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CAN CYSTEINE DIRECT TYROSINE IN SIGNAL TRANSDUCTION FOR ENVIRONMENT-ORIENTED GENE CONTROL?

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ABSTRACT

Signals are transduced from the cell surface to the nucleus through phosphorylation and dephosphorylation chain reactions of cellular proteins at tyrosine and serine/threonine. Recent evidence suggests that the signal generated through the protein modification at cysteine by oxidation/reduction crosstalks to the protein phosphorylation/dephosphorylation-linked one. I propose that the cysteine-oriented signal potentially directs the tyrosine-oriented one and this mechanism underlies the environment-oriented control of internal signaling for gene expression.

Key Words: Signal transduction, Oxidative stress, Protein tyrosine kinase, Reactive oxygen intermediate, Redox, Heavy metal

INTRODUCTION

Living organisms are created and function through signal exchange between individual genes in the cells at distinct differentiation stages. Signal exchange can occur between any two different genes to bring about their expression, promotion or suppression, with proteins and other organic and inorganic molecules as the messengers. The signals are transduced from cell-surface receptors to the cell nucleus through chain reactions of these messenger molecules, similar to serial on/off switches. These apparently internal, closed reactions for signal transduction are likely to be affected by environmental stresses, which externally control cellular functions. Little is known about the environment-linked control of gene actions at the molecular level. I will discuss the potentially crucial role of oxidization/reduction (redox)-linked protein cysteine modification in environment-oriented gene control.

INTERNAL SIGNAL TRANSDUCTION FOR GENE CONTROL

The ligand-mediated receptor crosslinkage, which depends on the complementarity of the structures of ligands and receptors, works as the first on-switch for the intracellular signal transduction.[1)] This initial event is followed by forced interaction of intracellular compartments of the receptors and molecules that intracellularly associate with the receptors. The receptors may work by themselves as protein tyrosine (or serine/threonine) kinases (PTKs) or

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phosphotyrosine (or phosphoserine/phosphothreonine) phosphatases (PTPases), or associate intracellularly with non-receptor type PTKs. Once these PTKs and PTPases are functionally mobilized or activated by receptor crosslinkage, they phosphorylate or dephosphorylate substrate proteins including other PTK molecules at tyrosine. This might cause conformational change in the substrate molecules, thereby regulating their functions. Protein phosphorylation and dephosphorylation at tyrosine also medicate association or dissociation of the two molecules that bear phosphorylated or dephosphorylated tyrosine and the specific acceptor domain for the phosphorylated tyrosine, named src homology-2 (SH2).[2–8] Activation of tyrosine kinases, which should occur after receptor crosslinkage, is frequently followed by activation of serine/threonine kinases such as protein kinase C (PKC)[9,10] and mitogen-activated protein kinases (MAPKs).[11,12] Phosphorylation and dephosphorylation of signal elements finally control the activities of transcription factors and cell cycle regulating elements.

OXIDATIVE STRESS AND SIGNAL TRANSDUCTION

Interaction between cell surface receptors and their ligands, which initiates the intracellular signal delivery, is principally an internal event, except when the ligands are derived from the environment. Animals are, however subject to a number of environmental forces or stresses such as oxygen/oxidants, heat, ultraviolet rays, heavy metals, food and microorganisms (Fig. 1). Animal cells use oxygen by stepwisely reducing it to produce ATP, and a number of reactive oxygen intermediates (ROIs) are generated during this process. Many of the environmental stresses on the animals become oxidative stress because they promote generation of ROIs and related metabolites. For example, large amounts of ROIs are produced in phagocytes that have ingested invaders from the environment. This suggests that the internally regulated signal delivery may be modified by the oxidative stress or ROIs. The oxidative stress and ROIs are known to directly affect proteins, lipids and DNA for molecular damage.[13] Recent studies have, however, revealed that different types of oxidative stress, caused by oxidants, nitric oxide-generating agents, alkylating agents, ultraviolet rays and heavy metals, all share in promoting or inhibiting the tyrosine phosphorylation of cellular proteins[14–22] (Table 1). This suggests that the signal transduction elements are the target of the oxidative stress for modification.

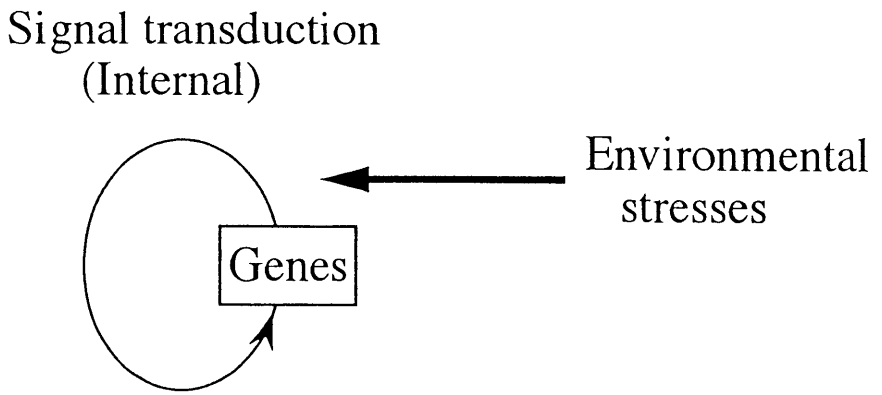

Fig. 1. Internally delivered signals that regulate expression and amplification of genes are subject to control by environmental stresses.

Table 1. Recent reports on cysteine-oriented regulation of signal transduction.

Event	Agent	Suggested mechanism	Ref.
Promotion of protein tyrosine phosphorylation	Oxidant	Inactivation of PTPase	14, 15
	Heavy metal, NO	Modulation of PTPase activity	16, 17
	Heavy metal, MAA	Cell surface triggering	18
		Promotion of ligand-dependent signal	19
Inhibition of protein tyrosine phosphorylation	Oxidant	NS	20–22
Activation of receptor PTK (Ltk in ER)	Oxidant/IAA	Aggregation of Ltk by S-S bond	24
Activation of nonreceptor PTK (c-Src, Lck, c-Abl)	Ultraviolet ray	NS	49
	IR	NS	50
	Oxidant	NS	51, 52
	NO	Promotion of PTPase activity	17
	Heavy metal	Aggregation of cell surface receptors by S-Hg-S bond	25–27
	Heavy metal	SH-modification of Src	54, 55
Inactivation of nonreceptor PTK	Harbimycin, NEM	SH-modification of Src	45, 46
	NEM	blocking interaction between CD4 and Lck	47
Inactivation of PTPase	Heavy metal/oxidant	SH-modification of PTPase	42–44
Inactivation of PKC	NO	SH-modification of PKC	48
Activation/inactivation of transcription factors (AP-1, NF-kB, steroid receptor)	Oxidant	Activation in vivo	60–62
		Inhibition in vitro	63
	Thioredoxin/Ref-1	Activation through reduction of S-S bond	67–69

PTK: protein tyrosine phosphatase
PTPase: phosphotyrosine phosphatase
PKC: protein kinase C
NO: nitrogen oxide-producing chemical
MAA: monoiodoacetic acid
IAA: iodoacetamide
NEM: N-ethylmaleimide
IR: ionizing radiation
ER: endoplasmic reticulum
NS: not specifically explained

CYSTEINE-ORIENTED VS. TYROSINE-ORIENTED PROTEIN MODIFICATION

Among several reactive residues on proteins, thiol (SH)-groups of cysteines are the central target of the redox reaction.[23] The cysteine-oriented redox reaction on protein molecules alters their status and structure through two mechanisms. First, it crosslinks two or more peptides with intermolecular S-S bonds. Second, it induces conformational changes in the molecules with intramolecular S-S bonds for adjacent SH-groups on the molecules. Both types of redox-linked protein modification are comparable to those of a tyrosine-mediated one, resulting from inter- and intra-molecular bonding between the phosphorylated tyrosine and its acceptor sequence on

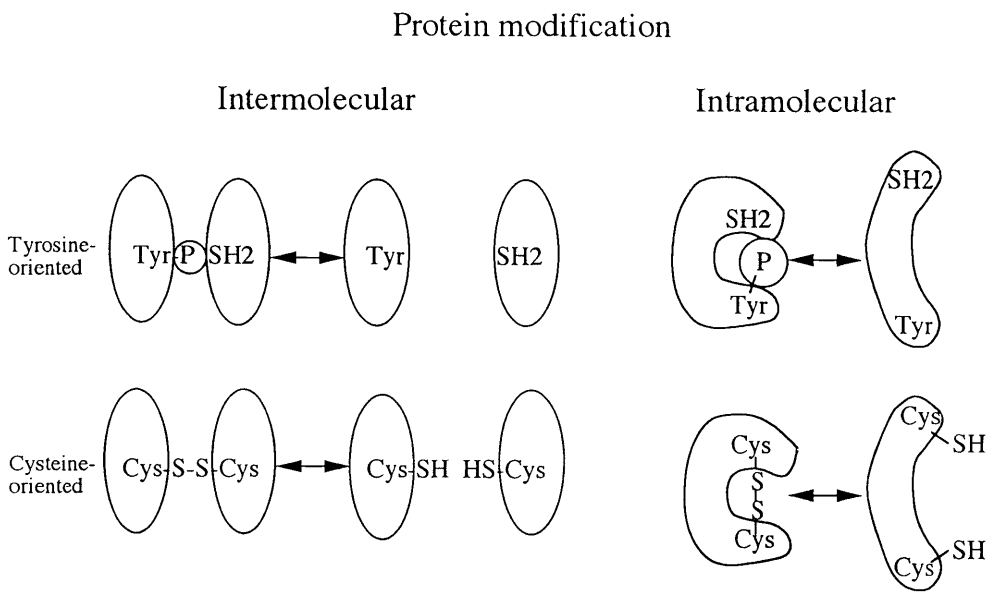

Fig. 2. Cysteine-oriented vs. tyrosine-oriented conformational changes of signal elements.

the SH2 domain[4,8] (Fig. 2). The redox-linked cysteine-oriented protein modification therefore, probably fullfils the condition for working as an on/off switch of the signal transduction, just as the tyrosine-oriented modification does.

CYSTEINE-ORIENTED RECEPTOR CROSSLINKAGE AND ACTIVATION OF PTKs

Ben-Neriah and his colleagues[24] reported that intracellularly inoculated oxidants and alkylating agents induced aggregation and activation of the receptor type PTKs in the endoplasmic reticulum. This was a result of the formation of intermolecular S-S bonds between the two PTK molecules, potentially catalized by protein disulfide isomerase. Independently, we provided evidence that the redox reaction might affect cell surface receptors for initiating signal transduction from the cell surface to the nucleus[18,25–27] (Table 1). The study was carried out using Hg^{2+}, a well-known SH-reagent with an extraordinally high association constant to cysteine SH-groups and able to replace the S-S bond with the functionally equivalent S-Hg-S bond.[28–32] We showed that exposure of murine T lymphocytes to Hg^{2+} in vitro induces high grade phoshorylation of cellular proteins at tyrosine, and activation of Lck kinase, a nonreceptor PTK of the Src family. These events were accompanied by aggregation of a number of cell surface proteins including CD3 (a signal transducing element in the T cell receptor complex), CD4, CD45 (receptor type tyrosine phosphatase) and Thy-1 (glycosylphosphatidylinositol (GPI)-anchored cell membrane protein).[25] Both tyrosine phosphorylation promotion of cellular proteins and aggregation of cell surface proteins seemed to be mediated by the reaction between SH-groups on cell surface proteins and Hg^{2+}, because reducing or SH-group-donating reagents neutralized the Hg^{2+} action. Evidence was further produced that the primary target of Hg^{2+} for promotion of protein tyrosine phosphorylation includes cell surface GPI-anchored proteins.[27] GPI anchored proteins are

known to associate with nonreceptor PTKs such as Lck and Src kinases across plasma membrane,[33] and to transmit signals for cell activation.[34–36] In earlier experiments, we co-cross-linked both T cell receptors and Thy-1 as a GPI-anchored protein with appropriate antibodies, and found that the two cell membrane proteins worked synergistically to deliver a high grade signal for extensive protein tyrosine phosphorylation and activation of Lck kinase.[36,37] Based on these observations, we speculated that heterogeneous crosslinkages of multiple transmembrane and GPI-anchored proteins through S-Hg-S bonds are capable of generating extraordinarily high grade signals to activate PTKs.[25] Correspondingly, Murakami et al.[38] recently showed that crosslinkage of interleukin-6 receptors (IL-6R) by IL-6 as an inflammatory cytokine induces dimerization of gp130 (the signal transducing element of IL-6R) through an S-S bond, and this dimerization is associated with activation of nonreceptor PTKs. By analyzing the mechanism of activation of the ret proto-oncogene[39,40] by multiple endocine neoplasia 2A mutations, Asai et al.[41] also showed that dimerization of the Ret kinase proteins through a *ret* mutation-linked S-S bond underlies their constitutive activation in the neopolasm. These reports support the view that dimerization or aggregation of cell surface receptor proteins through S-S bonds (or S-S bonds-replacing S-X-S bonds) is widely involved physiologically and pathologically in the initial mechanism of cell signaling.

PTPases AND PTKs AS THE TARGETS OF CYSTEINE-ORIENTED MODIFICATION

The S-S bond or S-Hg-S bond-mediated receptor crosslinkage may promote mutual interaction of intracellular elements that associate with the intracellular compartments of the cell membrane receptors for activation or regulation. The receptor crosslinkage, whether or not it is mediated by redox mechanism, might also promote production of ROIs in the cell, possibly through a protein phosporylation-dependent signaling. These ROIs could in turn affect the cell surface receptors and intracellular signal elements secondarily for the SH-modification as a potential signal amplifying mechanism. Thus, intracellular signal elements could be the targets of both intracellularly introduced oxidants and the secondarily generated ROIs (Table 1). It was first suggested that this signal pathway operated on PTPases,[15,42–44] known to be sensitive to SH-reagents in vitro for inactivation.[43] Herbimycin A and some alkylating reagents have also been shown to directly affect Src and Lck kinases for inactivation.[45,46] Furthermore, alkylating agents might inhibit the kinase activity by blocking the interaction between CD4 and Lck,[47] and nitric oxide or nitric oxide-generating agents could inactivate PKC in vitro.[48] These observations raised the possibility that nonreceptor PTKs and PKC are the direct target of the cysteine-oriented regulation. On the other hand, ultraviolet rays,[49] ionic radiation,[50] oxidants[51,52] and anoxia,[53] when live cells are subjected to them, have been shown to upregulate the c-Src, Lck and c-Abl kinases. The molecular mechanism of the kinase activation by oxidizing agents in vivo,[49–53] and the contrast with the inactivation by SH-reagents in vitro,[45,46] have not been well-explained. Recently, we demonstrated for the first time that the catalytic activity of Src kinase is upregulated by the SH-modification of the kinase protein with Hg^{2+} in a cell-free system.[54,55] Correspondingly, Veillette et al.[56] reported that Lck kinase contains conserved cysteines crucial for enzymatic activity. Taken together, we speculate that the Src kinase bears the target structure for appropriate SH-modification to either upregulate or downregulate the kinase activity depending on the conditions.

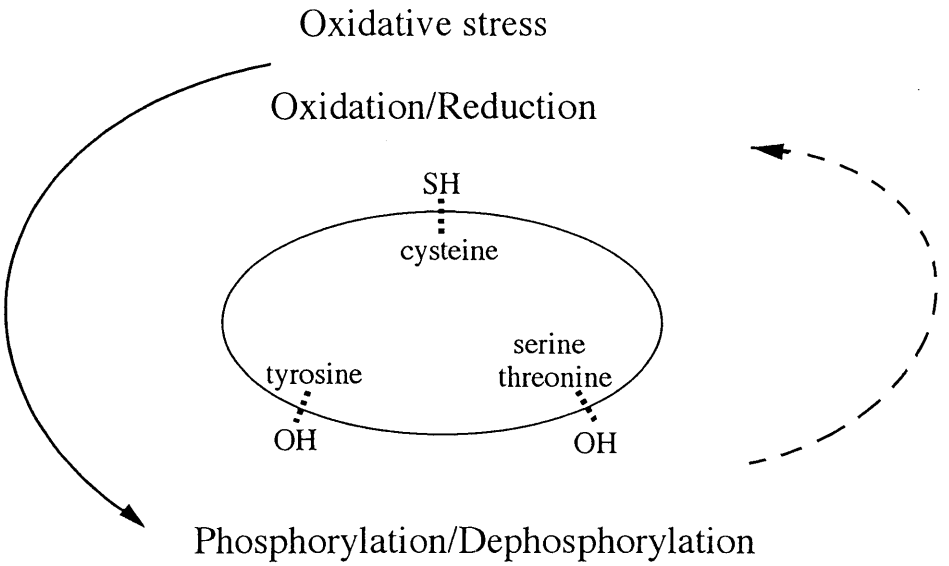

Fig. 3. Cysteine can direct tyrosine and serine/threonine on proteins in signal transduction.

RELATION BETWEEN CYSTEINE-ORIENTED AND TYROSINE-ORIENTED SIGNALS

For the Hg^{2+} model, we examined the relation of the redox-mediated cysteine-oriented regulation to the phosphorylation/dephosphorylation-dependent tyrosine-oriented control of Src kinase activity. Interestingly, activation of Src kinase by Hg^{2+} occurred independently of the regulation through phosphorylation/dephosphorylation of C-terminal Tyr-527 (the known regulatory site[2–5]) of c-Src kinase, selectively promoting Tyr-416 (the known autophosphorylation site) phosphorylation without definite change in the phosphorylation level of Y527.[54,55,57] We therefore proposed that the cysteine-oriented regulation could be upstream from the tyrosine-oriented one.[54,55] In other words, the cysteine may direct the tyrosine for cellular signal transduction (Fig. 3). This hypothetical principle underlies the action mechanism of the environmental stress controlling internal signal transduction.

THE SECOND WAVE OF THE CYSTEINE-ORIENTED SIGNAL

The signal initially triggered by the cysteine-oriented mechanism should further drive the tyrosine-oriented and the serine/threonine-oriented signal pathways. They include the phosphorylation and activation of MAPK[11,12] and stress-activated protein kinases (SAPK/JNK)[58,59] of the MAPK family, which phoshorylates and regulates transcription factors. Change in the activity of transcription factors is therefore expected following the oxidative stress-induced activation of PTKs. It has indeed been reported that transcription factors such as NF-kB could be activated in association with changes in the cellular redox status caused by oxidative stress.[60–62] The DNA binding activity of NF-kB was shown, however, to be inhibited by treatment with oxidizing and alkylating agents in vitro.[63] The stress-provoked signals induce production of stress proteins, such as superoxide dismutase[64] and thioredoxin,[65,66] which protect the cells from overly

high oxidative stress. The stress proteins could also provoke the second wave of redox signal that regulates the action of transcription factors, such as AP-1,[67] NF-kB[68] and corticosteroid receptors.[69] This potentially switches the inactivated form bearing the S-S bond on conserved cysteins to the activated form with reduced SH-groups.

In concert with the sophisticated phosphorylation/dephosphorylation-dependent regulatory mechanism, the first and second stages of redox-linked control could ultimately decide the levels of gene expression and expansion for physiological and pathological cell growth and death.[25,70–72]

The view presented in this paper is mainly based on results of our recent study, obtained in collaboration with Drs. M. Pu, A.A. Akhand, M. Kato and K. Ohkusu in the Department of Immunology and Dr. M. Hamaguchi in the Laboratory of Molecular Pathology, Research Institute of Disease Mechanism and Control, Nagoya University School of Medicine.

CONCLUDING REMARKS

In conclusion, I propose that the cysteine-oriented conformational change of signal elements works as a molecular on/off switch for signal transduction. This is an alternate to the known tyrosine-oriented or serine/threonine-oriented modification, and may even direct these for environmentally regulated internal signal transduction. In other words, the cysteines on proteins might be an open window to receive the "wind" of the environment, which promotes or regulates cellular functions.

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