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The relationships among biological membranes and signaling mediators. II. How do the receptors find and identify their targets?

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Summary. Although many components of the signaling pathways are known, nevertheless, how mitogen-activated protein kinase signaling pathways relay, integrate and transmit signals from a diverse range of intracellular and extracellular stimuli to nuclear DNA remains an open question for now. How do RNA polymerase or DNA polymerases find and identify their targets avoiding fruitless searching through megabases of non-target DNA? In this paper I review how this sequence searching may be done. I described an alternative perception mechanism of intracellular and extracellular stimuli and transduction them to nuclear DNA using as a messenger (a receptor) the short DNA strands i.e. primers. I believe that so-called receptors (transcription factors) are synthesized simultaneously with membranes and stored in the rafts of membranes upon their damage by chemical or physical factors. Liberated during membrane destruction these receptors (signaling molecules), called by us as gene keys, conjoin with the template DNA strands at the promoter (gene locks) through non-covalent bonds with A-T base pairs. Thus, the gene key dictates when, where and what specific genes are transcribed.

Keywords: DNA, membranes, primers, receptors, signaling.

Introduction. The existence of living cells depends on their ability to receive exogenous or endogenous signals and to respond to them in a suitable way. It is generally accepted that all biological processes in the living systems are controlled by divers signal transduction pathways which are highly spatially organized. Many cellular substances are considered to be signaling molecules that are sensed by the receptors attached to the cell membranes. Signaling through the receptors is essential for cell survival. Although many components of the signaling pathways in the living systems are known, the understanding how cells convert extracellular or intracellular signals into the required cellular responses have remained elusive. In this paper I consider the nature of receptors and their possible signal transduction pathways.

How strong is the evidence that membranous proteins are related to signaling molecules? There are numerous endogenous and exogenous substances that influence growth and development of animals and plants species. Endogenous growth regulating substances in animals are called hormones and natural plant growth regulators are called phytohormones.

While the available data provide a good evidence for a role of membrane in the signaling and response pathways, there are some limitations that can be drawn from them. For instance: How many growth substances are produced by living creatures? The number of biologically active substances is several times greater than the number of moving proteins presented within the plasma membrane and other cellular membranes. In fact, only a number of natural substances are estimated at around 500 000 [10]. Moreover, there are enormous quantities of

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diverse synthetic chemicals which can influence the growth, development and physiological state of living organisms and their number are growing day by day.

Cellular proteins as receptors are usually determined after visible biochemical/physiological changes i.e. in the next time when radioactive growth regulators were introduced to the cells. From this is not clear whether growth regulators were combined with membrane receptors before or after initiation of biochemical reactions.

It is suggested that from 20 to 30 % of the proteins encoded in the human genome are membrane proteins which are implicated in signal transduction [17]. The following question arises: How many mobile proteins are presented in the plasmalemma and other cellular membranes and what are they?

As mentioned above the number of growth regulating chemicals exceed the number of membrane proteins. From this information follows that dozens or hundreds of diverse growth regulating agents may use the same protein as the receptor, but this is in contradiction with the specificity of biological action, first of all, hormones and phytohormones. Moreover, from rational point of view is nonsense to synthesize so many possible receptors, for example to xenobiotics or poisons to be killed by them. In any event, mechanisms must exist to control the specificity, as well as the intensity, of action of diverse growth regulating substances.

The G-protein-coupled receptors: What is important and what is not. Usually the typical receptor consists of three functional modules: a sensory histidine kinase, a histidine phosphotransfer protein, and a response regulator [11]. There is some evidence that eukaryotic MAP kinase cascades transduce received stimuli to responses via reversible phosphorylation of a MAP kinase by a MAPK kinase and a MEK kinase [12]. Nevertheless, how mitogen-activated protein kinase signaling pathways relay, integrate and transmit signals from a diverse range of intracellular and extracellular agents to nuclear DNA remains an open question today.

By imagine very complicated and in most cases incomplete signaling pathways researches did not explain the detail mechanism of recognizing appropriate genes, integration with RNA-polymerase and initiation transcription.

Meanwhile, there is funny happening in the theory of receptors: some proteins present the receptors whereas other growth regulating agents. In accordance to several publications a set of low molecular peptides act through a G protein-dependent pathway. Among them only cyclic peptides possess a broad range of biological activities [13].

How does this new complex reconcile with the biochemical properties of foregoing substances? If such large protein receptors are combined with growth regulating agents, especially with other peptides/proteins, they should have serious impediments to moving within the cytosol to nucleus.

How does the receptor with DNA- or RNA-polymerase recognize the needed for transcription genes? It is well known that RNA polymerases act in a time and tissue-dependent way, but where they are located in the cell and why only several genes are transcribed during life of the cell is not well understood. Is also unclear how RNA polymerase finds appropriate chromosomes and target genes within them. How are chromosomes identified when making a transcription reaction? RNA polymerase or DNA polymerases must find their targets by implying mechanisms that avoid fruitless searching through megabases of non-target DNA. Below we review of how this sequence searching is done.

In bacteria DNA replication occurs at polymerization rates of about 500 nucleotides per second and about 50 nucleotides per second in mammals [1]. It is clear that such speed and accuracy cannot be achieved by a single DNA polymerase.

The human genome encodes 15 different DNA polymerases [2]. What do all these polymerases do in the cell? One might hypothesize that the great number of DNA polymerases may take part in the DNA synthesis simultaneously. Similar phenomenon may take place in the RNA synthesis.

An essential difference between RNA polymerase and DNA polymerase is that the latter requires a primer to initiate nucleic acid synthesis, whereas the former does not [18].

Nevertheless, in vitro transcription by purified RNA polymerase II requires the addition of several initiation factors that are called general transcription factors [5].

In bacteria the genome may consist of approximately 2,000 to 6,000 genes, whereas in higher plants and animals there may be up to 50,000 genes [4]. The human genome includes approximately 30 000 genes [14, 16]. The number of genes in Arabidopsis is estimated about 26 000 [*Arabidopsis*, http://www.Arabidopsis.org], in the Medicago and Lotus more than 40 000 genes are documented [3], in the poplar genome is estimated to be over 45 000 [15].

If each gene or a cluster of genes should be transcribed using its own receptor hence the next question arises: What role do receptors in reality play in the transcription?

In this connection we proposed that so called nuclear receptors are DNA fragments which linear sizes may correspond to the linear sizes of the nucleosomal triplet [6, 7]. We believe that these fragments of DNA are also termed in the literature as DNA primers. TATA box is essential part of each DNA primers. May they be considered to be real receptors?

During life of the cell the hundred acts of transcriptions occur. The paradox is how such a number of proteins are located within the nucleus. How are they coordinated?

Today the available evidence suggests that there are genomic and non-genomic responses to biotic and abiotic stimuli. In this connection the next question arises: Are there receptors different or a single receptor triggers responses in both responses? How complex are signaling pathways?

We postulate that non-genomic responses are initial reactions to growth regulating chemicals and physical factor actions which result in loss of membrane integrity [9]. When destructive processes in the membranes take place receptors are liberated. Then the following question also arises: Why only peptide substances present receptors? Are there another substances having similar functions?

Earlier we reported that signaling molecules can be presented by short DNA (about 80-100 bp) strands. Such short DNA strands (and also possibly RNA strands) can correspond to

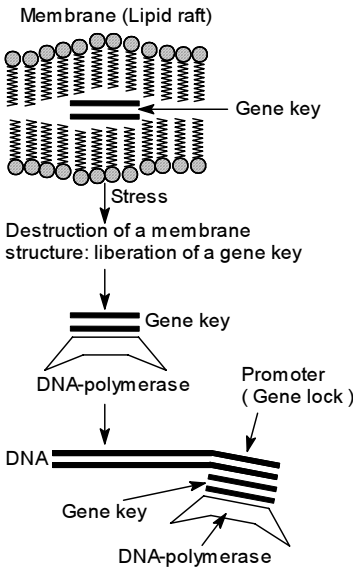

Fig. 1. Schematic representation of the scenario for the receptor formation and replication initiation. Gene key before contact with the promoter (gene lock) may be conjoined with DNA-polymerase or appropriate unknown proteins that are disposed at the membrane or in the cytoplasm.

the molecules known as primers. We believe that so-called receptors (i.e. primers) are synthesized preliminary and stored in the lipid raft domains and caveolae of the membranes upon their breakdown by chemical or physical factors.

The receptors (named by us as gene keys) liberated by this way conjugate with the template DNA strand at the promoter through non-covalent bonds (Fig. 1).

From described above information follows that biochemical agents and physical factors do not contacts with DNA and have a function to serve as destructive factor that liberates the gene key from lipid rafts of the plasmalemma and other membranes of the cellular diverse compartments. The liberated gene key conjugates with DNA- or RNA-polymerases. Such complex is able to conjoin with the appropriate promoter at the gene/cluster (Fig. 2) [6, 7] to start, for example, replication (or transcription).

The gene lock (at the promoter) and the gene key (the receptor, a sigma factor) are collinear, but the base pairs within the sigma possess inverse position (Fig. 2B). Between the TATA box and TA bp are disposed CG base pairs. Hence, only coincidence TA bp between two fragment of DNA (within a promoter and a sigma factor) permits to form stable hydrogen

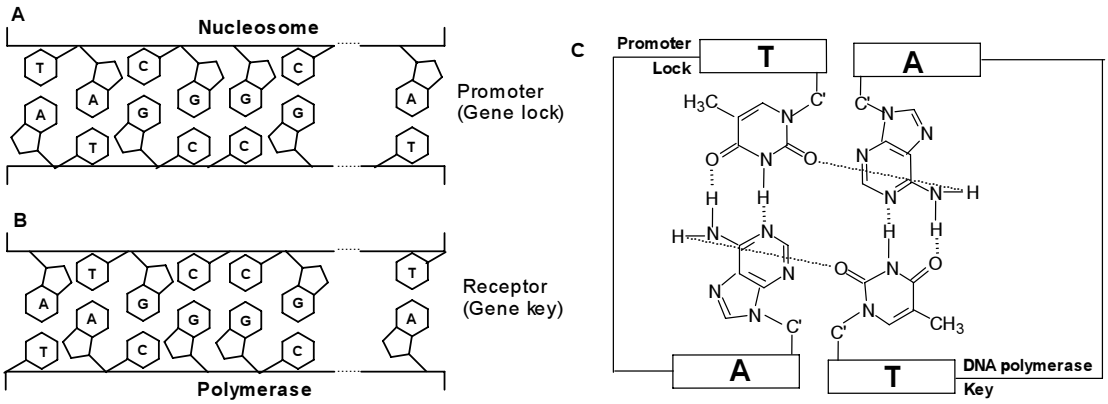

Fig. 2. The fragment of a promoter (A) and a sigma factor (B) that contain AT bp. Between the TATA box and TA bp can be disposed GC or CG base pairs. The formation of hydrogen bonds between TA base pair of the promoter and AT base pair of sigma factor (C). The hydrogen bond is formed between the hydrogen at N6 of the adenine and the oxygen at C2 of the thymine.

bonds and initiate replication (or transcription). Thus, the gene key dictates when, where and what specific genes are transcribed.

In our model of DNA package in the chromosome DNA is disposed between to layers of nucleosomes [8]. In the case if chromosomes are encored to nuclear envelope the transcription proceed only from one (intra-nuclear) strand of DNA. Also before transcription the intra-nuclear strand of DNA may be deleted (or moved) and newly DNA strand will be synthesized complementary to old DNA strand. The deleted strand of DNA will be served as the template for RNA synthesis. This means that the strand of DNA from the side of nuclear envelope will not be changed during a full life of the cell.

It also is non-logical phenomenon when intracellular natural growth regulating chemicals (synthesized in different compartment of the cell) should move to the plasmalemma (or any nearest membrane) to combine with the receptor and then come back to target compartment (or a nucleus) to initiate respective chemical reactions.

How growth regulating substances find their proteins at the membranes? And at last when growth regulating chemicals identify and conjoin with proteins at the membrane what happens to them? What chemical changes occur into both structures? These questions remain unanswered.

Conclusion. In our view, however, the current understanding of the initial molecular mechanism action of the physical and chemical factors on the living systems is very limited. It is unclear whether growth regulating substances directly bind to nuclear DNA or regulate gene expression by interacting with DNA-binding proteins. The experimental data suggest the growth regulating factors have overlapping yet distinct functions and thus should have different downstream targets. It also remains unclear how the replication machinery selects and recognizes target genes. We can expect that identification and further examination of the transcription factors will be essential to unraveling the roles of A-T nucleotide base pairs in the replication and transcription processes.

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Відношення між біологічними мембранами і сигнальними медіаторами.
II. Як рецептори знаходять та ідентифікують свої мішені

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Резюме. Хоча багато компонентів сигнальних шляхів відомі, усе ж як міоген-активовані білково-кіназні шляхи сприймають, інтегрують, передають сигнали з різного роду внутрі- й екзо-клітинних стимулів до ДНК залишається на сьогодні відкритим питанням. Як РНК чи ДНК полімерази знаходять та ідентифікують свої мішені, ми-

наючи непродуктивні пошуки метабаз не мішеней ДНК? У статті розглянуто, як цей пошук може відбуватися, описано альтернативний механізм сприйняття внутрі- й екзо-клітинних стимулів і трансдукцію їх в ядро з використанням як посередника (рецептора) коротких ниток ДНК, тобто праймерів. Вважаємо, що так звані рецептори (транскрипційні фактори) синтезуються одночасно з мембранами, де і зберігаються до пошкодження останніх хімічними чи фізичними факторами. Вивільнені під час руйнування мембран рецептори (сигнальні молекули), названі нами генні ключі, зв'язуються з нитками ДНК на промоторі (генний замок) нековалентними зв'язками з A-T-парами. Таким чином, генний ключ диктує, коли, де і які специфічні гени транскрибуються.

Ключові слова: ДНК, мембрани, праймери, рецептори, сигнальність.

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