REVIEW

Phylogeny and evolution of chemical communication: an endocrine approach

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ABSTRACT

The present review assesses the phylogenetic history of information molecules (bioregulators pheromones, hormones, neuroactive compounds), receptors, transducers, second messengers) in uni- and multicellular organisms.

Transitional stages between contemporary endocrine secretions including hormones and neuroactive materials, and primogenial exocrine compounds (pheromones) are proposed. Several hypotheses have been developed to explain the origin and evolution of bioregulator/receptor units.

Finally, how these primordial information molecules have either been co-opted or have changed their function during the course of biological evolution is analysed.

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INTRODUCTION

Successful biological evolution may be considered to have, in large part, resulted from chemical communication using messengers such as food signals and toxins; these may be regarded as the primary conduits for information in the biosphere. The emergence of multicellular organisms, such as vertebrates, necessitates the differentiation and specialization of multiple chemical signalling systems organized in a network to carry the diffusible signal from one cell to the other (Fig. 1) either between or within several compartments.

The existence of an endocrine system as such conceptually demands multicellularity, but the actions of bioregulators are not restricted to the classical concept of hormones as endocrine signals. The tenor that 'compounds secreted by endocrine structures (glandular or nervous cells) and transported to other parts of the body by way of the blood-stream (or other fluids), where they evoke physiological responses' no longer applies.

Indeed, many bioregulators such as pheromones, paracrine substances, and growth factors do not originate from defined glandular or nervous structures and are delivered to their sites of action in many ways (Fig. 1). Chemical communication is central to evolutionary history; many types of information molecules including bioregulators, receptors, transducers, effectors and second messengers are almost universally present. Uni- and multicellular organisms are likely to share biosynthetic and functional mechanisms in terms of their chemical communication.

The use of phylogenetic perspectives to reconstruct evolutionary history has become increasingly important and provides solutions to basic questions in the area of chemical communications. Bioregulators, secretory cells and target tissues have left no palaeontological record, but the evolution of chemical communication can be extrapolated from comparative analyses of extant organisms.

In addition, the living record is of course far richer and more extensive than the fossil one, because cells today retain important information about their past in terms of amino acid sequences of their proteins and in the composition of nucleic acids (RNA, DNA).

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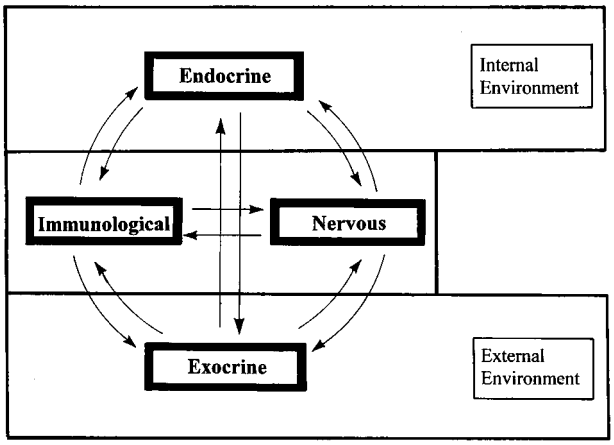

FIGURE 1. Interactions among chemical signalling systems and their respective environments. Bioregulators travelling through internal (blood stream, haemolymph) or external (water, air) environments are called hormones (or neurotransmitters, etc.) and pheromones respectively. In some cases, a bioregulator acts simultaneously as hormone and pheromone as occurs in the interaction between host and parasite (see Interspecific Communication).

Thus, a phylogenetic analysis of the distribution, biological functions and mechanisms of action of informational molecules is a logical way to gain insight into evolutionary trends and history.

INTRASPECIFIC COMMUNICATION

The ubiquity of bioregulators throughout the biosphere suggests that these compounds predate truly hormonal roles, and chemical communication took place among unicellular organisms prior to the emergence of Metazoa.

Two primary secretory systems may be recognised depending on the routes they take following release. One type involves the synthesis and release of materials which have actions within the organism itself (contemporary endocrine system). The second type of secretion includes pheromonal systems in which the secretion leaves the organism, either as liquid or gas, to affect the function of a second organism (primordial exocrine system).

The variety and ubiquity of pheromonal molecules (cyclic nucleotide, amino acids, small peptides, large proteins, alkanes, ketones, terpenoids, steroids; Table 1, Fig. 2) renders it likely that such substances in unicellular organisms evolved into the hormones of multicellular organisms.

The earliest phylogenetic example of intraspecific communication at cellular organization level is the aggregation process (Bonner 1971, Schapp 1984) of unicellular organisms of the same species in which there is directed migration towards a region of higher concentration of pheromone (paracrine signal) (Table 1).

In addition, some pheromones are produced in the same cell in which they exert their effects, referred as to an autocrine signal; this type of self-stimulation is seen in the protozoan *Euplotes raikovi* (Vallesi *et al*. 1995). There are two kinds of pheromone–receptor interactions in this species: (i) autocrine pheromone receptors (cell division) and (ii) paracrine pheromone receptors (mating behaviour). In the first type of interaction the pheromones cause mitogenic proliferation of the same cells from which they were secreted. In the second type of interaction these cells interrupt their vegetative cycle and are triggered towards mating behaviour when non-self co-specific pheromones act on paracrine pheromone receptors.

There are broad paradigms for the evolution of the chemical communication involved in these biological responses: (i) the same information molecule can develop more than one physiological function; (ii) the autocrine and paracrine functions appeared prior to the origin of endocrine function; (iii) reciprocal interaction between members of the same species began in an asocial environment (without colonies and without co-operative interactions) preceding the development of multicellular organisms.

Chemical communication ranges from prokaryotes responding to a wide variety of environmental chemical signals to the complex endocrine regulatory processes in multicellular organisms. Certainly, the ubiquity of the vertebrate information molecules (Table 2) is a product of natural selection–adaptation events.

It is, however, important to distinguish between characteristics selected for one function (adaptation process) from those originally selected for one function but subsequently employed by chance in an extension of the initial function (exaptation process) (Gould & Vrba 1982).

A particularly good example of this is seen in invertebrate and vertebrate metamorphoses, spectacular biological processes regulated by a variety of hormones (Bentley 1976, Stoka 1987). In this process, juvenile hormone (sesquiterpenoid, Insecta) and prolactin (polypeptide, Amphibia) act as juvenile agents, although they are also involved in reproductive functions (Bern & Nicoll 1969, Highnam & Hill 1977). The ancestral insects and amphibians were essentially aquatic, metamorphosis

TABLE 1. Ubiquity and chemical diversity of pheromones

Organism	Effect	Chemical diversity	Reference
Algae			
Volvox carteri	Cellular differentiation	Glycoprotein	Starr & Jaenicke (1974)
Ectocarpus siliculosis	Sexual attraction	Ectocarpen (Fig. 2)	Muller *et al.* (1971)
Fucus serratus	Sexual attraction	Fucoserraten (Fig. 2)	Muller & Jaenicke (1973)
Fungi			
Dictyostelium lacteum	Aggregation	Pteridine derivative	Van Haastert *et al.* (1982)
Polysphondilium violaceum	Aggregation	Glorin (Fig. 2)	Shimomura *et al.* (1982)
Allomyces sp	Sexual attraction	Sirenin (Fig. 2)	Machlis *et al.* (1968)
Achlya bisexualis	Cellular differentiation and sexual attraction	Antheridiol (Fig. 2)	Barksdale (1967)
Achyla bisexualis	Idem	Oogoniol (Fig. 2)	McMorris *et al.* (1975)
Saccharomyces cerevisiae	Idem	Alpha and a factors (Fig. 2)	Wilkinson & Pringle (1974); Duntze *et al.* (1970)
Mucor mucedo	Cellular differentiation	Trisporic acid (Fig. 2)	Meselson & Radding (1975)
Protozoans			
Euplotes raikovi	Cell division and mating behaviour	Polypeptides	Vallesi *et al.* (1995)
Arthropods			
Bombyx mori (Insects)	Sexual attraction	Bombycol (Fig. 2)	Butenandt (1963)
Apis mellifera (Insects)	Sexual attraction	9-oxo-decenoic acid (Fig. 2)	Butler (1967)
Balanus balanoides (Crustaceans)	Aggregation	Protein	Crisp & Meadows (1962)
Fishes			
Carassius auratus (goldfish)	Sexual behaviour	17,20 β-dihydroxy-4-pregnen-3-one	Dulka *et al.* (1987)
Fugu niphobles	Sexual attraction	Tetrodotoxin	Matsumara (1995)
Amphibians			
Cynops pyrrhogaster	Sexual attraction	Sodefrin (Fig. 2)	Kikuyama *et al.* (1995)
Reptiles			
Thamnophis sirtalis parietalis	Sexual attraction	Long chain C_{29}-C_{37} unsaturated methyl ketones	Mason *et al.* (1989)
Mammals			
Sus scrofa	Sexual attraction	Androgen steroids	Melrose *et al.* (1971)
Homo sapiens	Sexual behaviour	Androstenol (steroid)	Gustavson *et al.* (1987)

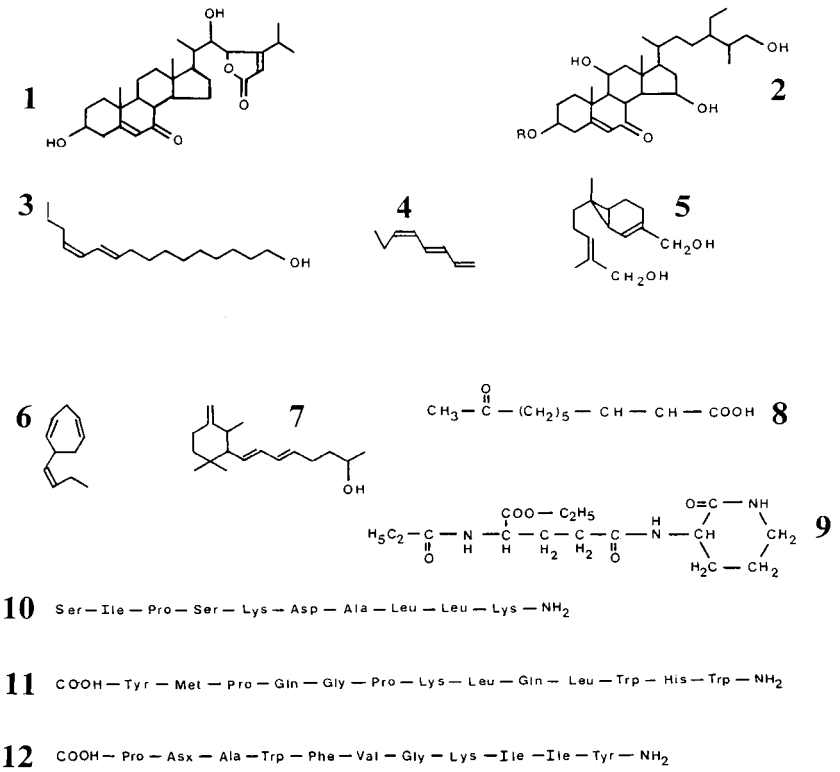

FIGURE 2. Lipid-type and peptide-type structures of exocrine bioregulators (pheromones). 1, Antheridiol; 2, oogoniol; 3, bombycol; 4, fucoserraten; 5, sirenin; 6, ectocarpen; 7, trisporic acid; 8, 9-oxo-decenoic acid; 9, glorin; 10, sodefrin; 11, alpha-factor; 12, a-factor. Alpha-factor from *Saccharomyces cerevisiae* has structural homology and functional analogy with a vertebrate-type hormone (see Table 2).

being an adaptation process to permit aquatic and terrestrial life styles (Toms 1984).

Can the primordial functions of juvenile hormone and prolactin be identified?

Insect metamorphosis is linked to sexual maturation and the primordial function of juvenile hormone was probably to regulate the reproductive cycle of the ametabolous ancestors (Apterygota) of winged insects. The most primitive contemporary insects, Apterygota, display virtually no metamorphosis and juvenile hormone acts only on reproductive tissues.

In contrast, prolactin may be seen as an osmoregulatory hormone in fish. Thus, the morphogenetic and regulatory effects of the juvenile hormone and the later physiological attributes of prolactin (milk secretion in mammals, crop-milk production in birds, water-drive behaviour in amphibians) are secondarily evolved, when juvenile hormone and prolactin were used by other tissues or species.

The acquisition of new advantageous functions by a determined bioregulator might be an opportunistic process in which novel structures (receptors, transducers, effectors) and new domains of an old bioregulator developed new physiological functions in other tissues and/or species. This kind of evolutionary opportunism characterizes the phylogeny of chemical communication (exaptation process).

INTERSPECIFIC COMMUNICATION

Evolutionary opportunism is commonly seen in the interactions between hosts and parasites. Examination of these relationships reveals that in some cases there are indeed effects on the hormonal regulation of development. Several studies (Table 3) described cellular receptors of host hormones in parasites which can respond differently with respect to their hosts (heterotrophic effects).

With respect to the effect of host hormones on parasites (Table 3) there is evidence for the hypothesis that some bioregulators originally served

as a defence molecule: (i) vertebrate-type steroid hormones are present in insects (Schildknecht *et al*. 1966, 1967) and steroid derivatives occur in coelenterates (Sturaro *et al*. 1982), and they act as defence mediators towards other species; (ii) sterols and steroids have antimicrobial effects (Buetow & Levedahl 1964); (iii) host hormones can inhibit the growth of parasites (Loose *et al*. 1983, Schar *et al*. 1986, Stoka 1996); (iv) several phytocompounds disrupt insect development (Stoka *et al*. 1987*a*).

Examples of transitional stages in the evolution of defensive and hormonal functions may also be noted: (i) a neurotoxin is a male attractant pheromone in vertebrates (Matsumara 1995); (ii) enterotoxins induce steroidogenesis in adrenocortical cells (Donta & Moon 1974); (iii) cholera toxin β-chain and vertebrate glycoprotein hormones (thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone) share structural similarities (Kurosky *et al*. 1977); (iv) the finding of chemical signalling systems in plants that play dual roles as growth regulators and as a defence mechanism against pathogens (Chang *et al*. 1993, Chen *et al*. 1993).

Heterotrophic effects can also be viewed as (i) an adaptive strategy of parasites that renders it advantageous to co-ordinate their physiological functions with the physiological stage of their host. For example, 20-OH-ecdysone acts as a moulting hormone in insects, but can also stimulate growth and sexual differentiation in the protozoan parasite *Trichonympha sp*. (Cleveland 1959); (ii) a mechanism for pre-existing hormones to evolve in other target tissues or species (exaptation process). In unicellular organisms vertebrate-type bioregulators affect their growth (Table 3), while in multicellular organisms they are involved in sexual activity and metamorphosis (Baulieu *et al*. 1978, Geuns 1978, Stoka 1987, Stoka *et al*. 1987*b*). It is likely that receptors originally triggered one physiological response after interacting with a specific bioregulator, but subsequent changes (e.g. coupling with different transducers and effectors) allowed the development of additional physiological responses. This adaptive acquisition, with an old bioregulator playing a new function, is perhaps the most important consequence of transitional stages of change.

EVOLUTIONARY ASPECTS OF EXOCRINE AND ENDOCRINE SYSTEMS

Transitional stages are, phylogenetically, key points during the divergent evolution of uni- and multicellular organisms. In an evolutionary sense primitive pheromones are a transitional stage between the most primitive (food signals, toxins) and the most advanced (hormones, neuroactive compounds) chemical signals.

Current data are in agreement with the hypothesis that pheromones are ancestors of present-day hormones and that they arose very early during the evolutionary history as local cellular signals in unicellular organisms (primordial exocrine system).

Essentially, these bioregulators act as autocrine and paracrine factors (Vallesi *et al*. 1995). For example, cAMP regulates the metabolism of prokaryotes (autocrine action) (Mackman & Sutherland 1965), and regulates many activities in unicellular eukaryotes (paracrine action) (Schapp 1984). Moreover, cAMP plays a dual role as intra- and intercellular messenger in *Dictyostelium discoideum* (Gerish 1987).

What are the origins of this dual role of cAMP?

If a bioregulator acts as inter- and intracellular messenger receptor transformations must be involved. For instance, the action of cAMP as second messenger suggests that an internalization process takes place, because in *Escherichia coli* cAMP is produced in the same cell in which it exerts its effects (autocrine action) and forms a complex with an extracellular specific protein, the cAMP-receptor protein (CRP) (Mackman & Sutherland 1965). This protein is homologous to protein kinases, which are intracellular cAMP receptors in unicellular eukaryotes (Weber *et al*. 1982, Saxe *et al*. 1991).

It is obvious that with the development of multicellularity, cAMP cannot act as a cell-to-cell messenger because of its intrinsic chemical instability, and can only carry information as an intracellular second messenger by means of cytosolic cAMP-dependent protein kinases (Weber *et al*. 1982).

The presence of membrane and cytosolic cAMP receptors suggests the development of transitional stages during evolution. Clearly, each form of cAMP/cAMP receptor unit is linked to a determined facet within the evolutionary triptych of bioregulator–receptor interaction including: (i) autocrine (membrane form of cAMP receptor, cAMP=first messenger); (ii) paracrine (membrane and cytosolic forms of cAMP receptor, cAMP=first and second messenger); (iii) endocrine (cytosolic form of cAMP receptor, cAMP=second messenger).

The transitional taxonomic level on the cAMP receptor evolution may be represented today by *Dictyostelium discoideum*, an intermediate stage displaying: (i) a level of organization that is

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TABLE 2. Ubiquity of information molecules with structural homology and/or functional analogy to those of vertebrates

	Binding protein for:	K_d	Bioregulator	G-protein	Reference
Organism					
Archaebacteria					
Halobacterium halobium	—	—	—	59 kDa	Schimz *et al.* (1989)
Eubacteria					
Escherichia coli	—	—	—	35 kDa	Ahnn *et al.* (1986)
	—	—	Insulin	—	Le Roith *et al.* (1981*a*)
	—	—	Somatostatin	—	Le Roith *et al.* (1985*a*)
Clostridium perfringens	—	—	Thyrotrophin	—	Macchia *et al.* (1967)
Progenitor cryptocides	—	—	Human choriogonadotrophin	—	Maruo *et al.* (1979)
Yersinia enterocolitica	Thyrotrophin	$4{\cdot}2 \times 10^{-8}$ M	—	—	Weiss *et al.* (1983)
Pseudomonas testosteroni	Testosterone	$6{\cdot}7 \times 10^{-9}$ M	—	—	Watanabe *et al.* (1973*a*)
Pseudomonas maltophilia	Human choriogonadotrophin	$2{\cdot}3 \times 10^{-9}$ M	—	—	Richert & Ryan (1977)
Fungi					
Dictyostelium discoideum	—	—	—	42 kDa	Leichtling *et al.* (1981)
Saccharomyces cerevisiae	—	—	—	54 kDa	Brock *et al.* (1985)
	17β-Oestradiol	$3{\cdot}0 \times 10^{-9}$ M	17β-Oestradiol	—	Feldman *et al.* (1982, 1984)
	GnRH	$1{\cdot}0 \times 10^{-6}$ M	Alpha factor homologous to GnRH	—	Loumaye *et al.* (1982)
Neurospora crassa	—	—	Insulin	—	Le Roith *et al.* (1980)
Trichophyton mentagrophytes	Progesterone	$6{\cdot}8 \times 10^{-8}$ M	—	—	Schar *et al.* (1986)
Paracoccidioides brasiliensis	17β-Oestradiol	$1{\cdot}5 \times 10^{-8}$ M	—	—	Loose *et al.* (1983)
Candida albicans	Corticosterone	$7{\cdot}2 \times 10^{-9}$ M	—	—	Loose & Feldman (1982)
Protozoa					
Trypanosoma cruzi	—	—	—	45 kDa	Eisenschlos *et al.* (1986)
Tetrahymena pyriformis	—	—	Relaxin	—	Schwabe *et al.* (1983)
	—	—	Insulin	—	Le Roith *et al.* (1980)
	—	—	Adrenocorticotrophic hormone, B-endorphin, somatostatin	—	Le Roith *et al.* (1983)
	—	—	Adrenaline, noradrenaline	—	Janakidevi *et al.* (1966)
Platyhelminthes					
Planarians	—	—	Dopamine, noradrenaline	—	Welsh & King (1970)
Annelids					
Glycera convoluta	—	—	Adrenaline, noradrenaline, dopamine, serotonine	—	Manaranche & L'Hermite (1973)
Mollusca					
Anodonta cignea	—	—	Insulin	—	Plisetskaya *et al.* (1978)
Mytilus edulis	—	—	Testosterone, 17β-oestradiol	—	De Longcamp *et al.* (1974)
Lymnaea stagnalis	—	—	Somatostatin	—	Grimm-Jorgensen (1983)

TABLE 2. *Continued*

	Binding protein for:		K_d	Bioregulator	G-protein	Reference
Echinoderms						
Pisaster ochraceus	—		—	17β-Oestradiol	—	Botticelli *et al.* (1960)
Arthropods						
Dytiscus marginalis	—		—	Oestrogens, androgens	—	Schildknecht *et al.* (1967)
	—		—	11-deoxycorticosterone	—	Schildknecht *et al.* (1966)
Calliphora vomitoria	—		—	Insulin	—	Duve *et al.* (1979)
Drosophila melanogaster	—		—	—	40 kDa	Thambi *et al.* (1989)
Urochordates						
Ciona intestinalis	—		—	Somatostatin	—	Falkmer *et al.* (1978)
Plants						
Pinus silvestris	—		—	Testosterone	—	Saden-Krekula *et al.* (1971)
Spinach, tobacco	—		—	Somatostatin	—	Le Roith *et al.* (1985*b*)
Wheat	—		—	Opioids	—	Zioudrov *et al.* (1979)

GnRH, gonadotrophin releasing hormone.

transitional between prokaryotic and multicellular organisms; (ii) *D. discoideum* has both membrane and cytosolic forms of cAMP receptors; (iii) in this species cAMP acts as a paracrine bioregulator (first messenger) and intracellular regulatory molecule (second messenger) (Table 4).

It seems likely that in the development of transitional stages such as *D. discoideum*, the earliest membrane receptors were the predecessors of cytosolic receptors. There are other examples of transitional stages; for example, progesterone, testosterone and gonadotrophins can act through both membrane and cytosolic receptors (Baulieu *et al*. 1978, Rao & Chegini 1983, Diez *et al*. 1984).

Briefly, contemporary endocrine bioregulators (hormones, neuroactive materials) were probably, in the past, exocrine information molecules (food signals, pheromones), and their contemporary clearly defined endocrine functions (sexual reproduction, developmental processes) are more a product of target tissue specialization (e.g. transduction mechanisms) than a consequence of changes in the structure of the bioregulators themselves.

These views are supported by several observations: (i) the most effective signals for the chemotactic response in *E. coli* (maltose, glucose, serine, aspartate) are used as nutritive molecules (Adler 1969) in contrast to more specialized bioregulators (neuroactive compounds, hormones); (ii) the receptors or binding proteins in unicellular organisms display clear threshold values for chemotaxis towards a particular bioregulator (Adler 1975), which resemble the dissociation constant (K_d) of vertebrate-type bioregulators (Table 2). For example the threshold value of reduced glutathione (GSH), a feeding response activator (Lenhoff 1968, Colasanti *et al*. 1995), in *Hydra littoralis*, a member of one of the most ancient phyla among multicellular organisms, the Coelenterates, is 10^{-9} M.

GSH has, phylogenetically, the characteristics of a transitional stage among food signals and contemporary bioregulators (hormones, pheromones) because: (i) it plays a dual role acting in *Hydra* both as a chemical signal for detection of food sources, and as a neuromodulator of behavioural feeding response; (ii) like contemporary bioregulators, GSH is not used as a nutritive molecule.

In addition, primitive peptides and ancestral lipid molecules (sterols, steroids) were probably all growth promoting factors as a consequence of their intrinsic nutritional value. For example, *Pseudomonas testosteroni* is a prokaryote capable of utilizing steroids as their only carbon source (Watanabe *et al*. 1973*a*,*b*).

It is thus important to note a plausible relationship among some enzymes involved in the

TABLE 3. Heterotrophic effects of some bioregulators

Bioregulator	Organism	Effects	Reference
17β-Oestradiol	Vertebrates	Maturation of accessory sexual tissues	Geuns (1978)
	Plants	Induction of flowering, sex determination	Geuns (1978)
	Opalina ranarum (Protozoan)	Cause encystment	El Mofty & Smyth (1964)
	Trypanosoma cruzi (Protozoan)	Growth inhibition	A M Stoka (unpublished observation)
	Coccidioides immitis (Fungi)	Growth stimulation	Drutz *et al.* (1981)
	Paracoccidioides brasiliensis (Fungi)	Growth inhibition	Loose *et al.* (1983)
Progesterone	*Trichophyton mentagrophytes* (Fungi)	Growth inhibition	Schar *et al.* (1986)
	Tetrahymena pyriformis (Protozoan)	Growth inhibition	Csaba & Fulop (1987)
	Xenopus laevis (Amphibia)	Induction of meiotic division	Baulieu *et al.* (1978)
Thyroxine	Mammals	Regulation of: (a) oxidative metabolism, (b) growth	Tata (1964)
	Amphibians	Regulation of metamorphosis	Tata (1964)
	Tetrahymena pyriformis (Protozoan)	Growth stimulation	Csaba & Nemeth (1980)
Prolactin	Mammals	Milk secretion	Bern & Nicoll (1969)
	Birds	Crop-milk production	Bern & Nicoll (1969)
Adipokinetic hormone	Insects	Lipid mobilization	Stone *et al.* (1976)
	Crustaceans	Pigment concentration	Stone *et al.* (1976)
Juvenile hormone	Insects	Regulation of: (a) reproduction, (b) metamorphosis	Stoka (1987)
	Trypanosoma cruzi (Protozoan)	Growth inhibition	Stoka *et al.* (1990); Stoka (1996)
	Mammals	Inhibition of testicular steroidogenesis	Vladusic *et al.* (1994)
20-OH-ecdysone	Insects	Moulting hormone	Stoka (1987)
	Trichonympha sp (Protozoan)	Growth stimulation and sexual differentiation	Cleveland (1959)

TABLE 4. Evolutionary transition of cAMP-receptors: from membrane to cytosolic environment

	Organism	Type of c-AMP receptor	c-AMP function	Interaction c-AMP/c-AMP receptor
Organization level				
*Prokaryote	*Escherichia coli*	Membrane form	First messenger	Autocrine
†Eukaryote unicellular	*Dictyostelium discoideum*	Membrane and cytosolic forms	First and second messenger	Paracrine
‡Eukaryote multicellular	Vertebrates and invertebrates	Cytosolic form	Second messenger	Endocrine

*Mackman & Sutherland (1965); †Gerish (1987); Saxe *et al*. (1991); ‡Weber *et al*. (1982).

metabolism of bioregulators, and the origin of receptors. An interesting point of view is that binding and catalytic activities of some cell surface receptors arose from primitive enzymes. This hypothesis is supported by the presence of some transitional stages among enzymes and receptors: (i) the transport of testosterone in membrane vesicles of *P. testosteroni* is coupled to membrane bound 3β- and 17β-hydroxysteroid dehydrogenases (Watanabe & Po 1976); (ii) a membrane form of guanylate cyclase in mammals acts as a receptor for atrial natriureic peptide (Chinkers *et al*. 1989); and (iii) the cytosolic receptor for salicyclic acid in plants has catalase activity (Chen *et al*. 1993).

Furthermore, some point mutations in the binding site of an enzyme enhanced its substrate binding capacity although they produced a catalytically non-productive species (Craik *et al*. 1985).

The catalytic and binding capacities of prokaryotes were probably employed in a different fashion to their contemporary hormonal ones in multicellular organisms and represented a vestige of Precambrian times (exaptation process). Some advantageous changes in the structure of prokaryotic enzymes involved in the metabolism of nutritive substrates (e.g. 3β- and 17β-hydroxysteroid dehydrogenases of *P. testosteroni*) may have 'filtered' through the natural selection process, and evolved into other functions such as autocrine, paracrine and/or endocrine signal receptors to facilitate regulation of intracellular processes. In this evolutionary scenario a different type of regulation may be considered. A bioregulator can be produced and act within the same cell (intracrine regulation) (O'Malley 1989). It is known that certain unicellular organisms biosynthesize steroid hormones and contain intracellular steroid receptors. Examples include *Saccharomyces cerevisiae* and *Candida albicans* (Loose *et al*. 1981, Loose & Feldman 1982, Feldman *et al*. 1982, 1984).

If the first unicellular organisms possessed few multifunctional molecules, probably nutritional compounds would serve both as metabolic and regulatory elements. It is possible then, that intracrine regulation could be a transitional stage between regulation through metabolic substrates (nutritive line) and hormonal modulation (regulatory line).

Primitive members of the contemporary steroid receptors family were possibly membrane enzymes which bound environmental nutritional molecules (steroids, sterols), and their prime regulatory function was to control intracellular metabolism.

Such relationships between food signals and contemporary bioregulators do not preclude other parallel original functions of bioregulators in defence functions.

The primary role of ancestral bioregulators in unicellular organisms was a nutritional one, but the adaptive radiation among molecules produced compounds that were inhibitors (e.g. toxins) of enzymes involved in the growth of competitive species.

PHYLOGENETIC ASPECTS OF BIOREGULATOR–RECEPTOR RELATIONSHIP

Some ligand–binder relationships (substrate–enzyme, antigen–antibody, bioregulator–receptor) must be restricted to an interaction with specific and small regional domains within their structures, as exemplified by insulin and its receptor (Pullen *et al*. 1976).

The chemical structure of bioregulators has been well conserved through evolution and there are high degrees of homology within the system (Table 2). In addition, the divergent functions of bioregulators with a similar structure (e.g. progesterone/cortisol) pose a number of questions including the following.

Have receptors evolved in a similar way? How similar and how different are receptors for different bioregulators?

A case in point is that the amino acid sequences of nuclear thyroid hormone receptors display homology with a similar domain of several steroid

hormone receptors (Weinberger *et al*. 1986). Thus, the relationship between steroid hormone receptors and thyroid hormone receptors suggests that all these receptor structures, including the v-erb-A oncogene product of the avian erythroblastosis virus (AEV), may be a superfamily of information molecules that have evolved in several divergent lines during their phylogeny. However, the existing homologies between the thyroid hormone receptors and steroid hormone receptors, vitamin D_3, aldosterone, cortisol, testosterone, progesterone or oestradiol did not seemingly compete (Weinberger *et al*. 1986). Obviously, the secondary, tertiary and quaternary structures of the thyroid hormone receptor increase specificity for thyroid hormone.

What is the origin of the bioregulator–receptor specificity?

The origin of cell specificity for a determined bioregulator depends on the critical period of receptor maturation which determines its binding properties (Blazquez *et al*. 1976) and which may be influenced or altered by the action of a similar chemical structure (Csaba 1980).

On the other hand, the superfamily of steroid receptors, besides receptors binding thyroid hormones, retinoic acid or steroid hormones, contains orphan receptors for which no ligand is known (O'Malley 1989, Escriva *et al*. 1997). It seems likely that the number of ancestral steroid receptors (enzymes?) was expanded by the presence of immature receptors which adopted different binding site configurations with high specificity for particular ligands, as occurs with the evolution of antibody catalysis (Wedemayer *et al*. 1997).

Another aspect involved in the origin of bioregulator–receptor interaction is the structural similarity between secreted bioregulators and chemical components of cells. It has been suggested that pheromones and hormones evolved from membrane components (Kochert 1978, Pfeffer & Ullrich 1985). Accordingly, there are two hypotheses to explain the origins of bioregulator–receptor units.

Hypothesis 1: polypeptide hormones are produced by proteolytic cleavage of membrane proteins of secondary lysosomes (Hales 1985)

The peptide hormone, epidermal growth factor (EGF), is cleaved from membrane protein (Kaback 1985). In addition, bioregulators and their receptors may evolve as a result of changes in the DNA by gene duplication and subsequent point mutational events. Some genes encoding receptor–bioregulator units began as single genes encoding single polypeptide molecules. Gene splitting and different gene expressions may have induced the synthesis of bioregulators and their receptors in different cell types (Niall 1982, Kaback 1985).

Hypothesis 2: the presence of a bioregulator is an essential condition for receptor formation (the theory of signal-imprinting, Csaba 1980)

During this process, there is likely to be initial contact between a chemical signal and a potential receptor region of the cell membrane, and induction of shape complementarity and hydrophobicity of the interacting surfaces may take place (Tainer *et al*. 1984).

However, it is not only the bioregulator–receptor unit which determines the quality of physiological responses under its control. Phylogenetically transducting systems involve trimeric G-proteins, identified in various prokaryotes (Ahnn *et al*. 1986, Schimz *et al*. 1989) and eukaryotes (Namba *et al*. 1983, Firtel *et al*. 1989, Whiteway *et al*. 1989).

In addition to the homologies among steroid receptors and thyroid hormone receptors (Weinberger *et al*. 1986), there is another type of homology – that of G-proteins. Key observations are: (i) the prostaglandin E receptor subtype EP3 and the tromboxane A_2 receptor belong to the large superfamily of receptors coupled to G-proteins which consist of seven transmembrane domains (Namba *et al*. 1983) and are isoforms of rhodopsin (Franke *et al*. 1990) and dopamine receptors (Monsma *et al*. 1989); (ii) the sequence and size variation among receptor domains whereby receptors bind G-proteins (Monsma *et al*. 1989) suggest widespread diversity within the G-protein family. Thus, different physiological responses of receptor isoforms are structurally based (Fig. 3).

In addition, there is other evidence for conservation and functional versatility of information molecules: (i) the pheromone signalling pathway in *S. cerevisiae* is activated by an heterologous transducting system including the human β-adrenergic receptor and the rat Gs-protein (King *et al*. 1990); (ii) *S. cerevisiae* has a primitive steroid receptor system analogous to the mammalian oestradiol receptor unit (Feldman *et al*. 1982, 1984); (iii) a bioregulator can interact with autocrine and paracrine receptors to accrue more physiological functions (Mackman & Sutherland 1965, Gerish 1987, Saxe *et al*. 1991, Vallesi *et al*. 1995); (iv) a receptor in combination with homologous bioregulators can regulate the same physiological response in a determined target tissue (Stone *et al*. 1976, Mordue & Stone 1976, 1977); (v) a receptor can bind different bioregulators and elicit varying patterns of gene activation but with the same effects

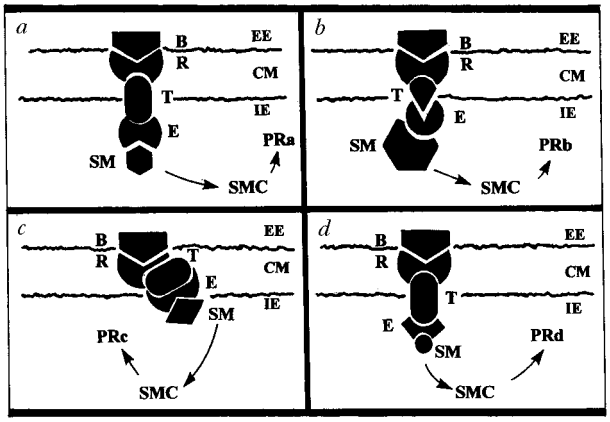

FIGURE 3. Different receptor isoforms can produce more than one physiological response. B, Bioregulator; R, membrane receptor; T, transducer (e.g. G-protein); E, effector (e.g. cyclases, phospholipases); SM, second messengers (e.g. cAMP); SMC, second messenger cascades (e.g. inositol triphosphate cascade); PR, physiological response; CM, cell membrane; EE, extracellular environment; IE, intracellular environment. The G-protein diversity probably results from multiple genes and/or alternative RNA splicing generating multiple isoforms from a single gene. Possible scenarios: (*a* and *b*) receptors are coupled to different transducers; (*a* and *c*) different domains of a determined receptor interact with the same transducer but with an analogous effector. This effector may react with the same or with a different second messenger; (*a* and *d*) receptor isoforms activate different effectors.

in a particular tissue (Yang *et al*. 1996, Paech *et al*. 1997).

SOME HYPOTHESES TO EXPLAIN THE EVOLUTION OF INFORMATION MOLECULES

A basic question is: were contemporary bioregulators (hormones, neuroactive compounds, growth factors, pheromones) primary chemical messengers such as food signals and defensive compounds?

Sterols have probably played an important function as growth bioregulators in the Precambrian period, $1000–3000 \times 10^6$ years ago. Some evidence supports this hypothesis: (i) cellular life may have been present about 3000×10^6 years ago as fermenting bacteria (Schwemmler 1984); (ii) sterol biosynthesis existed at a very early stage in some organisms such as the Cyanobacteria (blue-green algae, *Nostoc*, *Spirulina*) (Nes & McKean 1977); (iii) some sterols participate in the activation of oocyte meiosis (Byskov *et al*. 1995) – a possible feature of ancestral bioregulators was the control of cell division; (iv) the presence of both binding and catalytic activities for vertebrate-type steroid hormones in aerobic bacteria (Watanabe *et al*. 1973*a*,*b*, Watanabe & Po 1976).

Indeed there are important differences in structural conservation during the phylogeny of information molecules. Some bioregulators such as steroid and thyroid hormones are rigorously conserved because they result from enzymic cascades and require almost absolute substrate specificity. In contrast others (somatomammotrophins, pituitary glycoproteins, secretins) are direct gene products and will tolerate change providing the 'active' (receptor activating) portion of the peptide is not changed and variation can be tolerated (Henderson 1997).

In some cases, the structural evolution of information molecules produced a functional versatility of a hormone. For example, prolactin has acquired different functions among vertebrates: (i) osmoregulatory activity in fish; (ii) growth-promoting activity in tetrapods; (iii) metamorphosic actions in amphibians, and (iv) lactogenic activity for neonatal nutrition in mammals and birds (Bern & Nicoll 1969).

Correlated with these physiological acquisitions, comparative studies on structural and functional bioregulator–receptor interactions reveal that both prolactin and prolactin receptors have undergone considerable changes (White & Nicoll 1979), although there is no strict relationship between functional versatility and the number of chance substitutions in the structure of informational molecules.

A most striking example in this area is the structural change found in naturally occurring insulins among vertebrates. These insulins have different affinities for the insulin receptor in mammalian tissues (Muggeo *et al*. 1979) and they differ 50- to 100-fold in their potency (Blundell *et al*. 1972).

At the level of specificities among ligands and binders, some bioregulator–receptor units seem to be in a transitional evolutionary stage according to their cross reactions, as occurs with arthropod neurohormones (Fig. 4, Table 5). In contrast, other bioregulator–receptor units such as the glucagon/glucagon receptor unit (Blundell & Humbel 1980) display maximum specificity. It is thus possible that different bioregulator–receptor units in a particular species evolved at different rates and times, as occurred with some morphological characteristics, a process termed mosaic evolution (De Beer 1954); there are several examples for different molecules (Wilson *et al*. 1977). From a functional point of view, the prolactin/prolactin receptor unit has evolved more rapidly than the insulin/insulin receptor unit

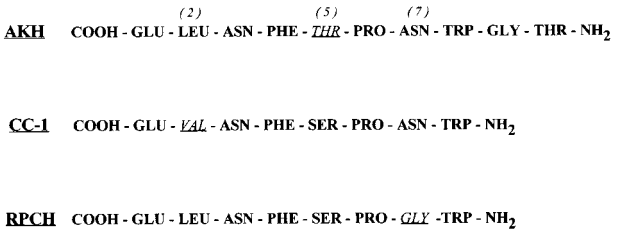

FIGURE 4. Structural homologies among arthropod neurohormones. The primary structures of red pigment concentrating hormone of prawn (RPCH, Crustacea, octapeptide), adipokinetic hormone of locust (AKH, Insecta, decapeptide) and periplanetin of cockroach (CC-1, Insecta, octapeptide) suggest that they evolved from a common precursor by gene duplication and subsequent point mutational events (*); then they diverged functionally. *AKH: Serine (SER) — Threonine (THR) (amino acid position 5); CC-1: Leucine (LEU) — Valine (VAL) (amino acid position 2); RPCH: Asparagine(ASN) — Glycine (GLY) (amino acid position 7).

as judged by the number of known physiological functions acquired during vertebrate evolution.

In addition, the taxonomic distribution of insulin and prolactin differs (Fig. 5) and does not correlate with their functional versatility. In this regard, it may be that chemical communication arose many times during phylogeny and primordial information molecules, with functional and/or structural characteristics different to present-day bioregulator–receptor units, may have appeared and disappeared as a result of changes in the expression of genetic information (point mutations, gene duplication, alternative splicing, transpositions, etc.). The very nature of the regulation of genetic transcription would indicate that the genome of contemporary biota must contain DNA regions with information for potential unexpressed bioregulator–receptor units, and therefore the specific group of present-day information molecules may be only one of several lineages.

Indeed, these non-translated DNA regions (introns) are, in terms of genetic information, the remnant of phylogenetic history and the building blocks of current and future evolution.

This hypothesis is supported by ectopic endocrine activity of patients with neoplasia. It was observed that the occasional disruption of normal cell differentiation can produce nonendocrine tumours, capable of synthesizing a great amount of hormones (e.g. phaeochromocytoma) (Baylin & Mendelsohn 1980). Such complicated and fascinating processes reflect a regression towards a primordial cellular stage; under such conditions it seems that there is a return to a cellular pluripotentiality for the biosynthesis of information molecules. Such potential information molecules may arise from 'hidden' sequences of DNA regions (introns) and developed unknown (extinct or new) bioregulator signalling mechanisms. Evolution in a sense can go into reverse to re-express earlier chemical communication systems.

It is also plausible that present-day chemical communication systems may be superior to primordial systems, but they may not necessarily always be so under some conditions.

CONCLUSIONS

The inherent similarity of mechanisms involved in bioregulator–receptor interactions reveals that glandular and nervous systems (endocrine systems) may have a common origin from a pheromonal system (primordial exocrine system) of unicellular organisms.

This hypothesis can explain many phenomena: (i) vertebrate-type hormones acting in unicellular organisms as pheromones that cause regulatory effects (Tables 2 and 3); (ii) the presence of vertebrate-type bioregulators (gastrin, somatostatin, prolactin) in classical exocrine fluids such as saliva, gastric juice and other humoral secretions (Le Roith

TABLE 5. Effects of arthropods neurohormones

	Pigment concentrating activity in erythrophores (*Crustacea*)	**Hyperglycaemic activity in fat body cells** (*Insecta*)	**Adipokinetic activity in fat body cells** (*Insecta*)
Neurohormone			
†Adipokinetic hormone (AKH)	Yes*	Yes*	Yes
‡Periplanetin (CC-1)	—	Yes	Yes*
§Red pigment concentrating hormone	Yes	—	Yes*

Cross-reactions (*) were observed among arthropod neurohormones. Probably binding and transduction activities of neurohormone receptors could have evolved independently. The evolution of arthropod neurohormones and their receptors seems to be an example in which specificity has not reached a maximum (*transitional or immature stage*).
†Mordue & Stone (1976, 1977); Stone *et al*. (1976); ‡Hanaoka & Takahashi (1976); §Mordue & Stone (1976, 1977); Ferlund (1974).

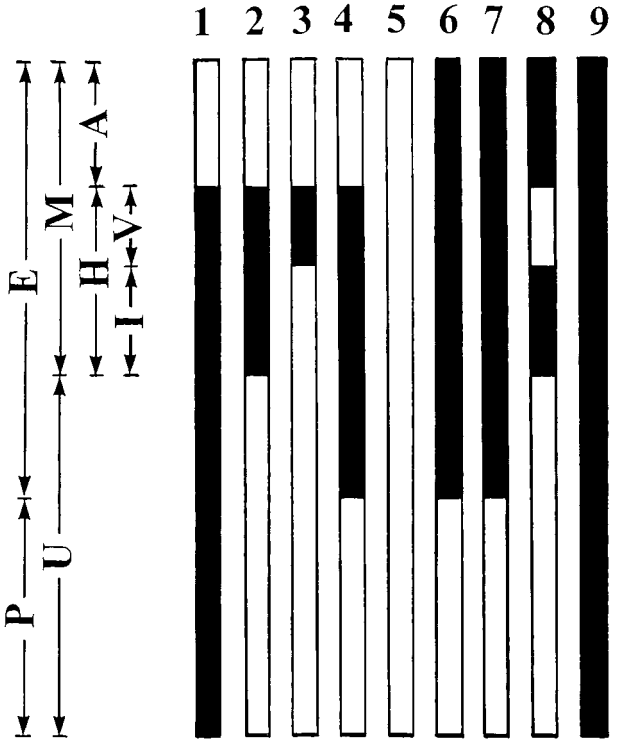

FIGURE 5. Conservation of some bioregulators during biological evolution. Some properties of bioregulator–receptor units suggest that they may have evolved by selection of some other function, and then diverged functionally. Certainly, the conserved specific domains of bioregulator–receptor units and their presence in different taxonomic groups are consistent with a natural selection event to ensure survival and reproduction of new life forms (adaptation), but in certain cases (Riedel *et al*. 1986) their final physiological function is largely unknown (exaptation). ■ Present-day distribution of different bioregulators through different taxonomic levels. 1, Insulin; 2, glucagon; 3, prolactin; 4, noradrenaline; 5, potential but not expressed bioregulators; 6, juvenile hormone and juvenile hormone analogues. Juvenile hormone activity has been found in protozoans (Fisher & Sandborn 1962), arthropods (Judy *et al*. 1973), vertebrate tissues (Gilbert & Schneiderman 1958, Williams *et al*. 1959) and plants (Slama & Williams 1965, Bowers & Nishida 1980)); 7, oestradiol; 8, 20-hydroxyecdysone; 9, somatostatin. □ Extinct or unexpressed pathways of bioregulator biosynthesis in contemporary species. P, Prokaryotes; E, eukaryotes; U, unicellular; M, multicellular; H, heterotrophs; A, autotrophs; I, invertebrates; V, vertebrates.

et al. 1986); (iii) the ubiquity of insulin in extrapancreatic tissues of mammals (brain, liver, cultured lymphocytes and fibroblasts) (Rosenzweig *et al*. 1980), insects (Duve *et al*. 1979), annelids (Le Roith *et al*. 1981*b*) and unicellular organisms (Le Roith *et al*. 1980, 1981*b*); (iv) the overlap of brain and gut bioregulators in mammals (Zimmerman 1979) and insects (Duve & Thorpe 1981); (v) the biosynthesis of gastrointestinal hormones in neural, endocrine and paracrine cells (Grosman 1979); (vi) gonadal steroid hormones acting as defensive agents (Schildknecht *et al*. 1966, 1967, Loose *et al*. 1983, Schar *et al*. 1986); (vii) the presence of specific binding of insulin to the unicellular alga *Acetabularia mediterranea* (Legros *et al*. 1975); (viii) the ubiquitous distribution of thyrotrophin releasing hormone in the animal kingdom (Henderson 1997) and plants (Morley *et al*. 1980, Jackson 1981).

It is suggested that endocrine secretory mechanisms (glandular, nervous) have a common origin and are products of phylogenetic and evolutionary processes from unicellular organisms (primordial exocrine bioregulators) to multicellular organisms (contemporary exocrine and endocrine bioregulators).

On the other hand, receptor binding activities seem to have arisen as a result of modifications in the structure of enzymes. Within this hypothesis a divergent evolution is possible since enzyme binding activity need not always co-evolve with its intrinsic catalytic activity. Such an evolutionary mechanism enables receptors to be created without the development of additional binding sites (Stone *et al*. 1976, Shemshedini & Wilson 1990, Lee *et al*. 1992).

It is not surprising that receptor evolution, as with other evolutionary characteristics, required millions of years during which some transitional stages (e.g. hormonal receptors with catalytic activity) predominated. Enzymes are thus the key to understanding the origin and evolution of both enzymes and receptor binding activities. Some basic questions are obviously raised: how similar are the binding regions of receptors and enzymes for a particular bioregulator? Is the origin of receptor and enzyme binding activities, for a particular bioregulator, a product of convergent or divergent evolutionary processes?

Although evidence is scanty, it is possible that by mutational and natural selection processes a bioregulator–receptor unit arose through initial divergent evolution followed by a convergent process. Convergent and divergent evolution have been noted in some eukaryotic phosphorylases (Hwang & Fletterick 1986). Figure 6 illustrates possible sequences within the evolutionary history of bioregulator–receptor units.

The evolutionary development of chemical communication systems reflects parallel evolution of all information molecules (bioregulators, receptors,

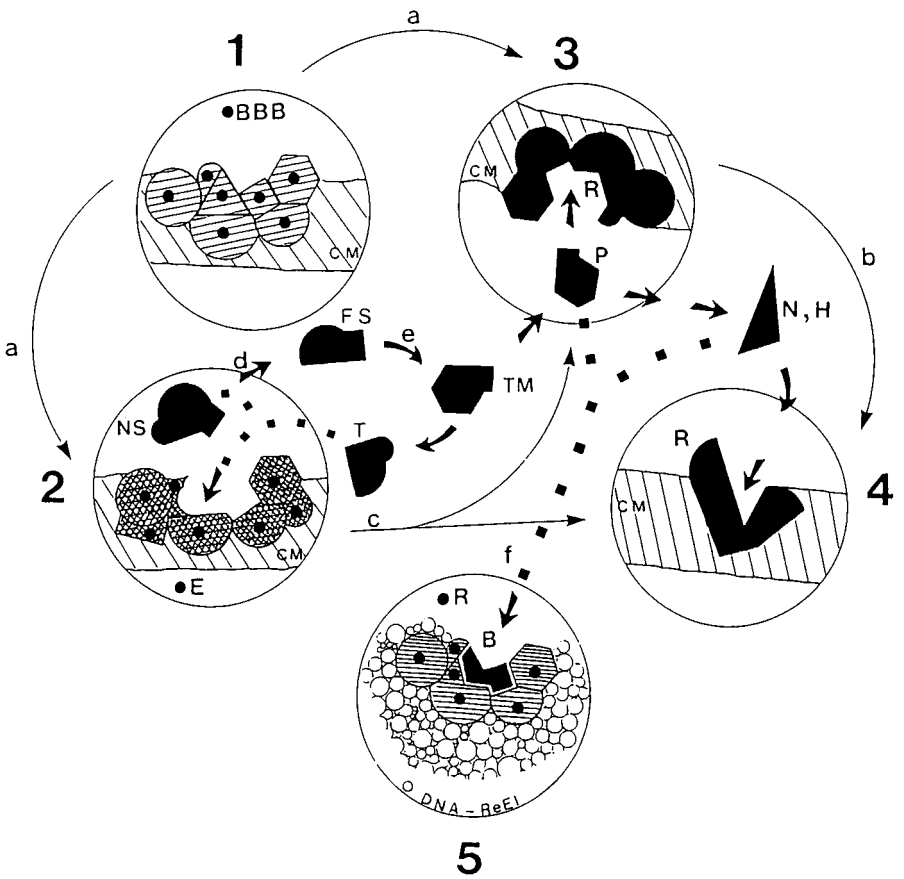

FIGURE 6. Hypothetical sequence of evolutionary transitions from a potential signal-receiver unit to bioregulator–receptor interaction. Evolutionary stages: 1, potential signal-receiver unit; 2, substrate–enzyme interaction; 3, pheromone–membrane receptor interaction; 4, neurotransmitter or hormone-receptor interaction; 5, bioregulator (pheromone, hormone, neurotransmitter)–nuclear receptor interaction. Initially, in this schematic representation, a potential signal-receiver unit (stage 1) may have evolved into two divergent models: (i) enzyme (membrane form)–substrate (nutritive line) (stage 2); (ii) membrane receptor–pheromone (regulatory line) (stage 3). After this divergent process, a convergent evolution is possible; for instance the receptor binding activities arose from enzymes involved in the metabolism of nutritive substrates as a result of: (i) point mutations in the binding site (transition c substrate specificity enhancement and catalytic activity diminution) (Craik *et al*. 1985); (ii) altered binding specificity may also influence interactions of some molecules (nutritive substrates, transition molecules) with membrane receptors which acquire a pheromonal status. On the other hand, these transition molecules may have evolved into toxins which may serve as enzyme inhibitors or enzyme activators. Transitions: a, origin of signal-receiver units through genetic or imprinting mechanisms; b, origin of endocrine bioregulators (neurotransmitters, hormones, etc.) through the development of multicellularity; c, hormonal and pheromonal receptors arose from primitive enzymes; d, probable relationship between nutritive substrates and food signals; e, transition molecules (pheromone-like compounds) arose from advantageous changes in the structure of food signals; f, receptor internalization process: from membrane receptors to soluble intracellular receptors. BBB, biological building blocks; CM, cell membrane; DNA-ReEl, DNA-response element, R, receptor; H, hormone; P, pheromone; NS, nutritive substrate; E, enzyme; B, bioregulator (hormone, pheromone, etc.), TM, transition molecule; FS, food signal; T, toxin; N, neurotransmitter.

transducers, enzymes, second messengers, second messenger cascades, etc.), which are sometimes co-ordinated and essential processes (adaptation) and are sometimes opportunistic and random events (exaptation).

Despite extensive accumulation of information over the last 20 years, many important gaps remain in our knowledge of phylogenetic relationships and genetic processes therein.

Virtually nothing is known about: (i) the effects of mutations in different kinds of genes (multiple genes, simple non-overlapping genes) that can be quite different according to their primary function, (ii) whether it is possible to describe the exact physico-chemical conditions and frequencies of new adaptations and exaptations.

Molecular endocrinology is now at a stage when some of these and other questions can be addressed. The author expects that this review will stimulate new routes of thinking in research teams that work in the area.

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