Biochemistry (Moscow), Vol. 70, No. 4, 2005, pp. 391-405. Translated from Biokhimiya, Vol. 70, No. 4, 2005, pp. 476-492.

REVIEW

Molecular Mechanisms of Hormonal Activity. II. Kinase Systems. Systems with Intracellular Receptors. Transactivation of STS*

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Received February 16, 2004
Revision received April 12, 2004

Abstract—Hormone receptors and other components, functional mechanisms, and biological role of analyzed signal transduction systems (STS) are described. The recently revealed module principle of the structure and STS transactivation providing diversity and plasticity of regulation are highlighted. STS activities are significantly changed in many diseases. Novel promising pharmaceuticals targeted to certain components of STS increase in number from year to year. The data published by the beginning of January 2004 are summarized in this review.

Key words: signal transduction systems, receptors, tyrosine kinases, protein kinases, transactivation

4. TYROSINE KINASE SIGNAL TRANSDUCTION SYSTEMS

4.1. General characteristics of tyrosine kinase STSs. Kinase STSs encompass both receptor and non-receptor (intracellular) serine/threonine and tyrosine protein kinases. Except for PIP_3, all of the components of these systems are proteins. Tyrosine kinases (TKs) are specific proteins phosphorylating tyrosine (but not serine or threonine) residues in proteins. They are essential components of two different STSs. The obvious difference is that in the first system the receptor is a transmembrane protein with two functional domains: extracellular receptor and intracellular tyrosine kinase domain, whereas in the second system this receptor has no catalytic activity, but interacts with tyrosine kinase (independent protein). There are known 91 TKs including 59 RTKs and 32 nRTKs (65 and 35%, respectively) [1, 2]. Their antagonists are PTPs encoded by 100 genes [3]. In 1986, R. Levi-Montalcini was awarded with the Nobel Prize for discovery of nerve growth factor (NGF), one of hormones involved in this system.

Both TK types are associated with various *disorders*, such as inflammation, diabetes, increased proliferation in psoriasis and endometriosis, and vessel wall remodeling associated with atherosclerosis, hypertension, and particularly neoplasia. The latter are diseases of aberrant (impaired) regulation [4]. As a rule, they develop from activating mutations in genes encoding STS components, particularly TKs themselves or alternative splicing. As a result, TKs become constitutively active. Carcinogenesis is also caused by either gene amplification or overexpression. Thus, 70% known oncogenes and protooncogenes are various TK genes, whose mutations are found in half of human cancers. So, the term "carcinogenic TKs" has been introduced. They become important as targets of cancer therapy. Three major medication types are used: low-molecular-weight TK inhibitors, such as gefitinib (Iressa), imatinib (Gleevec), and erlotinib (Tarceva);

Abbreviations: ACT) PKB (protein kinase B); CBP) CREB-binding protein; CM) calmodulin; EPK) extracellular signal-activated protein kinase; GF) growth factor; GPCR) G-protein-coupled receptor; HRE) hormone-reactive (response) element; IL) interleukin; JNK) Jun N-terminal kinase; MAPK) mitogen-activated protein kinase; nRPK) non-receptor protein kinase; nRTK) non-receptor tyrosine kinase; PI) phosphatidylinositol (phosphoinositide); PI3-K) phosphatidylinositol-3-kinase; PIP_3) phosphatidylinositol 3,4,5-trisphosphate; PK) protein kinase; PM) plasma membrane; PP) protein phosphatase; PTP) protein tyrosine phosphatase; RPK) receptor protein kinase; RTK) receptor tyrosine kinase; SAPK) stress-activated protein kinase; STAT) signal transducers and activators of transcription; STS) signal transduction system (pathway); TF) transcription factor; TGF) transforming growth factor; TK) tyrosine kinase; TNF) tumor necrosis factor.

* Part I of this review, "Molecular Mechanisms of Hormonal Activity. I. Receptors. Neuromediators. Systems with Second Messengers", was published in *Biochemistry (Moscow)*, Vol. 70, No. 1.

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monoclonal antibodies, such as transtuzumab (herceptin); and antisense oligodeoxynucleotides against the oncoprotein mRNAs, such as oblimersen (Genasense, Genta). These drugs cause cell cycle arrest, induce apoptosis, and inhibit both tumor angiogenesis and metastasis. Their decided advantage is better tolerance due to low dosage allowable because of cascade signal amplification in STS [5, 6].

4.2. Systems with receptor tyrosine kinases (RTKs). These systems (see Part I, Table 1, 4B) include: 1) RTK, or tyrosine kinase receptors arising in multicellular organisms, with extracellular hormone binding domains and single transmembrane and intracellular catalytic domains; 2) serine/threonine PK complex cascades transmitting signals into the cell and then into the nucleus (Fig. 1). Hormone binding with RTK induces their dimerization (alternatively, they are dimers initially, like insulin receptors), mutual activation, and cross-phosphorylation of intracellular domains, which stabilizes active conformation of the receptors. Docking sites appear on cytosolic domains for RTK binding with multiple protein substrates, which being phosphorylated by the same catalytic domains transmit the signals forth. RTK substrates are protein adapters, TF-STAT, and some important enzymes such as phospholipase C-γ, PI3-K, and PTPs [2, 7, 8]. One of them, PTP1B, is specific for insulin RTK substrates. RTK activation by cytokines, GFs, and circulating hormones results in accumulation of reactive O_2 species, which oxidize the SH-group of PTP cysteine and inhibit the enzyme, thus elevating substrate phosphorylation; both GSH and thioredoxin restore PTP activity [3].

RTKs are activated by insulin and multiple GFs, such as insulin-like, epidermal, and various fibroblast GFs, platelet GF, macrophage colony-stimulating factor (M-CSF), vessel endothelium GF (VEGF, receptor Flk-1), hepatocyte GF (receptor Met), stem cell factor (receptor c-kit), dendrite cell GF (receptor Flt-3), glia-derived neurotrophic factor family (GDNF, receptor Ret), as well as by cytokines and STH (somatotropic hormone). The RTK system mediates multiple effects of these hormones: metabolic changes (particularly caused by insulin), stimulation of proliferation and differentiation, cell migration and survival (which is very important for human longevity), and a variety of morphogenesis types (angiogenesis, hematopoiesis, neurogenesis, epithelial tubulogenesis, spermatogenesis, etc.) [2, 8-10].

The RTK VEGF and stem cell growth factor inhibitor SU 5416 and the anti-VEGF monoclonal antibody bevasizumab (Avastin) are used in clinical practice. One of the virulence genes of *Yersinia* (including the etiological agent of plague) encodes PTP [8].

Signal transduction from RTK inside the cell is realized by **three main signal transduction pathways**: via phospholipase C-γ, Ras–MAPK, and PI3-K–ACT. Some RTKs also activate TF STAT and NF-κB (see below).

Unlike phospholipase C-β, **phospholipase C-γ** is commonly activated by GF and RTK rather than by hormones with GPCR and the protein $G_{q/11}$. Nevertheless, both phospholipases C are functionally identical: they cleave PIP_2 into inositoltrisphosphate (IP_3) and diacylglycerol (DAG) with possible further involvement of Ca^{2+} and protein kinase C (PKC) [8].

Ras–MAPK cascade (see Fig. 1), the second STS transmitting signals from RTK inside the cell, is most important for GF and arose in unicellular eucaryotic organisms. *Ras proteins* play the role of switches from RTK to MAPK. They are components of a superfamily numbering more than 100 members of small GTPases, which are monomeric [11], unlike trimeric G-proteins (see Part I). Ras is fixed at the inner side of the PM as a result of farnesylation (attachment of a hydrophobic C_{15}-fragment, which is also used in cholesterol synthesis). GTP binding is another circumstance of Ras activity. The signals from RTK induce transition from GDP-Ras to its active form, GTP-Ras, as a result of GDP-GTP exchange. Alternative pathways of Ras modulation are DAG, PKC, and Ca^{2+}/CM-PK. Activated Ras controls proliferation, differentiation, and survival of all eukaryotic cells. Raf, a serine/threonine PK initiating MAPK cascade, is an effector for Ras, and Rap, a related protein, is a physiological modulator for Ras. Another family of small GTPases, the Rho family, participates in hormone effects as well [2, 8, 11-13].

Ras was the first oncogene found in humans. Its mutations resulting in its constitutional activation are found in 30% of malignant tumors, particularly in leukemia and pancreas cancer [13]. Pharmaceuticals like tipifarnib (Zarnestra) have been developed for depressing Ras activity via farnesyl transferase inhibition [5, 14].

MAPK-cascades (see Fig. 1) discovered in 1990-1994 comprise several STS, each being formed by sequential phosphorylating and cross-activating protein kinases: MAPK kinase kinases (MAPKKK, MKKK) → MAPK kinases (MAPKK, MKK) → MAPK, the first and the third are serine/threonine PK, and the second is PK of dual specificity (tyrosine and threonine phosphorylation) [9, 15-17]. In a classical *MAPK/ERK-cascade*, the first PK is Raf, the second is MAPK/extracellular signal regulated kinase kinase (ERKK or MEK1/2), and the third is ERK1/2 (p44/42 MAPK or MAPK with molecular mass of 44/42 kD). The latter is partially translocated into the nucleus in the form of mono- and dimers and directly or indirectly (mediated by other cytosolic and nuclear serine/threonine PKs) stimulates pyrimidine nucleotide synthesis, phosphorylates both TFs (CREB, c-Fos, c-Myc, STAT, lipophilic hormone receptors, etc.) and the co-integrator p300 [18], and induces coupled phosphorylation and acetylation of H_3 histone, thus inducing chromatin structure alteration and gene activation. Ribosome recruiting via activation of the translation initiation factor 4E (eIF4E) and phosphorylation and activation of other

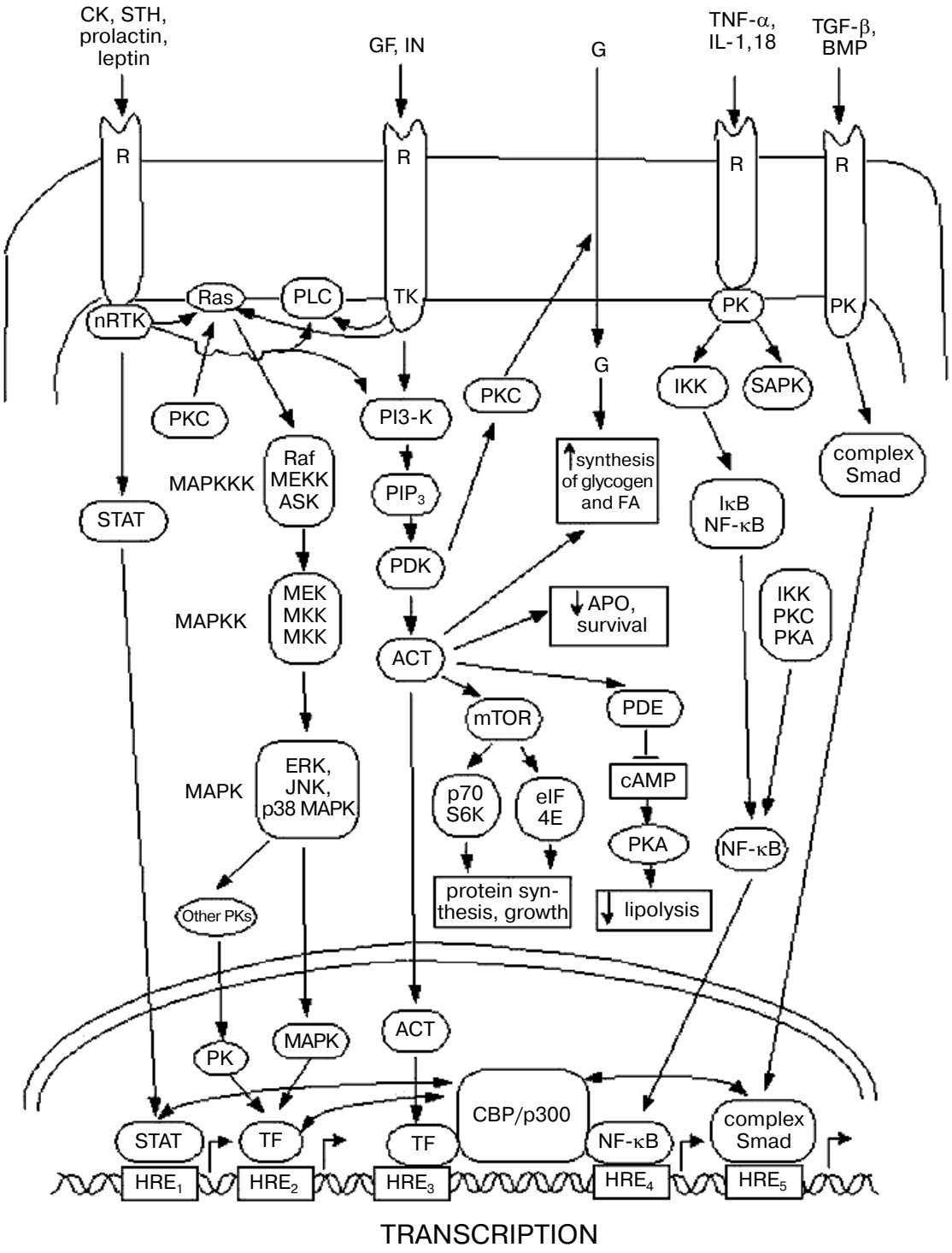

Fig. 1. Kinase signal transduction systems without second messengers. Abbreviations: CK) cytokines; STH) somatotropic hormone; GF) growth factors; IN) insulin; R) receptor; nRTK) non-receptor tyrosine kinase; Ras) small G-protein Ras; PLC) phospholipase C; TK) tyrosine kinase; PKC) protein kinase C; STAT) signal-transducers and activators of transcription; MAPKKK) MAPK kinase kinase; MAPKK) MAPK kinase (common terms for three cascades); Raf, MEKK, and ASK) ERK, JNK, and p38 MAPK kinase kinases; MEK, MKK, and MKK) ERK, JNK, and p38 MAPK kinases; PI3-K) phosphatidylinositol-3 kinase; PIP_3) phosphatidylinositol-3,4,5-trisphosphate; PDK) PIP_3-dependent kinase; ACT) protein kinase B; mTOR) mammal target for rapamycin (PK); p70S6K) 70 kD S6-ribosomal kinase; eIF4E) eucaryotic initiation factor 4E; G) glucose; FA) fatty acids; APO) apoptosis; PDE) cAMP phosphodiesterase; PKA) protein kinase A; TNF) tumor necrosis factor; IL) interleukin; PK) protein kinase; IKK) IκB-kinase; IκB) NF-κB inhibitor; NF-κB) nuclear factor kappa-B; SAPK) stress-activated protein kinase; TGF) transforming growth factor; BMP) bone morphogenetic proteins; Smad) TFs transducing signals of TGF family; TF) transcription factor; HRE) hormone-reactive (responding) factor; →, activation; —|, inhibition; ↔, interaction; ↑, increase; ↓, decrease; ┌→, transcription.

PKs (such as ribosomal S6-kinase p90, whose substrate is the small subunit protein S6) stimulate protein synthesis. Transcription of cyclin D1 also increases, and its complex formation with Cdk4 (cyclin-dependent PK4) is facilitated, thus stimulating cell growth. This results in enhancement of proliferation, differentiation, embryogenesis, morphogenesis (including angiogenesis and anaplerosis), and survival of all cells, plus synaptic plasticity, behavior, learning, and memory in the brain. The system is activated not only by GF, insulin, and mitogens, but also by differentiation factors, STH, GPCR hormones-stimulators, and cell–cell and cell–matrix interactions [15, 16, 19].

The SAPK1/JNK-cascade (Jun-N-terminal kinase) consists correspondingly of MEKK1/4 (or TGF-β-activated kinase, TAK) → MKK (SEK) 4/7→ JNK (SAPK1) and acts in the same way: JNK phosphorylates and activates TF c-Jun (AP-1 component) in the nucleus. The cascade is activated by inflammatory cytokines (TNF-α, IL-1, and IL-18), pro-apoptotic Fas-ligand, GFs, GPCR hormones-stimulators, cellular (via small GTPases Rho) and oxidative stress, γ and UV irradiation, ischemia, toxic compounds, and bacterial infection and induces both inflammation and apoptosis, as well as cellular growth and differentiation [2, 8, 16, 20]. JNK is a potent effector of neuronal apoptosis or degeneration [21].

The p38 MAPK-cascade consisting of the apoptotic signal-regulating kinase-1 (ASK1) or TAK → MKK3/6 → p38 MAPK (38-kD MAPK, SAPK2) is activated by inflammatory cytokines, Fas-ligand, stress, and UV irradiation. This cascade is functionally close to the previously described one. It stimulates inflammatory cytokine translation, leukocyte migration, activation and degranulation of inflammatory cells, facilitates apoptosis, etc. [2, 8, 16, 22, 23].

In general, the MAPK/ERK-cascade functions in normal situations; it is stimulated chiefly by mitogens, insulin, and insulin-like and platelet GF and fulfills basically physiological functions. Two other cascades preferentially function in pathological conditions, weakly react to mitogens, but define reactions to harmful and toxic influences, stress, ischemia, infection, cytokines, and other inflammatory agents. Nuclear translocation of all MAPKs only occurs under prolonged activation of the system and determines genome effects including long-term and stable adaptation and morphogenesis. All these systems are necessary for survival: knockout of their genes induces embryonal or early postnatal death [15]. The necessity of intrasystem contra-regulations is obvious. Unlike c-Jun, TF JunB does not enhance but decreases proliferation. However, PPs and dual specificity PPs play the main role. They decrease both extent of phosphorylation and activity of MAPK-cascades even in the continuous presence of activating stimuli [24].

The role of these cascades is great *in pathology* as well. Excessive activity of ERK results in myocardial hypertrophy, and that of JNK/SAPK in more hazardous dilatational cardiomyopathy [15], and it plays a key role in inflammation [20]. SAPK-cascades participate in chronic inflammation pathogenesis, stroke, exhaustion in diabetes, and in side effects of cancer chemotherapy [25]; they are important in development of inflammatory-induced syndrome of multiple organ dysfunction [26]. p38 MAPK inhibitor is effective in arthritis, septic shock, and myocardial damage; it reduces chemotaxis in monocytes [27]. JNK inhibitors may be useful in inflammatory, vascular, neurodegenerative, and metabolic diseases and cancer. The lethal factor of anthrax toxin selectively inhibits MEK activity. New ERKs 5/7 activate matrix metal-dependent proteinases and facilitate invasiveness in de-differentiated cells. MEK-ERK 1/2 activity increases in many cancers and human leukemia [15].

The PI3-K–ACT cascade discovered in 1991-95 is the third STS transmitting signals from RTK into the cell (see Fig. 1). PI3-K is a very important PM lipid kinase activated by RTK, nRTK, G-protein βγ-dimer, B- and T-cell receptors, and integrins. It catalyses the reaction:

$$PIP_2 + ATP \rightarrow PIP_3 + ADP.$$

In this reaction, the fourth phosphate is incorporated at position 3 of the inositol ring. PI3-K is activated by Ras as well. PIP_3 is a membrane second messenger. It recruits, binds, and activates 3-phosphatidylinositol-dependent PK1 (PDK1), which phosphorylates and activates ACT (PKB) and PKA, PKG, atypical PKC isoenzymes, and Sgk (see section 6). ACT modulates activities of a variety of its substrates/effectors by phosphorylation. PDK1 and ACT are serine/threonine PKs. The general sequence of events for the system is as follows [2, 8, 28, 29]:

$$PI3\text{-}K \rightarrow PIP_3 \rightarrow PDK1 \rightarrow ACT\ (PKB) \rightarrow \rightarrow effector\ proteins.$$

This results in *protein synthesis and cell growth stimulation* via the key translation activators: two PKs (mTOR and ribosomal p70 S6K) and initiation translation factor 4E [29, 30]. ATP deficit results in inhibition of both ACT and mTOR via AMP-kinase and lessening of protein synthesis, thus decreasing ATP expenses. This system is regarded as the main regulator of cell growth and size. It accelerates the cell cycle and proliferation, augments viability, prevents apoptosis (by inhibition of its activators BAD, p53, etc.), stimulates $NO^{\cdot}$ synthesis in endothelium, and influences cell migration and establishment and maintenance of cell polarity [2, 29]. Excessive activity of hormones activating this STS is prevented by PP2A, PP2B (calcineurin), PP MAPK, and the main negative regulator of the system PTEN (PP of chromosome 10) inhibiting PIP_3 by phosphate cleavage [31, 32]. However, PPs stimulate some proteins: CD45 activates nRTK SRC, and calcineurin activates NFAT [8].

RTK is a receptor for **insulin**; it involves coupled intracellular STS and stimulates cellular proliferation and differentiation via insulin receptor substrate (IRS) proteins. This is preferentially realized through the MAPK/ERK cascade [2, 9]. However, unlike insulin-like GF-1, insulin also possesses a unique and potent effect on all metabolism types. These effects are generally realized through the PI3-K–ACT system. Recent data suggest the following mechanism. PDK 1/2 activates atypical PKC isoenzymes, which induce translocation of the transporter protein GLUT-4 from cytoplasm into the PM, wherein it provides glucose influx into the cell. ACT inhibits glycogen synthase kinase (GSK-3), thus deinhibiting glycogen synthase and ATP-citrate lyase and enhancing both glycogen and fatty acid synthesis. The ACT system increases both protein synthesis and cell growth and decreases apoptosis [2, 33]. ACT activates phosphodiesterase 3B as well, thus decreasing cAMP concentration and PKA functioning and, as a result, inhibiting lipolysis [2]. Genomic effects of GFs and insulin realized via Ras–MAPK and PI3-K–ACT systems are rather similar (insulin is now often called a GF), but insulin regulates expression of a large number (more than 150) of genes [34]. Note, IRSs participate in effects of STH, leptin, and some ILs as well [9]. ACT and AMP-kinase enhance, and PKC, mTOR, and GSK-3 decrease IRS phosphorylation, that is, positive and negative feedback loops are involved [35]. Suppressors of cytokine signaling (SOCS) inhibit effects of insulin and insulin-like GF [35, 36].

Some *medical aspects* have already been developed [2, 8]. This system is important for immune responses and functioning of professional phagocytes [37]. In malignant tumors both mutations activating PKs involved in the system, with constitutive activation of these PK, and mutations inhibiting PPs, particularly PTEN, increasing PIP_3 concentration and activity of the same PKs are observed [32, 38]. Rapamycin (mTOR inhibitor) and cyclosporin A (calcineurin inhibitor) are immunosuppressors. Insulin resistance in diabetes type II is associated not only with decrease in receptor amounts, but also with decrease in functioning of the PI3-K–ACT system, whereas the Ras–MAPK system maintains its activity [39].

The structure of this STS is notable: binding of hormones with RTK can result in *parallel or preferential actuation of three independent modules (blocks)*: C-γ phospholipase, Ras–MAPK, and PI3-K–ACT. Metabolic effects of insulin are more pronounced than that of GFs: insulin realizes relatively specific actuation of one of the modules, namely PI3-K. This is probably associated with the inhibition of the Raf–ERK cascade by ACT [2]. *A module principle of the structure* is applied in other STS as well: nRTK and GPCR also actuate the Ras–MAPK and PI3-K–ACT systems; cAMP–PKA and Ca^{2+}–CM-PK independently activate TF CREB; phospholipase C (albeit different isoenzymes) is involved in the PI system as well as in RTK. This provides diversity and flexibility of STS (see section 7).

4.3. System with non-receptor (intracellular) tyrosine kinases (nRTK). This is a phylogenetically ancient STS (it also arose in unicellular eukaryotes) (see Fig. 1), which can be called a system with TK-coupled receptors as well, on the analogy of GPCR (No. 3A in Table 1 of Part I), and is the main STS for cytokines. These receptors are usually called cytokine receptors [10], but circulating hormones, such as STH, prolactin, leptin, and erythropoietin, also act via these receptors. The STS consists of: 1) receptor on the outer surface of the PM with one transmembrane domain; 2) nRTK with intracellular localization (in cytosol or anchored on the inner PM side); 3) latent cytoplasmic TF. STAT proteins are the TF for the *hematopoietic* family, the largest cytokine family. Hormone binding with receptors induces their dimerization, nRTK recruiting and activation, receptor phosphorylation by nRTK, and binding of STAT proteins with subsequent phosphorylation by the same nRTK. Dimer of phosphorylated STAT dissociates from the receptors and translocates into the cell nucleus (via diffusion through nuclear pores) in which it docks onto a regulatory DNA site (5′-$TTN_{5\text{-}6}$AA-3′ [40]) and interacts with co-integrators CBP and p300 (see section 6), thus inducing gene transcription [2, 8, 41, 42].

The system includes eight nRTK families: JAK (Janus-TK) transmit signals from STH, prolactin, leptin, erythropoietin, interferons, interleukins and other cytokines important for cell growth and differentiation, angiogenesis, hematopoiesis, survival, early adaptation, and inflammatory and immune responses; SRC-kinases, which are functionally similar to JAK, participate in cell growth regulation, hematopoiesis, and B- and T-lymphocyte functioning; FPS participate in differentiation, ABL are involved in growth inhibition, and SYK in hematopoiesis and immunoreception in B- and T-lymphocytes and natural killers. JAK knockout experiments have demonstrated their vital necessity. Seven STATs have been found already, and distinct specialization is typical for them as well: for instance, STAT-1 transmits signals from α- and γ-interferons and ILs; STAT-3 from leptin, ciliary neurotrophic factor (CNTF), IL-6, -10, -11, and G-CSF; STAT-5 from STH, prolactin, erythropoietin, GM-CSF, IL-2, -3, -7, -9; and STAT-6 from IL-4 and -13 [2, 8, 43].

Main features of this mechanism: 1) necessity of phosphorylation of receptors for their activation; 2) direct and rapid (minutes) transduction of hormone signal from receptors in PM into the cell nucleus, directly to the genes; 3) realization of these events by activation, phosphorylation, and translocation into the nucleus of initially latent cytosolic TF STATs. nRTKs transmit signals of hormones (cytokines, STH, leptin) through other important STS as well: Ras–MAPK and PI3-K–ACT, which, besides their direct action, phosphorylate and activate STAT in turn [42].

Intrasystemic negative feedbacks include ubiquitin–proteasomal degradation of STATs, their deactivation by PP, action of protein inhibitor of activated STATs (PIAS), and induction of suppressors of cytokine signaling (SOCS) by cytokines and other hormones. SOCS bind and inhibit JAK, interfere with the interaction between CK and receptors and, as a result, decrease effects of cytokines, erythropoietin, STH, leptin, and insulin [35, 44, 45]. SOCS deficiency leads to exacerbation, aggravation, or transition to chronic form of inflammation, and complete knockout of the SOCS gene is lethal [46].

Hormones of this group regulate not only inflammatory and immune reactions. This is well known for STH, prolactin, and leptin. However, metabolic and other effects are typical for cytokines as well, especially for IL-6 family: IL-6 and -11, cardiotrophin-1, CNTF, etc. They induce acute phase reactions and influence hematopoiesis, regeneration of hepatocytes and neurons, and fertility [2].

Insufficiency of this system leads to defects of listed functions, for instance, immunodeficiency and agammaglobulinemia, and their *redundancy* tends to result in immune and inflammatory diseases including severe chronic processes (rheumatoid arthritis, colon inflammation, glomerulonephritis, allergic inflammation), osteoporosis, and multiple sclerosis. nRTK and STAT are activated in many malignant processes including various leukemia and colorectal cancers [2, 8, 47]. Due to the tight correlation of signaling pathways of leptin and insulin, resistance to both is typical for obesity [44]. Constitutively active nRTK BCR-ABL, which is encoded by a chimeric gene resulting from conjugation of chromosomes 9 and 22, eventuate to the development of chronic myeloid leukemia [2]. Novel cytokine-suppressant anti-inflammatory drugs (CSAID) are currently being developed. The blocker of STH receptors pegvisomant (Somavert) avails in acromegaly [48].

5. PROTEIN KINASE SIGNAL TRANSDUCING SYSTEMS WITHOUT SECOND MESSENGERS

These systems mediate effects of noticeably less amount of hormones. Unlike the preceding ones, they use serine/threonine PKs, but in the same two variants: either as protein kinase receptors or as intracellular enzymes coupled with independent receptor.

5.1. System with receptor protein kinases (RPK). This STS realizes effects of hormones belonging to the **transforming GF-β (TGF-β) superfamily.** It uses two transmembrane receptors (RPKs). Their extracellular domain binds hormones, and the intracellular one acts as serine/threonine PK. A number of latent cytosolic TFs *Smad* are involved in this system as well. Endoglin (CD105 protein) is a co-receptor of TGF-β [49]. Formation of hormone–receptor complex induces dimerization of the main receptors and phosphorylation of one receptor by another with following phosphorylation of receptor-regulated R-Smad by RPK (see Fig. 1). Common Co-Smad4 binds the phosphorylated R-Smad, and this complex (with R-Smad2/3 for TGF-β and activin and with R-Smad1/5/8 for bone morphogenetic protein) is translocated into the cell nucleus, in which it interacts with a regulatory DNA site and, in participation of the co-integrator CBP/p300, modulates genome processes. Binding to α_2-macroglobulin of blood plasma and inhibitory I-Smad6/7 and SANE proteins (Smad antagonistic effector) and SNIP1 (nuclear protein interacting with Smad) prevent excessive activity of the system. SNIP1 inhibits CBP/p300 proteins. The described STS is a simple and rapid (minutes) route of signal transduction from hormones of PM into the nucleus via activation of latent cytoplasmic TF Smad [2, 50-53]. The TGF-β family activates TAK and further activates other STS: JNK, p38 MAPK, and PI3-K–ACT [2, 50, 51].

TGF-β is a pleiotropic hormone with a key role in tissue morphogenesis and growth, particularly in conjunctive tissue including bones, and in reproductive organs as well. It stimulates embryogenesis, fibroblast proliferation, angiogenesis, protein synthesis and remodeling of intercellular matrix, and anaplerosis by inhibition of MAPK-ERK cascade, intensely retards division of epithelial and other cells including hematopoietic ones [49, 54, 55], and decreases inflammation and immune reactions. TGF-β overexpression induces fibrosis [55]. It retards early-stage growth of cancer cells, but later they become resistant and synthesize it themselves, because TGF-β stimulates malignant angiogenesis and suppresses immune reactions [56]. Inactivating mutations of some Smad facilitate carcinogenesis [52, 57] and impair anaplerosis and bone reparation [49, 55, 58].

Bone morphogenetic proteins (BMP) are necessary for embryogenesis of various tissues, tissue homeostasis, osteogenesis, and bone reparation and remodeling [53]. BMP and TGF-β induce expression of Id, inhibitors of differentiation and stimulators of proliferation, which play an important role in angio-, neuro-, and osteogenesis [53, 59]. Anti-Müllerian hormone induces male type differentiation in embryogenesis.

5.2. System with non-receptor protein kinases (nRPK). IL-1 and especially **TNF cytokine families** induce immunity development, hemopoiesis, and morphogenesis, and in pathology immune reactions, inflammation including severe chronic diseases, septic shock, neurodegeneration, bone resorption, cachexia, and apoptosis [60]. Antagonists of these cytokines are already employed successfully in therapy [61, 62]. Their STS are different from the hematopoietic one in the participation of serine/threonine PKs rather than TKs (see Fig. 1). The *TNF family* (TNF-α, Fas-ligand) receptors use cytoplasmic ASK-1 (see section 4.2) and NF-κB-inducing kinase

(NIK). The first activates SAPK/JNK system, and the second activates TF NF-κB. *Receptors for IL-1* and *-18* act via a kinase associated with IL-1 receptor (IRAK), and then through the same systems NF-κB and SAPK [2, 8, 63]. Both cytokine families activate apoptosis as well through proteins with death domains and caspases. IL-1 and TNF activate all three MAPK-cascades as well, especially JNK and p38 MAPK [9, 64]. Some ILs, chemokines, GFs, and circulating hormones (STH, angiotensin II, platelet activation factor, NGF, etc.), ceramide, PKC activators (phorbol esters), antigens, viruses, and oxidants also actuate the NF-κB system [65].

TF NF-κB is a dimeric protein, but in cytosol it is bound to IκB inhibitor, and so it is in latent ("dormant") state. A number of PKs (NIK, MEKK, and probably PKC and ACT) phosphorylate and activate specific IκB-kinase (IKK), which in turn phosphorylates IκB resulting in its dissociation and rapid (minutes) decomposition by proteasomes. The released NF-κB dimer is phosphorylated and activated by a number of PKs (IKK, NF-κB-activating kinase, atypical PKC, PKA, and GSK-3) resulting in its translocation into the nucleus, interaction with co-integrators CBP/p300 and DNA HRE, and stimulation of transcription of 300 genes [9, 65-67]. Note that NF-κB activation is realized by two-step phosphorylation: it leads to removal of bound IκB inhibitor first, and then to direct activation of NF-κB itself.

NF-κB induces inflammatory cytokines and enzymes, chemokines, adhesion molecules, and acute phase proteins. It is an immediate early *mediator of inflammation*, innate and acquired *immune reactions*, *development of viral infection*, *ischemia–reperfusion*, *and adaptation* and important factor of *survival* and inhibition of apoptosis, which participates in terminal differentiation and proliferation [64-66, 68]. Excessive reaction of NF-κB is usually limited by its induction of IκB and by natural antioxidants (glutathione system and vitamins). However, since primary inflammation induces NF-κB, which facilitates secondary inflammation development, a vicious circle can arise. In acute inflammation, this leads to local complications (ulceration and bone resorption) and septic shock, and in the chronic case to severe and progressive forms of asthma, rheumatic arthritis, intestinal inflammatory disease, and chronic cardiac failure. Knockout of genes encoding various NF-κB subunits results in either immunodeficiency and infection processes or perinatal death [65, 66]. Inhibition of the NF-κB system plays an important role in the therapeutic effect of glucocorticoids and a series of non-steroid anti-inflammatory agents [69, 70]. Carcinogens activate NF-κB, and in some tumors it is constitutionally active [71].

5.3. Summary on kinase systems without second messengers. Thus, receptors of various *cytokine families* (sections 4.3 and 5.2) are devoid of inner kinase activity and, hence, for realization of hormone activity they need to *recruit various cytoplasmic protein kinases*: *nRTK* for most cytokines (hemopoietic family) and serine/threonine *nRPK* for TNF and IL-1 families. Signal transduction *into the nucleus* is mainly (most often) realized by *phosphorylation*, activation, and subsequent *translocation* of initially latent *cytosolic TFs*: STAT and NF-κB, respectively. In contrast, *GF receptors* (sections 4.2 and 5.1) are *receptor kinases* (kinase receptors): TKs for most GFs and serine/threonine PKs for TGF family. Signals of the latter are also transduced into the nucleus by initially latent cytosolic TF Smad, after their phosphorylation, but *effects of most GFs are realized differently—via translocation into the nucleus* of the latter *PKs* in serine/threonine protein kinase cascade and *phosphorylation of resident nuclear TF* type AP-1. The effect of many TFs is associated with co-integrators CBP and p300. Moreover, *phosphorylation* of *key translation proteins*, namely initiation factor eIF4E and ribosomal protein S6, occurs in cytosol.

This enables formulation of the following generalizations: 1) *primary perception of hormone signal* by the systems without second messengers in most cases *requires TK involvement*, and serine/threonine PKs much more rarely fulfill this function; 2) *not only TKs*, *but also consecutive cascade of serine-threonine PKs participates* in signal transduction; 3) *GFs and cytokines differ* from each other by main (most often used) mechanisms of their effect on the cell; 4) *two mechanisms of hormone signal transduction* from cytoplasm directly into the nucleus are realized: *translocation of hyaloplasmic TFs* (STAT, Smad, and NF-κB) which are *already activated by phosphorylation* and *translocation of serine/threonine PKs themselves with the activation via phosphorylation of resident nuclear TFs* (CREB, AP-1, c-Fos, c-Myc, etc.). The third conclusion is substantially limited herein by the fact that both GFs and cytokines share the main STS of each other (see section 7). Decrease in signal transduction is often met as well: JNK decreases IRS activity and stimulates Smad, and ERK inhibits the latter; STAT and Smad mutually inhibit each other.

6. SIGNAL TRANSDUCTION SYSTEMS WITH INTRACELLULAR RECEPTORS

The action of lipophilic hormones (as well as all others) begins from binding to their *receptors*. These are proteins composed of 500-1000 aminoacyl residues with three important functional domains: hormone- and DNA-binding and modulatory (activating) ones. The second domains are highly homologous, whereas others are substantially different. Most steroid hormones have only one receptor type, whereas estrogens and iodothyronines have two receptor types. In spite of different initial localization of receptors for steroid (cytosol) and other lipophilic hormones (nucleus), all these receptors are called *nuclear* and are affiliated to a large superfamily of *nuclear receptors*. In humans, this family is represented

by 48-49 receptors (including isoforms), and only one-third of these are receptors for lipophilic hormones [2, 8, 72].

Lipophilicity of hormones facilitates their easy penetration across hydrophobic proteolipid PM. Hence, their receptors are located inside the cell, in cytosol, or directly in the nucleus. They are associated with other proteins. Binding of hormone entering the cell with its receptor induces dissociation of these proteins, and the *active hormone–receptor complex* can further join in the nucleus to the distinct *HRE*—a regulatory DNA element which is functionally analogous to a transcription enhancer or silencer, but participates in higher-level (i.e., hormonal) regulation. Genes regulated by various hormones usually have several HREs. Together with enhancers and silencers, the overall extension of regulatory DNA sites several (up to 5-10) times exceed that of encoding ones. Note that the abundance of regulatory DNA sites in eukaryotic genes has given birth to the term "gene brain", and the gene itself has deserved the name "smart gene" [73]. However, regulatory sites of genes comprise only a minor part of the genome.

It was recently revealed that regulation of transcription is considerably more complex. TFs do not act solely, but they are "gathered" by a number of other regulatory proteins—*co-activators* and *co-repressors* recruited into this complex. The primary co-regulators are mainly the proteins p160. Particularly, co-activators of steroid receptors (SRC), modulating proteins of thyroid and vitamin D receptors (TRAP/DRIP), nuclear co-repressor (NCoR), RIP140 (receptor interacting protein), and silence mediator of retinoid and iodothyronine receptors (SMRT) are most important. Such a composite complex of regulatory DNA elements and protein factors (TFs, co-regulators) is called *hormone-reactive unit (HRU)*. Universal proteins CREB-binding protein (CBP, called by the name of its first recognized partner, CREB) and p300 protein interact with this unit. The complex of hormone, receptor, co-activators, and co-integrators interacts with basal transcriptional machine, thus making it effector of hormonal signals. *Co-integrators (integrating co-activators)* transmit to the latter the signals of both intracellular and extracellular regulators, such as classic TFs and many TF-mediating hormone effects. The complexes of lipophilic hormones with their receptors represent the ligand-bound TFs [2, 72-77] (see Fig. 1 in Part I and Fig. 4 in [78]).

Another important mechanism of these co-integrators is enzymatic. *Histone acetylation* is very important for transcription. In non-activated chromatin, N-terminal lysine residues of histones are free and positively charged, and therefore they are firmly bonded to phosphate "backbone" of DNA. CBP/p300 co-integrators and some co-activators possess internal activity of histone acetyltransferase (HAT); other HATs are also recruited into the complex. Lipophilic hormones induce HAT. Phosphoacetylation (acetylation and phosphorylation) of histones remodels chromatin, releases the template, and makes it active and ready for transcription. Methyl transferases PMRT1 and CARM1 participate in histone remodeling. TFs are acetylated as well [19, 72, 75-77].

There are substantial differences between two groups of lipophilic hormones in subcellular localization of their inactive receptor complexes, character of proteins associated with the receptor, and HRE structure. *Steroid hormone receptors* are localized in cytoplasm in the form of inactive associates with chaperones; after dissociation, the hormone–receptor complex relocates into the nucleus and interacts with HRE. Phosphorylation by MAPK, ACT, and CM-PK is important for the receptor functioning: it results in increased activity of estrogen receptors and decreased activity of glucocorticoid ones [2, 72]. *Receptors for iodothyronine and active forms of vitamins D and A (calcitriol and retinoic acid)* are always localized inside the cell nucleus (nuclear resident receptors) in association with *co-repressors* (inhibitory proteins). These associates are not only passive, but also inhibit transcriptional activity in the absence of hormones. Histone deacetylase activity (HDAC) of repressors and HDAC recruited by these repressors facilitate this process resulting in shielding of matrix by histones; it becomes inaccessible for transcription. Free hormone must penetrate not only the cell, but the nucleus as well [72-74, 79-81]. After dissociation of co-repressors, the hormone–receptor complex interacts with corresponding HREs of DNA. All of them consist of 15 nucleotides with two inverted or direct repeats of six nucleotides each. In most steroid hormones HRE contain the sequence 5′-AGAACANNNTGTTCT-3′, in estrogens 5′-AGGTCANNNTGACCT-3′, in other hormones 5′-AGGTCANNNAGGTCA-3′ [40, 73].

It should be particularly emphasized that co-regulators and co-integrators play an active role: they not only inhibit or activate, but modify hormone action. So, the effect of lipophilic hormones can differ in different cells and organs. This is one of the mechanisms providing tissue specificity of hormones. It has allowed development of synthetic selective modulators of estrogen receptors (SERM), which act as antagonists in mammary glands and agonists in bones and the cardiovascular system [82]. Mutations in co-regulators of female sex hormones or their changed expression induce deviations from normal receptor functioning and can participate in breast cancer progression [83].

The described sequence of events explains in general the regulation of transcription by lipophilic hormones. They alter synthesis of nucleic acids and proteins, proliferation, and other genomic processes. Alterations in pregnancy represent a typical example. Enormous quantities of estrogens synthesized by the placenta, the largest endocrine gland, via the described mechanism induce protein synthesis in the uterus (whose mass increases from 50 g to 1.1 kg) and its remodeling with significant

improvement of contractile properties. At the time of delivery the placenta is removed from the body, the amount of estrogens drops, and the mass of the uterus gradually decreases almost to the pre-pregnancy level, and its properties approach the initial state. Similar alterations occur in mammary glands as well, preparing them for lactation and then to regression.

Steroid hormone and iodothyronine receptors are found in mitochondria as well. Hormone–receptor complexes increase via their HRE the transcription of respiratory chain enzymes encoded by nuclear and mitochondrial genes and the synthesis of these enzymes, and if necessary, also mitochondriogenesis [84, 85].

Estrogens act via their two receptors, ER_α and ER_β. The former dominate in classic targets: uterus, placenta, liver, cardiovascular system, and bones, whereas the latter dominate in prostate, testes, ovaries, endocrine glands, derma, lymphoid, and erythroid tissue; they are differently distributed in the brain [86]. Of these two iodothyronine receptor types TR_α are more important: their knockout induces progressive hypothyroidism, growth retardation, and death in the first 5 weeks, whereas TR_β control regulation of TTH secretion, and their inactivation only results in hyperthyroxinemia and difficulty in hearing [74]. Retinoids act via two main receptor types, RAR and RXR, and each has three subtypes—α, β, and γ. All-*trans*-retinoic acid is a selective agonist for RAR, and the 9-*cis*-isomer is active against both RAR and RXR. Retinoids are necessary for differentiation of cells, particularly embryonic, hematopoietic, and epithelial, and they facilitate regeneration [87]. Moreover, nuclear receptors for triiodothyronine and calcitriol form active dimers with RXR [88]. Calcitriol decreases proliferation via its receptors, increases cell differentiation, and supports immunity [89].

A number of drugs active towards these STS are widely used *in medicine*. Synthetic glucocorticoids inhibit immunity and proliferation, and most importantly, they are very potent anti-inflammatory agents. This effect is due to both inhibition of enzymes synthesizing eicosanoids, cytokines, and adhesion molecules and induction of anti-inflammatory TFs (NF-κB, AP-1, and STAT), and also due to the competition of steroid–receptor complexes with TFs for co-activators and co-integrators of type p300 [90, 91]. However, few antagonists of lipophilic hormone receptors are known so far: spironolactone, cyproterone, and flutamide and its analogs. Then mifepristone appeared, which is an antagonist of progesterone, glucocorticoids, and androgens [8, 92]. It is used for prevention of pregnancy, induction of abortion, and stimulation of uterine contraction, but no selective antagonists for these receptors is known. Real progress has only begun recently. The best of already mentioned SERMs (raloxifene, arzoxifene) are effective in menopause to prevent fractures and cardiovascular pathology, and in recent time for prophylactic and treatment of estrogen-dependent breast cancer [82, 93]. Fulvestrant (Faslodex), a complete and "pure" blocker of estradiol receptors, which also accelerates their degradation, proved to be a valuable drug in therapy of this cancer type [94]. A selective blocker of aldosterone receptors, eplerenone, has been introduced in medical practice [95], and the first potent antagonist of iodothyronine receptors, NH-3, has appeared [96]. Retinoids are effective in acute promyelocytic leukemia, prostate cancer, and psoriasis [97, 98]. Calcitriol analogs possess anti-inflammatory (calcitriol and OCT are used against psoriasis), immunosuppressor, and anticancer properties [89].

However, lipophilic hormones exhibit not only late genomic effects developing usually from 30-60 min to hours (or even days), but also short-term (they are often called fast) effects developing in seconds or several minutes (less than 10): aldosterone effect on lymphocytes or vessels, glucocorticoids and neurosteroids on neurons, progesterone on ovaries and spermatozoids, estrogens on vessels and brain functions (emotional responses, cognition, neuroprotective action), calcitriol on epithelium [100-104]. Similar data have been obtained for iodothyronines and retinoids [104].

7. TRANSACTIVATION OF SIGNAL TRANSDUCTION SYSTEMS

Initially views on mechanisms of hormone action were close to the opinion that a distinct STS type always corresponds to each hormone type: systems with ionotropic (channel) receptors to neurotransmitters; systems with GPCR and second messengers to most circulating hormones and neuromodulators; systems with RTK–MAPK to GFs; systems with nRTK–STAT to cytokines; intracellular receptors (ligand-dependent TFs) to lipophilic hormones. A simple and clear pattern of hormonal signal transduction has been seemingly achieved in the second half of 1990s. The existence of systems with serine/threonine PKs without second messengers seemed to be a not very serious exclusion because they belonged to two limited groups of hormones. However, new facts gradually accumulated disrupting the rank, and then demanding to reject the paradigm on exclusivity of STS for each class of hormones (Fig. 2). This problem has become one of most fervent in the field of mechanisms of hormonal action. The switch of hormone signal transduction from one STS to another is called here **STS transactivation**, although this term is often used in narrowed situations (particularly for signal transduction from GPCR to the system of RTK–MAPK-cascades). The possible term "mutual switching" is not used.

It is now recognized that *RTK* transduce signals of GFs and insulin not only via MAPK, but also via phospholipase C (Ca^{2+} plays an important role in actualization of GF effects [105]), PI3-K–ACT system (this pathway is

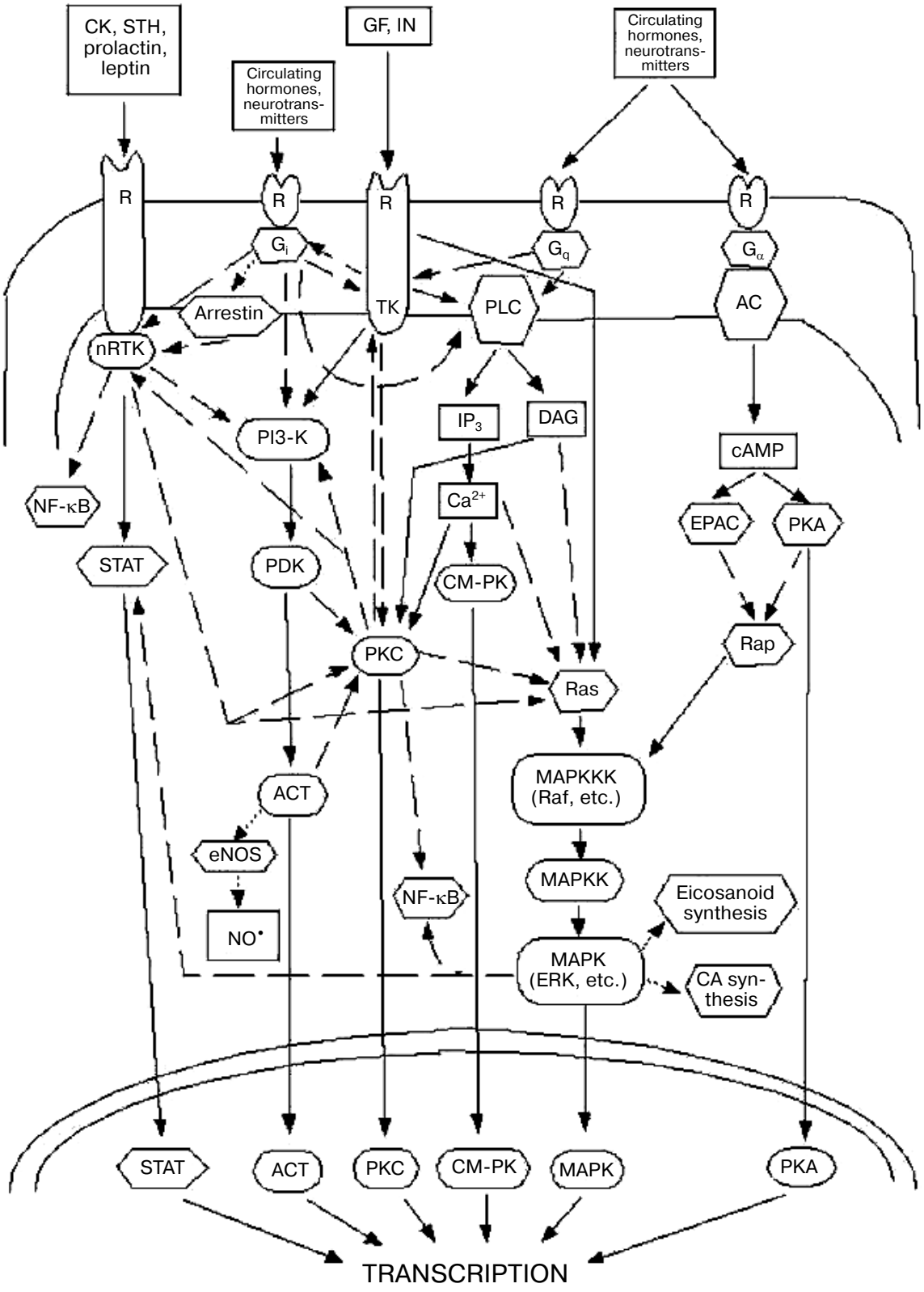

Fig. 2. Transactivation of the most important signal transduction systems. Abbreviations: eNOS) endothelial NO•-synthase; DAG) diacylglycerol; IP_3) inositol trisphosphate; CM-PK) calmodulin dependent PK; EPAC) exchangeable protein directly activated by cAMP; CA) catecholamines; ⟶, activation; ⇢, transactivation. For other abbreviations, see the first page of this manuscript and Fig. 1.

most important for insulin), and NF-κB. All these cascades of PK activate STAT [2, 17]. *nRTK* transduce the signals of cytokines, STH, prolactin, and leptin not only via STAT, but also via STS Ras–MAPK, PI3-K–ACT, TF NF-κB, and iNOS–NO˙ [2, 8, 106-108]. Suppressors of cytokine signals inhibit effects not only of cytokines, STH, and leptin, but also of insulin [35]. Hence, the mechanisms of GF, cytokines, and some hormones became strongly and mutually drifted together: in fact, STS with *RTK and nRTK transactivate each other*, *and they both activate NF-κB*, transducing the signals of nRPK.

Chemokines via GPCR, $G_{\alpha i}$, and $G_{\beta\gamma}$ activate phospholipase C, MAPK, PI3-K, JAK-STAT, and NF-κB, that is, all the main mechanisms realizing effects of RTK, nRTK, and nRPK [27, 109]. *GPCR activation* by circulating hormones and neurotransmitters of various chemical structure induces not only modulation of second messenger formation, but also transactivation of both RTK with subsequent involvement of MAPK, PI3-K, and NF-κB pathways and nRTK (SRC, JAK) [108, 110-113], and MAPK activation via cAMP–PKA–Rap–Ras pathways [2, 114] or directly cAMP–PKA–MAPK [115]. The indicated STS are recruited by subunits of G-proteins $G_{\alpha s}$, $G_{\alpha i}$, and $G_{\alpha q}$, $G_{\beta\gamma}$ dimer, Ca^{2+} both through second messengers – PKA, PKC, and CM-PK, as well as through direct action of $G_{\alpha i}$ and $G_{\alpha q}$ on nRTK SCR. Not only GPCR, but also *RTK and nRTK* activate PKC, and this transactivates other STS. Moreover, *receptors with 7-transmembrane domains* transduce hormonal signals without G-proteins as well: β-arrestins not only inhibit receptors, but also activate Ras–ERK cascade; receptors 5-HT_{2A} and AT_1 directly activate JAK–STAT. This fact even led to a supposition to reject the term "GPCR" pro "7-transmembrane receptors" [116]. The surface HIV protein gp120 induces Ca^{2+} accumulation, and the latter activates MAPK [117]. Angiotensin II, cAMP, and PKC induce translocation of fibroblast GF-2 and its receptor-1 into the nucleus, induction and phosphorylation of CREB and CBP, and transactivation of CRE, thereby stimulating tyrosine hydroxylase transcription and differentiation and neuronal plasticity [118]. There is now no doubt that a number of *circulating hormones and neurotransmitters* activating receptors with 7-transmembrane domains exert effects on biochemical as well as on genome levels: they stimulate gene expression, proliferation, differentiation, cell growth, myocardium hypertrophy, thickening of vessel walls, inflammation, and malignant transformation.

On the other side, the *MAPK* system activates CREB and key enzymes of catecholamine synthesis (tyrosine hydroxylase) and eicosanoids (phospholipase A_2), the hormones acting via GPCR [2]. *Insulin and insulin-like GF* via RTK activate the pathway G_i-protein–PI3-K–PKC–cAMP system [119]. Insulin via PI3-K increases endothelial NO˙ production and blood flow in parallel with glucose inflow and metabolism [120]. Vessel complications in diabetes are probably associated with the weakness of this effect of insulin.

The TGF-β–RPK system induces Ca^{2+}-signal [121], stimulates PKC [122, 123] either directly or via activation of phospholipase A_2 → increase of prostaglandin E_2 → GPCR stimulation [122]; MAPK p38 and JNK are activated as well [2, 123]. Bone morphogenetic protein stimulates the PI3-K–ACT system [124]. Antagonism between TF Smad and STAT is known [125]. Data on interaction of TGF-β with NF-κB and cAMP are ambiguous.

Short-term (seconds-minutes) effects of *lipophilic hormones* mentioned in section 6 are obviously of nongenomic nature. This is supported by the fact that transcription and translation inhibitors do not block them [100]. Final clarity has not been achieved in the elucidation of receptor mechanisms; they can be realized through intracellular receptors, as well as, more probably, via PM receptors. Estradiol-binding proteins are found in PM and reticulum, but their functional role has not been deduced [126]. The bulk of evidence suggests realization of these effects via "alien" STS: second messengers (cAMP, IP_3, and Ca^{2+}) and pathways (PKC, nRPK, RTK, Ras–MAPK, PI3-K, NF-κB, and eNOS) typical for circulating hormones and neurotransmitters, as well as for GFs and cytokines [2, 8, 100, 102, 104, 127-131]. On the other hand, *phosphorylation* of receptors of estradiol, progesterone, and androgens *by ERK and ACT* is important for their function, and progesterone (coupled with MAPK) enhances expression of GF receptors [2, 132]. Glucocorticoid–receptor complex competes with NF-κB for CREB and p300 and also induces IκB, resulting in inhibition of NF-κB [91].

Finally, *regulation of transcription* by hormones usually requires the recruitment of protein co-integrators CBP and p300 coupling activated TFs with the basal transcription machine (see above and Fig. 4 in [78]) and histone phosphoacetylation. Obviously, this is a common final chain for hormonal regulation of transcription. Mechanisms of *regulation of translation and cell growth* are well known for insulin and some GFs, but are less well understood for other hormones. Probably, they also include transactivation of MAPK-cascades – mTOR–ribosomal kinase p70 S6K and/or initiation translation factor 4E (eIF4E).

Figure 2 resembles a road map or telephone network. Its "rail junctions", the most active switchers of signal-transduction pathways, are Ras and PKC. The first accepts the signals from many systems and unidirectionally transduces them onto MAPK-cascades. The importance of PKC is wider: it realizes reception and transduction of multiple signals as well. This is possibly due to a large number (12) of PKC isoforms with their different properties, functions, and regulation [8, 133].

Thus, being at first exotics, the above said pattern proved to be the regularity. A more complete *generalizing*

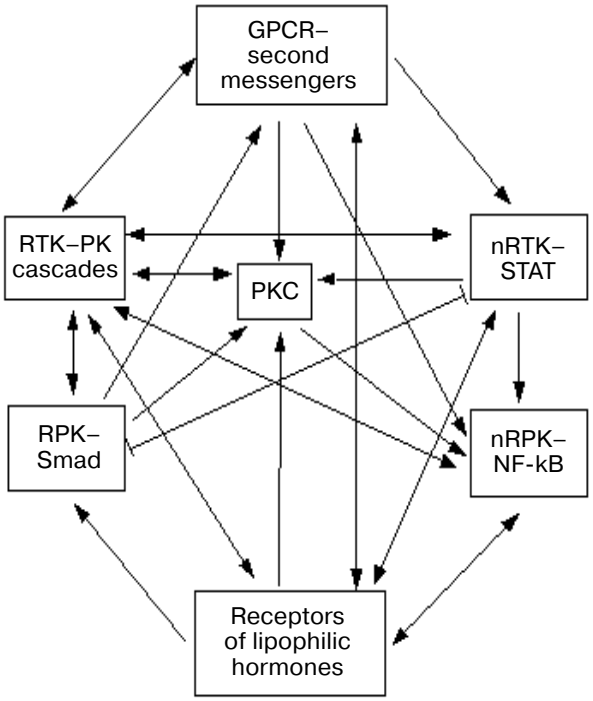

Fig. 3. Transactivation of the main signal transducing system types. Notation: ⟷, mutual transactivation; ⟶, one-side transactivation; ⊢⊣, mutual inhibition.

scheme (Fig. 3) displays 19 transactivations of 21 possible ones between STS pairs, and mutual transactivations are found in 2/3 of cases (mutual antagonism takes place in the STAT–Smad pair). *Obviously, it is time to transit from separate STS to their complexes, i.e., from pathways to networks*.

In general, mechanisms of hormone actions are diversified and complex; they recruit and transactivate various systems providing their diversity and flexibility. They widely use a module principle of organization and functioning. STS can be assembled from different modules, like a building or complex device are built up from standard units. This assumes usage of both "own" and "alien" STS as well, their "socialization" by hormones can be termed promiscuity.

Hence, during the last 3-4 years it has become evident that mechanisms of actions of various type hormones may be close, and sometimes identical, that the same hormone can effect via various STS, and different hormones via the same STS. This is still further evidence for the principal unity of all systems designed for intercellular regulation. Specificity of hormonal mechanisms is not absolute, but relative, like the specificity of enzymes. This fact supports again the necessity in their integration into one generality—hormones, similarly, distinct protein families are combined into superfamilies.

New facts provide a basis to form a new approach to the most difficult problem in hormonology: why such a multitude of very different effects of hormones is realized via a very limited number of STS? The possible answer is that mechanisms of hormonal activity include not single, but a rank of receptor types and/or subtypes, a rank of STS types/or subtypes. The existence of multiple forms in most signal proteins, the presence within these proteins of several domains specialized on different functions (we almost did not consider this) may play a major role. Various combinations of all these components ("assembly") can impart individual character to the effect of each hormone. The discovery of accessorial proteins significantly modifying the main mechanisms is yet another very important factor: there are adapter, isolating (insulator), supporting (scaffold), docking, and anchoring proteins [17, 134-136]. In a body, they can localize, "canalize" the signal transduction via necessary STS, and even inhibit a competing system, as it has been demonstrated for two related cascades—ERK and JNK [16, 17, 136].

The study of mechanisms of hormonal effects is developing very quickly and endows a continuing revolution in biology. It has been revealed during last 15 years that hormones regulate all vital processes—not only metabolism and functions of cells and organs, but template syntheses (transcription, translation) and other processes determined by genome (proliferation, growth, migration, differentiation, and survival of cells; adaptation, cell shock, apoptosis, and malignant transformation). General pathways of hormonal signal transduction into the cell nucleus have been discovered, transactivation of these systems has been established, and their molecular mechanisms have been identified. Obviously, the next task is the same deep insight into the regulation of mitochondrial functions.

Primary perception of hormonal signals occurs via various receptor types, and its transduction in the cell via hormone–receptor complexes (for lipophilic hormones), second messengers, and/or PK (serine/threonine and/or TK). Signal transduction to the genome involves proteins or protein complexes: receptors for lipophilic hormones (ligand-dependent TF), latent cytosolic TFs activated by phosphorylation (much more rarely by dephosphorylation), or PK activating resident TFs in the nucleus. STS in the cell are built and function according to the module principle providing their diversity and flexibility. The action of all hormones is complex, and various systems are recruited into its mechanisms.

There is good reason to believe that a consolidation has come to the head of all types of intercellular regulators — circulating hormones, neurohormones/neurotransmitters (neuromediators and neuromodulators), and tissue hormones (cell growth factors, cytokines, eicosanoids, amines, etc.) — into a parent and entire population "hormones". This statement is supported by

the principal unity of their biological functions and importance, particular features and properties, and molecular mechanisms of their action as well.

Achievements in the field of mechanisms of hormonal activity have become firmly established as a treasure of contemporary biology; they have become a necessary part of all new manuals on biochemistry and molecular biology ([7, 40, 73], etc.). They are widely used in its other branches, such as cellular biology, physiology, and pharmacology. The substances selectively effecting receptors and signal transduction systems in general, comprise more than 2/3 of contemporary drugs. Increasing use of ideas and achievements in the field of hormonal mechanisms in medicine is bright evidence for its adulthood. Effective introducing into practice never occurs until the readiness and possibilities mature in fundamental science.

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