Signal transduction through the cAMP-dependent protein kinase

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Abstract

Temporal cellular events responsible for hormonal activation of responses mediated by the cAMP-dependent protein kinase (PKA) have been studied in living cells. By selectively perturbing molecular function of Gs, the catalytic subunit of PKA (C), or the nuclear factor CREB, in cells through microinjection of inhibitory agents specific for these molecules or activated forms of these molecules, we have obtained evidence for a requirement for the function of each of these molecules in the hormonal stimulation of cAMP-regulated genes. Moreover, by introducing fluorescently labeled PKA subunits into these cells as molecular tracers, or by immunofluorescence of C subunit, we have observed biological translocation of C subunit from the cytoplasm to the nucleus during transcriptional activation and a quenching of this by the inhibitor molecule, PKI. The implications of these cellular and molecular events in the signal transduction of hormonal responses are discussed. (Mol Cell Biochem 127/128: 179-186, 1993)

Key words: protein kinases, signal transduction, transcription activity, phosphorylation, microinjection

Introduction

Cellular responses to extracellular signals are in many cases mediated through specific cell surface receptors and a variety of second messenger systems which are activated by ligand binding. One of the best characterized second messenger systems of this type is the cAMP-mediated system that results in activation of the cAMP-dependent protein kinase (cAPK) [1].

Many hormones act through cAMP-mediated systems to stimulate both metabolic functions and proliferation. These hormones bind to cell surface receptors of the seven transmembrane spanning domain class which are coupled to signal-transducing heterotrimeric G proteins. For hormones which act through elevations in cAMP, the receptors are coupled to Gs. Ligand binding activates Gs, which, in turn, activates adenylyl cyclase resulting in the generation of cAMP. The primary target of cAMP is the cAMP-dependent protein kinase (cAPK). In unstimulated cells, cAPK is found as an inactive holoenzyme consisting of a regulatory subunit dimer and two catalytic subunits [2]. Upon increased intracellular cAMP levels, the regulatory (R) subunits bind cAMP, inducing holoenzyme dissociation and the liberation of active catalytic (C) subunits. C subunit phosphorylates cytoplasmic and nuclear substrates.

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since a portion of C subunit enters the nucleus [3, 4]. Since cAPK activation results in pleiotropic effects on cellular metabolism, function and, in some cases, growth, both the activation and attenuation of C subunit activity must be carefully regulated.

There are multiple points of regulation of cAPK. Type I and II regulatory subunits (RI, RII) (reviewed in [5]) and PKI (reviewed in [6]) regulate the enzymatic activity and the intracellular distribution of C subunit [3, 4, 7]. In transfected cells, overexpression of C subunit is compensated for by increased RI levels and the formation of inactive type I holoenzyme [8]. The biological function of PKI remains unknown (for a recent review, see [6]) although its ability to inhibit C subunit and the identification of multiple forms of PKI [9, 10] suggest that it may also regulate C in vivo.

In addition to the regulation of kinase activity, cAMP-dependent phosphorylation events are regulated. One of the nuclear substrates of cAPK is the transcription factor CREB, whose transcriptional activity requires phosphorylation by cAPK [11]. Following phosphorylation, CREB is rapidly dephosphorylated by protein phosphatase type I (PPI) [12], which is, itself, regulated by cAPK. One of the inhibitors of PPI, inhibitor I [11] is stimulated through cAPK-mediated phosphorylation [13].

To assess the regulation of cAMP-mediated signal transduction in living cells, various components of this signaling pathway were manipulated and the effects on specific cellular responses were examined. Through perturbing the coupling of hormone binding to effectors, the intracellular distribution and level of C subunit expression and the expression of CREB, some of the potential molecular mechanisms governing the hormonal regulation of signal transduction have been revealed.

### Results

**Inhibition of cAMP-regulated gene expression by microinjection of a Gs-specific antibody**

A number of hormones, including thyrotropin (TSH), act through elevations in cAMP and the activation of cAPK in their respective cell types. The TSH receptor is a seven transmembrane spanning domain receptor which couples to Gs. In Wistar rat thyroid (WRT) follicular cells, TSH stimulates both gene expression and DNA synthesis. TSH specifically stimulates gene expression from promoters containing cAMP response elements (CRE’s) [14]. In WRT cells stably transfected with a CRE-regulated marker gene [15], TSH or 8BrcAMP stimulated gene expression (Table I). To assess the role of Gs in TSH-stimulated gene expression, a highly specific antibody raised to a unique peptide from the Gsα carboxyl terminus [16, 17] was injected into WRT CRE cells and the effects on TSH-stimulated gene expression were monitored. As reported previously [15], microinjection of the Gsα-specific antibody abolished TSH-stimulated gene expression. In contrast, the Gs antibody had no effect on gene expression stimulated by 8BrcAMP/IBMX which acts distal to the receptor. These results demonstrate that Gs is one of the initial components of cAMP-mediated signaling pathways and that its function is required for changes in gene expression stimulated by TSH.

### Table I. Modulation of TSH-induced gene expression by a Gs-specific antibody and PKI

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Cells injected with (N)</th>
<th>Uninjected (%)</th>
<th>Cells injected with (N)</th>
<th>Uninjected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gs antibody</td>
<td>PKI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH (10−9 M)</td>
<td>0</td>
<td>37 (208)</td>
<td>1</td>
<td>33 (222)</td>
</tr>
<tr>
<td>8BrcAMP/IBMX (1 mM)</td>
<td>86 (49)</td>
<td>85 (149)</td>
<td>14 (98)</td>
<td>80 (281)</td>
</tr>
</tbody>
</table>

*a* WRT CRE cells were rendered quiescent, then stimulated with the test agent for 6 h at 37°C. Following stimulation, the cells were fixed in 3.7% formaldehyde in PBS for 5 min at room temperature. The cells were subsequently permeabilized in 0.2% Tween/PBS for 2 min at room temperature, then stained with an FITC labeled anti-rabbit antibody to detect the rabbit anti Gs antibody or rabbit IgG coinjected with PKI. After antibody staining, the cells were incubated in Xgal (Moinkoth 1992) to assess β-galactosidase expression. Fluorescent cells were scored as expressing β-galactosidase (blue) or not (colorless).

*N* = number of cells analyzed.

Injected cells were compared to adjacent uninjected cells on the same coverslip.