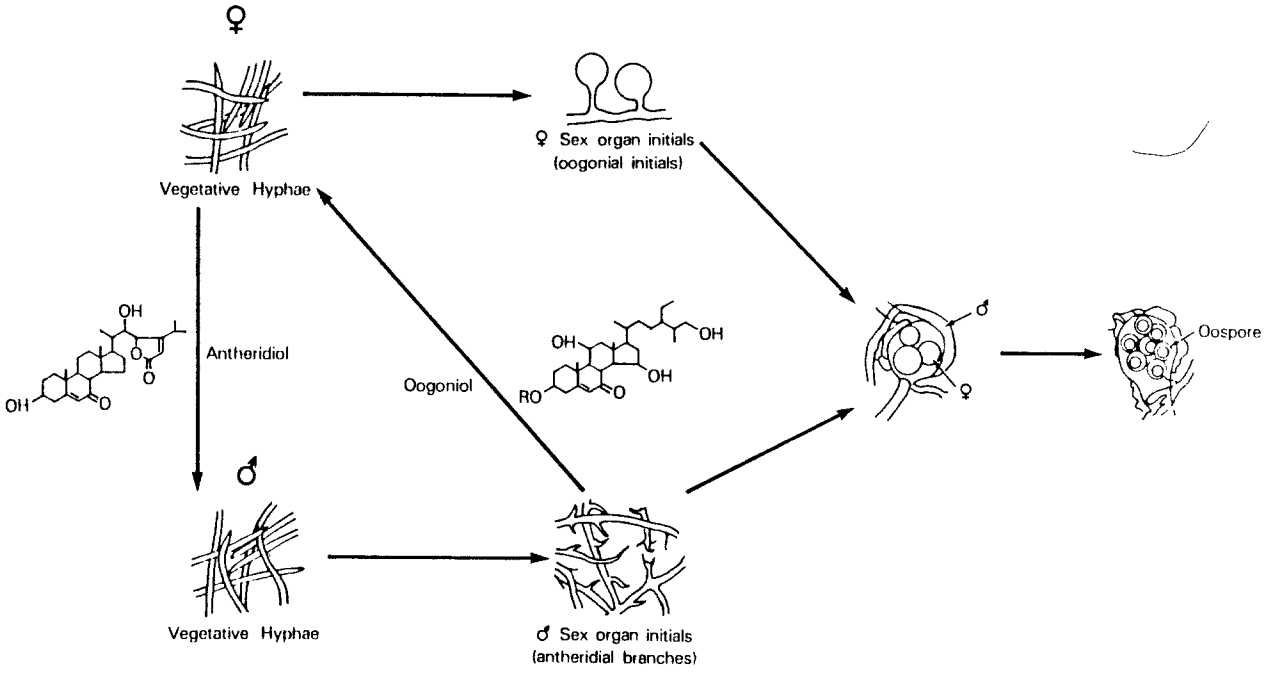


Figure 4. Sexual reproduction in the unicellular fungus, *Achlya ambisexualis*. Female continuously secretes antheridiol, a sterol pheromone, which induces in the male the formation of antheridial branches (sex organs) and secretion of oogoniol, a second pheromone, which causes the female to form sex organs. Antheridiol also attracts the male so it grows towards the female. Nuclei within both male and female sex organs undergo meiosis to form sperm and oospheres. A fertilization tube forms between male and female sex organs and male gametic nuclei pass into the oogonium. Sperm and oosphere fuse to produce zygotes ('oospores'). Under proper conditions, the zygotes then germinate and produce new diploid individuals. (Adapted from ref. 13).

not bind specifically to these yeast cells. The binding of labeled estradiol is of high affinity and can be competed for by estriol and progesterone with 25% of the affinity of estradiol; androgens, glucocorticoids, and the synthetic estrogen analog, diethylstilbesterol, have very low affinity[27]. Preliminary studies suggested that the physicochemical characteristics of the binding protein are similar to those described for the steroid receptors of vertebrates and the binding site can be destroyed by trypsin. The transformation of the organism from mycelial to yeast form, which is an early step in the infection of humans can be inhibited by estradiol at concentrations similar to those encountered in vivo in a dose dependent fashion. These investigators further speculated that with paracoccidioidomycosis (formerly known as South American blastomycosis), the disease with is caused by this organism is much more pathogenic in men than in women despite apparently equal exposure to the organism because in women, the endogenous estrogen inhibits a key step in the yeast that leads to infection.

Sex pheromones and receptor in a water mold. Achlya, a unicellular water mold, has two distinct sex types, designated male and female (fig. 4). The female secretes a pheromone, antheridiol, which affects the male in many ways including an enhancement of secretion of another pheromone, oogoniol, which has as its target the female cell. Both antheridiol and oogoniol are exceedingly similar in structure to the classic steroid hormones of vertebrates. Investigators have found in *Achlya* a soluble intracellular protein that binds antheridiol specifically but not oogoniol or other more distantly related steroids including the steroid hormones of vertebrates. Only the male cells contain the antheridiol binding protein; the female cells lack it. This binding protein has several of the peculiar physical and chemical characteristics that are typical of steroid receptors of vertebrates (fig. 5a, b)[38,39].

Opiate receptors in Amoeba. Feeding behavior or endocytosis of food particles by *Amoeba proteus* is highly regulated. Opioid alkaloids as well as opioid peptides in the nM range inhibit the endocytosis. This biological effect is antagonized by the bioactive stereospecific isomer of naloxone in a dose dependent manner, but not by its mirror image biologically inert form. These results suggest that the *Amoeba* has a specific receptor that elicits a biological response and has properties similar to those of the μ-type opioid receptor found in higher organisms. The existence of the receptor, its specificity, and its cellular connections have been demonstrated by these pharmacological experiments[17]. However, it has not yet been shown that this receptor acts physiologically to regulate normal feeding or other life processes in the organism. A similar approach has been used to detect other vertebrate-type receptors in the amoebae.

Prokaryotes

Chorionic gonadotropin binding sites in Pseudomonas maltophilia. Richert and Ryan found that ^{125}I-hCG binds to preparations of *Pseudomonas maltophilia* but not to other microorganisms, including *Pseudomonas aeruginosa* and other gram-negative rods[37]. The binding of the hCG was of high affinity with a single order of binding sites and a specificity which was quite similar but not identical with the hCG receptor of mammalian ovaries (fig. 6).

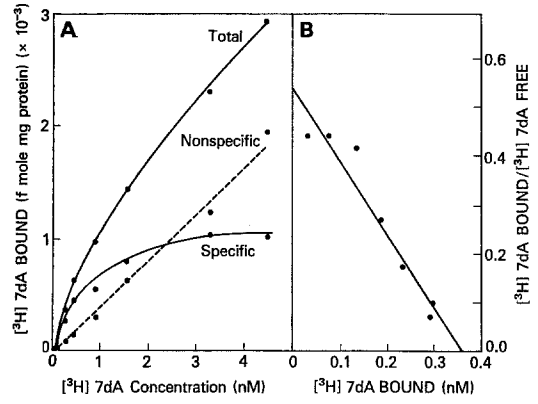


Figure 5a. Binding of [^{3}H] 7dA to *Achlya*. Mycelia of *Achlya ambisexualis* were minced with scissors and homogenized using a polytron. Homogenates were filtered through glass wool and centrifuged at 250,000 × g for 2 h. The supernatant was clarified by filtration through 0.22 μm membrane. 286 μg aliquots of cytosol protein were incubated at 0 °C for 1 h in the presence of [^{3}H]7dA, (a radiolabeled analogue of antheridiol) in the range of 0.1–4.5 nM. Similar incubations were performed in the presence of 50-fold molar excess of antheridiol. After the incubation period bound and free steroid were separated by dextran-coated charcoal. Non-specific binding is the amount of [^{3}H]7dA bound in the presence of 50-fold excess of unlabeled antheridiol. Specific binding is calculated by subtracting non-specific from total at each concentration (A). Scatchard analysis (B) revealed an equilibrium dissociation constant of 0.65 nM and maximum binding capacity of 1245 fmoles/mg protein (Adapted from refs. 38, and 39 with permission).

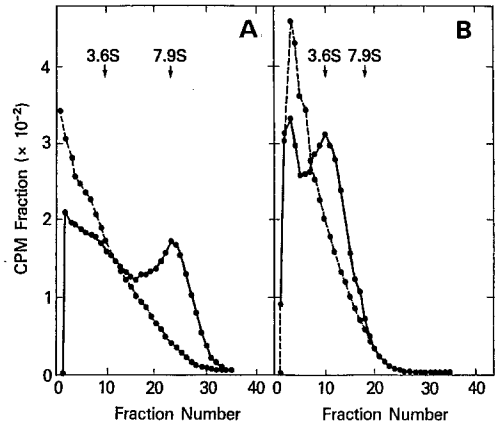


Figure 5b. Sucrose gradient analysis of [^{3}H]7dA binding in *Achlya* cytosol. *Achlya* cytosol containing [^{3}H]7dA, either with or without 200 nM antheridiol, were layered on 5–20% w/w sucrose gradients and centrifuged for 16 h at 150,000 × g. Sedimentation profile in low ionic strength gradients containing molydate (A) and high ionic strength gradients without molybdate (B). ●——● represents profile in presence of [^{3}H]7dA alone and ●---● represents profile in the presence of a 50-fold excess of unlabeled antheridiol. These results were interpreted as suggesting that the *Achlya* cytosolic binding protein can be observed as apparent aggregated (8S) and dissociated (4S) states and highly sensitive to the stabilizing action of low ionic strength and sodium molybdate. 3.65 arrow represents peak radioactivity from [^{14}C] ovalbumin and 7.95 from [^{14}C] aldolase. (Reproduced from ref. 38 with permission).

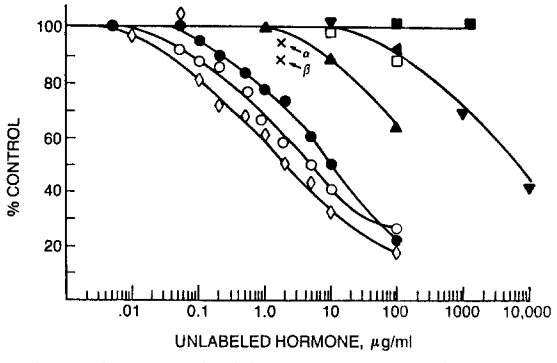


Figure 6. *Pseudomonas maltophilia* were grown to stationary phase in trypticase soy broth, sedimented by centrifugation, and resuspended in 40 mM Tris HCl, 10% sucrose. Bacterial pellets equivalent to 210 μg of bacterial protein were incubated with 2 ng of ^{125}I-hCG at 20 °C for 20 h in the absence or presence of unlabeled hormones. Incubations were ended by precipitating bound radioactivity with carbowax and centrifugation at 2000 × g. (◇) human luteinizing hormone; (○) human chorionic gonadotropin; (●) ovine luteinizing hormone, (×) human chorionic gonadotropin α and β subunits; (▲) ovine follicular stimulating hormone; (□) ovine prolactin; (▼) ovalbumin, ovomucoid, bovine gamma globulin; (■) sugars (glucose, galactose, mannose, maltose, D-glucosamine, N-acetyl-neuraminic acid); (□) KI. (Reproduced from ref. 37 with permission).

TSH binding proteins in bacteria. Using ^{125}I-labelled TSH, Weiss and co-workers detected binding sites in *Yersinia enterocolitica, E.coli,* and other gram-negative rods[51]. The binding of the labeled hormone was specific, i.e., unlabeled TSH competed better for the labeled TSH than did hCG, LH, and FSH, all of which are related to TSH; unrelated hormones did not compete at all. The binding of TSH to the bacteria was also inhibited by sera from patients with Graves' disease but not from sera from other people[12]. Since Graves' disease is thought to be etiologically caused by autoantibodies directed against the receptors for TSH on the thyroid gland, these investigators were able to show that enrichment of these antibodies inhibited TSH binding to the binding sites on the gram-negative organisms (fig. 7). These authors suggested the possibility that, in certain individuals, bacterial products (i.e., TSH-binding sites) may generate antibodies which crossreact with the endogenous TSH receptors of the thyroid gland. These antibodies in the absence of TSH may activate the thyroid cells causing them to overproduce their hormonal products resulting in hyperthyroidism in these patients.

Conclusion

Intercellular communication analogous to that found among vertebrates plays an important biological role in representative organisms at all levels of life, including microbes. Among the small number of well characterized intercellular messengers indigenous to microbial systems, several are structurally similar to messenger molecules of vertebrates. In addition, the molecules of intercellular communication typical of vertebrate systems – the extracellular molecules, their receptors, as well as their post-receptor intracellular components – show similarities to molecules of both eukaryotic and prokaryotic microbes; the normal function of these microbial components is, in most cases, not yet known. Overall, it appears that the extent of the overlap in the field of intercellular communication between vertebrates and non-vertebrates, as well as between multicellular organisms and unicellular organisms, is much more extensive than heretofore suspected.

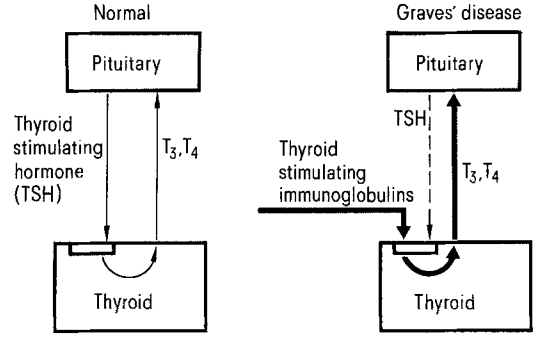


Figure 7. Hyperthyroidism associated with Graves' disease in humans is thought to be caused by 'autoantibodies' directed against the TSH receptor of the thyroid cells. *Yersinia enterocolitica* and *E. coli* have TSH-receptor-like material that bind ^{125}I-TSH in vitro, and this binding is inhibited by unlabeled TSH. Sera of patients with Graves' disease also inhibit the binding of labeled TSH to the bacteria. These studies have suggested that the TSH binding protein of bacteria and that of human thyroid membranes are homologous. Additional evidence is the production of anti-TSH receptor antibodies by rabbits injected with *Yersinia enterocolitica*. Interestingly, *E. coli, Proteus vulgaris* and *Klebsiella pneumoniae* share antigenic determinants with the nicotinic acetylcholine receptor and these authors have postulated a possible role of these bacteria in the pathogenesis of *Myasthenia gravis*, a disease causally related to autoantibodies directed against the acetylcholine receptor[45]. Similiarly, acute rheumatic fever which follows infection with *Streptococcus pyogenes*. Interestingly murine monoclonal antibodies to *Streptococcus pyogenes* react with skeletal muscle myosin[19].

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Development of hormone receptors: Conclusion

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Key words. Electrostatic complementation; evolution; genetic code; hydropathic complementation; receptor imprinting.

When I was asked to write the conclusion to this multiauthor review, I was somewhat reluctant at first, since the biochemistry of hormone receptors is far from my own field of research. However, I accepted this invitation because it is my personal experience that the view across borders into neighboring fields can bring new insight into one's own field of endeavor. I apologize if my sometimes unorthodox way of thinking about scientific problems and questions may now and then challenge traditional points of view, but possibly this is the true reason why I was asked to write this concluding review. Scientific data are traditionally looked at with the attitude that *anything that cannot be proved does not exist.* Historical development, however, has repeatedly shown that the attitude that *only those things which can be disproved do not exist* may hit the truth much better.

After reading these ten highly interesting expert reviews on specialized questions of hormone and receptor structure and function, phylogeny and ontogeny, imprinting and recycling, it would be most presumptuous of me to claim to present a final answer to the many open questions on how receptors may arise. Therefore, all those who expect the elaboration of a textbook-style theory on receptor development, based on classical points of view, will be disappointed. Research on receptor development is not ready yet for a classical theory. Classical theories give the impression that everything is already known and, owing to this impression, they inhibit the development of new insights and further research. *Man makes theories, but nature does as it very well pleases.* And because nature does as it pleases, a conclusion is nothing else but *the point where one gets tired of thinking.*

Even in science it is easier to swim with the stream (which scientist has not yet had this experience, especially when making a grant application?), but if you want to get to the source then you must frequently swim against the stream. So please forgive me, if I sometimes leave the treadmill of traditional thinking during my vague attempt to tie up some of the open ends. Remember, *you cannot pass someone, if you only keep stepping into his footsteps.*