Compensatory Regulation of RIA Protein Levels in Protein Kinase A Mutant Mice*

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The cAMP-dependent protein kinase holoenzyme is assembled from regulatory (R) and catalytic (C) subunits that are expressed in tissue-specific patterns. Despite the dispersion of the R and C subunit genes to different chromosomal loci, mechanisms exist that coordinately regulate the intracellular levels of R and C protein such that cAMP-dependent regulation is preserved. We have created null mutations in the RIβ and RIIβ regulatory subunit genes in mice, and find that both result in an increase in the level of RIA protein in tissues that normally express the β isoforms. Examination of RIA mRNA levels and the rates of RIA protein synthesis in wild type and RIIβ mutant mice reveals that the mechanism of this biochemical compensation by RIA does not involve transcriptional or translational control. These in vivo findings are consistent with observations made in cell culture, where we demonstrate that the overexpression of CaR in NIH 3T3 cells results in increased RIA protein without increases in the rate of RIA synthesis or the level of RIA mRNA. Pulse-chase experiments reveal a 4–5-fold increase in the half-life of RIA protein as it becomes incorporated into the holoenzyme. Compensation by RIA stabilization may represent an important biological mechanism that safeguards cells from unregulated catalytic subunit activity.

The cAMP-dependent protein kinase (PKA) is a key regulatory enzyme responsible for the intracellular transduction of a variety of extracellular signals and for the maintenance of numerous aspects of cellular homeostasis (1). The holoenzyme is composed of a regulatory (R) subunit dimer complexed with two catalytic (C) subunits. Two molecules of cAMP bind to each R subunit causing release of enzymatically active C subunits, which then modify the activity of target proteins by reversible phosphorylation of serine or threonine residues located within an appropriate consensus sequence (2).

Four R subunit isoforms and two C subunit isoforms of PKA have been characterized in the mouse (3). They are highly conserved among mammals, encoded by unique genes located on separate chromosomes, and show unique patterns of gene expression. The α-isofoms are expressed ubiquitously while β isoforms show more restricted patterns of expression. RIβ is induced relatively late in development and is highly expressed in neural tissues (4–6). RIIβ is expressed during embryogenesis in mouse brain, spinal cord, and liver (7). In adult mice RIIβ protein is most abundant in brain and brown and white adipose tissue, with lower expression in testis and ovary (8). Cβ is most abundant in the brain, but lower levels of Cβ mRNA are found in all tissues examined (9).

PKA holoenzymes can be separated by ion-exchange chromatography and analysis of a variety of mammalian tissues has revealed significant differences in the ratio of type I (RI-containing) to type II (RII-containing) holoenzyme (10). In rats and mice, brain and adipose tissue contain principally the type II holoenzyme, while heart and liver contain mainly type I. The ratio of type I to type II holoenzyme in individual tissues also varies across species. While mouse and rat hearts possess mainly the type I holoenzyme, beef and guinea pig hearts have principally the type II holoenzyme, with human and rabbit hearts showing equivalent amounts of both holoenzymes (11).

The type I to type II holoenzyme ratios can also change dramatically during cell development. Differentiation of Friend erythroleukemic cells results in a large increase in total PKA activity and a shift from equimolar amounts of type I and type II holoenzyme to a majority of RIIβ-containing holoenzyme (12). A similar selective increase in the RIIβ regulatory subunit occurs in differentiating ovarian follicles treated with estradiol and follicle-stimulating hormone (13). Selective increases in the RIA regulatory subunit and the type I holoenzyme occur during the differentiation of L6 myoblasts, which also show increases in total PKA activity (14). A similar phenomenon has been observed during the differentiation of 3T3-L1 cells (15).

Although the ratio of type I to type II holoenzyme varies in different cell types and stages of differentiation, total R and C subunit levels are thought to be equivalent in a variety of tissues (16). How this extremely tight coordination of R and C subunits is achieved in all tissues remains to be determined; however, experiments performed in cell cultures have revealed one potential mechanism (17, 18). The ubiquitous RIA subunit has been shown to be unstable when not associated with the C subunit in the type I holoenzyme. In Kin- cells that lack detectable C subunit, RIA subunits are rapidly degraded and the steady-state level of RIA is reduced (17, 19). In contrast, overexpression of the C subunit in NIH 3T3 cells elicits a coordinate increase in RIA protein (18).

In this report we show that loss of RIβ or RIIβ in gene-disrupted mice results in biochemical compensation by RIA with no change in RIA mRNA levels. We demonstrate in cell culture that this compensation is due to a decrease in the turnover rate of RIA protein when it associates with the C...
subunit. The capacity of RIs to compensate for changes in C subunit expression provides a mechanism to protect cells from unregulated C subunit activity during developmental and hormonally induced changes in PKA subunits.

EXPERIMENTAL PROCEDURES

Mice—Generation of RIIα and RIIβ mutant mice has been described (8, 20). Both mutant and wild type mice used in the experiments were age-matched and maintained on the same mixed C57BL/6 × 129SvJ genetic background.

Cell Culture—NIH 3T3 fibroblasts and COS7 cells (NIH 3T3 cells stably transfected with a plasmid containing the zinc-inducible metallothionein promoter driving expression of the mouse Caβ subunit) were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS). Exponentially growing cells in 10 cm plates were treated for 24 h with 90 μM zinc sulfate in DMEM containing 10% FBS and then harvested as described previously (18).

Western Blot Analysis—Brain and white adipose tissue were isolated from RIIα and RIIβ mutant and wild type animals, immediately placed in liquid nitrogen, and stored at −70 °C. Samples were thawed into homogenization buffer (250 mM sucrose, 100 mM NaPO4, pH 7.0, 150 mM NaCl, 1 mM EDTA, 4 mM EGTA, 4 mM dithiothreitol, 0.5% Triton X-100, 2 μg/ml leupeptin, 3 μg/ml aprotinin, 4 μM 8-Azaguanine (aDEAEBSF), sonicated, and centrifuged at 16,000 × g, and the supernatant was collected and assayed for protein concentration using a Bradford assay (Bio-Rad). Total protein (40 μg) was run on 10% polyacrylamide gels and transferred to nitrocellulose membranes. Blots were then blocked overnight and probed with affinity-purified polyclonal antibodies to RIs, Caβ, or RIIβ. Blots were then washed and incubated with horseradish peroxidase-conjugated secondary antibodies and visualized using the Amersham ECLTM system.

Translation Rate Determination—Wild type NIH 3T3 cells and COS7 cells were treated with 90 μM zinc sulfate for 24 h. Cells were then washed twice in labeling media (Hanks’ balanced salts solution, 5% containing equivalent total radioactivity were brought to a final volume of 1 ml with 2× labeling medium (DMEM) with 10% fetal bovine serum (FBS). Exponentially growing cells in 10 cm plates were treated for 24 h with 90 μM zinc sulfate in DMEM containing 10% FBS and then harvested as described previously (18).

Pulse-chase Experiments—After labeling of NIH 3T3 and COS7 cells for 1 h with 200 μCi/ml EXPRESSS5S protein-labeling mix, duplicate 10 cm plates were washed twice in DMEM, 10% fetal bovine serum and then incubated in DMEM, 10% fetal bovine serum plus 90 μM zinc sulfate containing 40 μg total protein from homogenates of cerebral cortex and hippocampus were run in each lane.

for 1 h, cells were harvested by washing twice in cold phosphate-buffered saline (20 mM NaPO4, pH 7.0, 150 mM NaCl) followed by addition of lysis buffer (250 mM sucrose, 25 mM Tris, pH 7.2, 25 mM NaCl, 5 mM MgCl2, 1 mM ADEAEBSF, 1% Triton X-100, 1% sodium deoxycholate). Plates were then scraped, transferred to Eppendorf tubes, sonicated, and spun for 1 h at 100,000 × g, supernatants were recovered and stored at −70 °C. To determine [35S]S incorporation into total protein, 2 μl from each sample was spotted onto Whatman GF/C filters, and protein was precipitated in 10% trichloroacetic acid, followed by three washes in 3% trichloroacetic acid/1% sodium pyrophosphate. Filters were then dried and counted in liquid scintillation fluid. Samples containing equivalent total radioactivity were brought to a final volume of 1 ml with 2× lysis buffer containing 100 mM NaCl and 40 μg total protein, were incubated for 2.5 h with affinity-purified polyclonal anti-RIs antibodies followed by 30 min with 3 μl of a 10% suspension of Protein A-Insoluble (Sigma). Reactions were then overlaid on a cushion of lysis buffer containing 1 mM sucrose and centrifuged to pellet the immunoprecipitates, which were stored at −70 °C. Pellets were resuspended and run on 10% SDS-polyacrylamide gel electrophoresis (PAGE) gels. Gels were fixed for 30 min in 10% methanol, 5% acetic acid, followed by a 30 min incubation in AmplifyTM. Gels were then dried and exposed to XAR™ Kodak film for 24 h. For determination of RIs translation rates in adipocytes, wild type and RIIβ mutant mice were sacrificed, and uterine fat pads were immediately isolated, weighed, and stored at −70 °C. Fat pads were homogenized in buffer (20 mM Tris, pH 7.6, 0.1 mM EDTA, 0.5 mM EGTA, 1% Triton X-100, 10 mM dithiothreitol, 5 mM magnesium acetate, 250 mM sucrose, 1 μg/ml leupeptin, 3 μg/ml aprotinin, 100 μg/ml soybean trypsin inhibitor, 0.5 mM ADEAEBSF, 100 μM ATP) and centrifuged for 30 min at 16,000 × g, and the supernatant were assayed for protein concentration using a Bradford assay (Bio-Rad). Samples diluted with homogenization buffer to a final concentration of 1–2 mg/ml were loaded onto a DEAE/PDLC column and eluted using a linear NaCl gradient from 0 mM to 250 mM. Fractions were collected and assayed for kinase activity in the presence and absence of 5 μM cAMP with Leu-Arg-Arg-Ala-Ser-Leu-Gly (Kemptide) as a substrate (21).

Solution Hybridization—The method used for measuring total amounts of RIs and Caβ mRNA has been described (9). Briefly, total nucleic acid samples isolated by proteinase K digestion and phenol/chloroform extraction were incubated with a single-stranded [32P]CTP-labeled RNA probe at 70 °C for 16 h. Following hybridization, samples were digested with RNase A and T1, precipitated in 10% trichloroacetic acid, and filtered onto Whatman GF/C filters. The amount of RNase-resistant probe was determined by liquid scintillation counting. RIs- and Caβ-specific mRNA in each sample was determined by comparison to a standard curve constructed with known amounts of M13 DNA containing the sense strand of the RIs and Caβ cDNAs. The results, calculated as picograms of RNA hybridized per μg of total nucleic acid, were converted to molecules/cell by assuming 6 pg of DNA/cell.

RESULTS

Compensatory Increase in RIs in Cerebral Cortex and Hippocampus of RIIβ Null Mutant Mice—We have previously reported that targeted disruption of the neural-specific RIIβ gene in mice results in deficiencies in hippocampal long term potentiation and long term depression (20, 22). Western blots using protein extracts from the cerebral cortex and hippocampus of RIIβ mutant mice were compared with age-matched controls to quantitate changes in RI isoforms. This analysis demonstrated a compensatory increase in RIs protein in both tissues (Fig. 1), whereas no changes were observed in C or RIIα isoforms (data not shown). Rls protein levels were determined by densitometry of Western blots from wild type and RIIβ mutant protein extracts. Densitometry analysis revealed an approximate 40% increase in RIs protein in both the cerebral cortex and hippocampus of RIIβ mutant mice (Table I). In order to address whether the increase in RIs protein was due to an elevation in transcription from the Rls gene, solution hybridization experi-
imments were performed using total nucleic acid isolated from cortex and hippocampus of wild type and RII μ mutant mice. This analysis revealed no change in RII α mRNA levels in mutant tissues (Table I).

**Disruption of RII β Leads to Increased Levels of RII α in White Adipose Tissue**—The RII β regulatory subunit is highly expressed in both white adipose tissue (WAT) and brown adipose tissue in mice. A targeted disruption of the RII β gene has been created that displays marked alterations in both WAT and brown adipose tissue metabolism (8). In order to address potential compensation by other regulatory subunits in mice carrying a null mutation in the RII β gene, Western blots were performed on WAT from wild type and RII β mutant mice. RII β mutant mice showed a complete loss of the RII β protein (Fig. 2A). Separate Western blots examining the levels of RII α and C α revealed a 3–4-fold increase in RII α protein in RII β mutant WAT, while C α protein was reduced by approximately 43% (Fig. 2B). RII α mRNA levels in total nucleic acid samples from WAT of wild type and RII β mutant mice were identical (Table II).

**Assembly of Type I Holoenzyme in RII β Null Mutants**—The large increase in RII α protein observed in WAT from RII β mutant mice suggests that the RII subunit has replaced RII β and formed a type I holoenzyme. HPLC analysis of WAT obtained from wild type mice revealed that the majority of PKA activity was associated with the type II holoenzyme together with a small free C subunit peak (Fig. 3). In contrast, WAT from RII β mutant mice contained only type I holoenzyme. Western blots using protein from HPLC fractions containing the type I holoenzyme peak confirmed the presence of RII α and C α in these fractions (data not shown). Peak activity fractions were also assayed in the presence of the heat-stable PKA inhibitor, PKI, which confirmed that all the kinase activity was PKA-dependent.

**The Rate of Translation of RII α Protein**—In order to address the mechanism of RII α compensation in RII β mutant mice, pulse-labeling experiments were performed in primary cultures of white adipocytes from wild type and RII β mutant mice. No significant difference was observed in the rate of translation of RII α protein between wild type and RII β mutant mice after a 1-h pulse (Fig. 4B). Western blots from the same extracts used to perform the pulse-labeling experiments confirmed that RII α protein was substantially increased in RII β mutant mice (Fig. 4A). This implies that the increased RII α protein must be due to stabilization of the protein.

**Altered RII α Stability in a Cell Culture Model of RII α Compensation**—Loss of either RII β or RII β would result in an excess of C subunit over R unless a compensatory mechanism exists to maintain the R/C balance. The observed increase in RII α appears to be a response to this imbalance. We used a cell line stably transfected with the C subunit to characterize further the mechanism of RII α compensation under conditions where the C subunit is expressed in excess of R. We have previously shown that when C is overexpressed in NIH 3T3 cells there is a specific increase in RII α with no change in RII β subunits (18). This increase in RII α resulted in the appearance of new type I holoenzyme, analogous to the results shown in Fig. 3 for RII β mutant WAT (18, 23). We therefore used these C α-overexpressing 3T3 cells for metabolic labeling studies to determine the mechanism of RII α compensation. Wild type NIH 3T3 fibroblasts and C α3T3 cells stably expressing a zinc-inducible expression vector for the mouse C α catalytic subunit (18) were treated with 90 μM zinc sulfate for 24 h and subsequently analyzed by Western blotting for RII α and C α. Zinc treatment of C α3T3 fibroblasts resulted in a 27-fold increase in C α protein compared with values obtained from wild type 3T3 cells (Table III). As previously observed (18), an elevation in RII α protein was also seen upon overexpression of C α (Fig. 5A). Western blot analysis of a range of protein dilutions from C α3T3 cells and wild type 3T3 cells showed a 4-fold increase in RII α protein (Table III).

Solution hybridization experiments demonstrated that mRNA levels for RII α remained constant despite the elevation in RII α protein (Table III). The increase in RII α protein could be due to either an elevated rate of translation or a stabilization of RII α protein. In order to determine the mechanism, zinc-treated NIH 3T3 and C α3T3 cells were labeled for 1 h with [35S]methionine followed by immunoprecipitation of RII α protein. The rate of synthesis of RII α protein in wild type NIH 3T3 and C α3T3 cells was equivalent (Fig. 5B). Western blot analysis from the same extracts confirmed that C subunit protein was indeed overexpressed in the zinc-treated C α3T3 cells as ex-

### Table I

| mRNA level* | Protein level* |
|-------------|----------------|
| Cortex      | Hippocampus    | Cortex | Hippocampus |
| molecules/cell | densitometry units | molecules/cell | densitometry units |
| Wild type  | 29 ± 2.2 | 45 ± 4.0 | 44 ± 1.0 | 63 ± 7.5 | 62 ± 7.4 |
| RII β mutant | 29 ± 2.2 | 63 ± 7.5 | 62 ± 7.4 |
| % change  | −3.4% | +2.4% | +4.0% | +4.1% |

*Solution hybridization results represent the averages ± S.D. of four wild type and four knockout animals.

### Table II

| mRNA level* | Protein level* |
|-------------|----------------|
| RII α       | Ca             |
| Wild type   | 32 ± 3.6 | 165 ± 28 | 45 ± 40 | 101 ± 47 |
| RII β mutant | 32 ± 6.4 | 185 ± 5.3 | 149 ± 26 | 58 ± 8 |
| % change  | 0% | +12% | +231% | −43% |

*Solution hybridization results represent the averages ± S.D. of three wild type and three knockout animals analyzed by making dilutions of the mutant samples and comparing them by Western blot with wild type samples using laser scanning densitometry.

Not significant.

* p < 0.05.
Pulse-chase experiments were performed to determine the half-life of RIA protein in wild type NIH 3T3 cells and C3T3 cells. The half-life of RIA in wild type NIH 3T3 cells was approximately 3.5 h as measured by immunoprecipitation of 35S-labeled RIA protein from cell extracts after a cold chase with unlabeled methionine. In contrast, the half-life of RIA protein in zinc-treated C3T3 cells was 13.5 h (Fig. 5C). This represents a 4-fold increase in the half-life of the RIA protein upon overexpression of Ca and is in good agreement with the 4-fold increase in RIA protein observed in this experiment.

DISCUSSION

The ability of mammalian cells to assemble and regulate multisubunit protein complexes usually relies on some type of autoregulatory loop. Whereas bacteria frequently solve the problem of coordinate regulation by utilizing multigene operons controlled by a single promoter, in higher animals the genes encoding interacting subunits are dispersed, frequently onto different chromosomes. Nevertheless, expression from these genes generally results in stoichiometric levels of protein subunits. The problem of coordinate regulation becomes crucial when an excess of one of the subunits might lead to undesirable biological effects in the cell. The PKA holoenzyme system is an example of such a case in which an excess of catalytic subunit could result in unwanted biological effects and reduce the ability of the cell to regulate activity by cAMP. Four separate regulatory and two catalytic subunits are produced, sometimes within the same cell, and yet most tissues are able to maintain an equimolar ratio of R and C subunits (16).

In this report we have artificially perturbed the expression of RIA and RIB subunits using targeted gene disruption in mice and examined the compensatory mechanisms that regulate R/C subunit balance in neurons and adipose tissue. In neurons of RIA mutant mice, levels of RIA protein are stabilized against proteolysis when assembled as a holoenzyme and stabilized by interaction with C subunit. Stabilization of RIA protein observed in this experiment.

Previous studies have shown that the R and C subunits are stabilized against proteolysis when released from the holoenzyme complex. Chronic activation of LLC-PK cells with cAMP can lead to the loss of more

![HPLC profile of PKA from wild type and RIIβ null mutant WAT.](image)

**FIG. 3.** HPLC profile of PKA from wild type and RIIβ null mutant WAT. 2 mg of total protein from WAT homogenates from wild type (top) and RIIβ mutant (bottom) mice was resolved by HPLC on anion-exchange chromatography, and proteins were eluted with a linear salt gradient. Individual fractions were assayed for PKA activity using Kemptide as the substrate (closed circles). Fractions containing peak kinase activity were also assayed in the presence of 5 μM PKI peptide to demonstrate that the kinase activity was PKA-specific (open circles). Both panels show HPLC profiles from one wild type and one RIIβ mutant mouse and are representative of three independent experiments run on different mice all with similar results.

![Pulse-labeling analysis of RIA synthesis in adipocytes.](image)

**FIG. 4.** Pulse-labeling analysis of RIA synthesis in adipocytes. Adipocytes from wild type (+/+, n = 2) and RIIβ mutant (+/−, n = 2) WAT were isolated and pulse-labeled for 1 h as described under “Experimental Procedures.” A, Western blot analysis of the cell homogenates used for immunoprecipitation of RIA in panel B. B, each cell pellet was homogenized, and samples containing equivalent total trichloroacetic acid-precipitable counts were used to immunoprecipitate RIA protein with a polyclonal affinity-purified RIA antibody. Immunoprecipitates were run on SDS-PAGE gels and analyzed by autoradiography to assess the level of newly synthesized RIA.

| mRNA and protein levels in zinc-treated NIH 3T3 and C3T3 cells |
|---------------------------------------------------------------|
| **mRNA level** | **Protein level** |
| RIA | Ca |
| RIA | Ca |
| mRNA molecules/cell | densitometry units | mRNA molecules/cell | densitometry units |
| NIH 3T3 | 105 ± 2 | 60 ± 3 | 106 ± 2 | 56 ± 3 |
| C3T3 | 107 ± 2 | 12,100 ± 100 | 118 ± 960 |
| Fold increase | 1 | 200 | 30 |

*a Solution hybridization results represent the averages of duplicate plates plus the range.

*b Protein levels were determined by making serial dilutions of the C3T3 extracts and comparing them by Western blotting with duplicates of NIH 3T3 cells using laser scanning densitometry.
trichloroacetic acid-precipitable counts were incubated with a polyclonal affinity-purified RI affinity-purified conalbumin antibody. FBS containing 4 mM L-methionine and 90% of wild type and C α-Insoluble. Immunoprecipitated RI activity and the important role played in this process by RI capacity to maintain cAMP-mediated control of C subunit ac-
toformation of first type II and then type I holoenzyme (6, 25).

The total amount of free C subunit is rate-limiting with respect to increased formation of type I holoenzyme. This suggests that there is an ordered assembly of first type II and then type I holoenzyme. In contrast, overexpression of RI subunits does not alter the amount of type II holoenzyme nor does it result in increased formation of type I holoenzyme. This suggests that the total amount of free C subunit is rate-limiting with respect to formation of first type II and then type I holoenzyme. In contrast, overexpression of RI subunits does not alter the rate of degradation of free C subunits.

Emerging from these studies is an appreciation of the cell’s complement of C subunit within 12.5 h (24).

What are the rules governing the assembly of type I and type II holoenzymes in vivo? Experiments in cell culture have shown that C subunits preferentially assemble with RII subunits rather than RI subunits (25, 26). NIH 3T3 cells and wild type WAT express both RI and RII subunits. However, when holo-
zymes are separated by ion-exchange chromatography only the type II holoenzyme is observed (8, 23). When NIH 3T3 cells are programmed to overexpress exogenous C subunit, the formation of new type I holoenzyme occurs (18, 23), suggesting that there is an ordered assembly of first type II and then type I holoenzyme.

In contrast, overexpression of RI subunits does not alter the amount of type II holoenzyme nor does it result in increased formation of type I holoenzyme. This suggests that the total amount of free C subunit is rate-limiting with respect to formation of first type II and then type I holoenzyme (6, 25).

Emerging from these studies is an appreciation of the cell’s capacity to maintain cAMP-mediated control of C subunit ac-
tivity and the important role played in this process by RIα. A simple model describing the dynamic assembly of R and C subunits is depicted in Fig. 6 using the example of WAT from wild type and RIIβ mutant mice. In adipocytes, the RIIβ sub-
units preferentially associate with C, leaving a pool of free RIα that is rapidly degraded. Type I holoenzyme is only formed when the level of C subunits exceeds the level of RI subunits (in this case caused by the loss of RIIβ). In this situation RIα can successfully compete for binding to the pool of free C subunits and is therefore stabilized in a holoenzyme complex. Preferential binding of RII subunits to C probably does not arise because of intrinsic differences between RI and RII sub-
units in their affinity for C, as these affinities have been shown to be quite similar (27). We propose that the phenomenon occurs as a result of a lower $K_a$ for cAMP-dependent activation of the RI holoenzyme compared with the RII holoenzyme. Free RI subunits have been shown by numerous investigators to have a higher affinity for cAMP than do RII subunits. Published values for the $K_a$ of RI-cAMP binding range from 0.1 (28) to 1 nM (29). In contrast, higher $K_a$ values for RII-cAMP binding are consistently reported, ranging from 4 (30) to 6 nM (31). We have shown that the apparent $K_a$ for cAMP activation of RIα holoenzyme is about 4-fold lower than that for RIIβ holoenzyme when measured in cell extracts (8). Given the enhanced sensitivity to activation of RI-containing holoenzyme, we predict that C subunits would shift preferentially to the RII-containing holoenzyme complex until the RII binding capacity of the cell is

![Fig. 5. Stabilization of RIα protein in Co overexpressing NIH 3T3 cells.](image)

![Fig. 6. Model for RIα compensation in RIIβ mutant mice.](image)
When the concentration of free C subunit increases due to the loss of RI
b or RII
b, RI
a rapidly responds to this perturbation via protein stabilization in a holoenzyme complex, thus protecting the cell from unregulated C subunit activity and rescuing the C subunit from rapid proteolysis. This biochemical adaptation provides a very effective mechanism for regulating the ratio of type II to type I holoenzyme formed in a given tissue and for maintaining regulation when C subunit levels change.

Modulation of RI
a turnover rate may represent an important biological mechanism for maintaining equivalent amounts of R and C subunits. Loss of this ability to maintain cAMP-dependent regulation of C subunit activity during the process of cellular differentiation could have catastrophic consequences, a phenomenon that we have recently observed in mutant mice lacking RI
a altogether. RI
a null mutants display early embryonic lethality with severe developmental abnormalities.

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