RET Finger Protein Is a Transcriptional Repressor and Interacts with Enhancer of Polycomb That Has Dual Transcriptional Functions*

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RET finger protein (RFP) belongs to the large B-box RING finger protein family and is known to become oncogenic by fusion with RET tyrosine kinase. Although RFP is reported to be a nuclear protein that is present in the nuclear matrix, its function is largely unknown. Here we show that RFP interacts with Enhancer of Polycomb (EPC) and strongly represses the gene transcription. Yeast two-hybrid assays revealed that the coiled-coil domain of RFP was associated with the EPC A domain and the carboxyl-terminal region of EPC. In addition, both proteins were co-precipitated from the lysates of human cells and mostly colocalized in the nucleus. Using the luciferase reporter-gene assay, we found that they repress the gene transcription activity independent of the differences of enhancers and promoters used, although the repressive activity of RFP was much stronger than that of EPC. The coiled-coil domain of RFP and the carboxyl-terminal region of EPC were most important for the repressive activity of each protein, whereas the EPC A domain had the transcription activating ability that is unique as the Polycomb group protein function. These results suggested that RFP may be involved in the epigenetic gene silencing mechanism cooperating with Polycomb group proteins and that EPC is a unique molecule with both repressive and transactivating activities.

RET finger protein (RFP) belongs to the large B-box RING finger protein family, members of which contain a tripartite motif consisting of a RING finger, a B-box, and a coiled-coil domain with three defined helices (1–5). In addition, it contains a specific carboxy-terminal region known as the RFP domain (6). RFP mRNA was detected in a variety of human and rodent tumor cell lines as well as in male germ cells at high levels (1). It was also shown that RFP is associated with the nuclear matrix (7) and is expressed in the nuclei of various cells, including peripheral and central neurons, hepatocytes and adrenal chromaffin cells (8), and partially colocalizes with PML and int-6 (9, 10).

There are over 200 members of the RING finger protein family reported to date, including PML, BMI1/Mel-18, RING1, and KAP-1 (11–13). RING finger proteins are thought to play roles in the formation and architecture of large protein complexes that contribute to diverse cellular processes such as oncogenesis, apoptosis, development, and ubiquitination (11, 14). BMI1/Mel-18 and RING1 belong to Polycomb group proteins (15, 16), and especially RING1 was reported to form the Polycomb group protein complex with HPC2, BMI1, and HPH and repress the gene transcription (16, 17). In addition, PML, BMI1/Mel-18, RING1 and RFP are all involved in oncogenesis by the formation of oncogenic fusion proteins (1, 18–22) or by deregulating the expression levels of certain oncogenes (17, 23, 24).

Polycomb group proteins have been initially identified in Drosophila as being involved in the maintenance of the correct expression pattern of homeotic genes. Polycomb group proteins form the chromatin associated-protein complexes that are involved in the epigenetic gene silencing and in the maintenance of cell type specificity (25–27). The homologues of Polycomb group proteins were also found in other vertebrates and invertebrates such as human, mouse, chicken, Xenopus, and Caenorhabditis elegans (28). On the other hand, epigenetic gene activation mechanism is known to be mediated by distinct protein complexes consisting of trithorax group proteins.

Enhancer of Polycomb (EPC) is a unique member of the Polycomb group proteins (29). Although mutations in E(Pc), the Drosophila homologue of EPC, exhibit no homeotic transformations, they enhance homeotic mutations by other Polycomb group genes, such as Pc, Pcl, ph, Sce, Scm, and sxc (30, 31). Mutations in E(Pc) also function as the strong suppressors of position-effect variegation (PEV) that is another epigenetic gene silencing mechanism and is associated with the heterochromatin formation (32, 33). Among Polycomb group genes, only E(Pc) and Enhancer of zeste (E(z)) were shown to function as Su(var)/s (suppressors of PEV) (29). Interestingly, E(z) was reported to have both transcription activating and repressing functions and can be classified as both Polycomb and trithorax group proteins (34). In contrast to most of other Polycomb group proteins, no interacting proteins of EPC have been found so far (28). These results suggest that EPC may have a unique role in the gene silencing mechanisms mediated by Polycomb group proteins.

Here we report that RFP interacts with human EPC and that...
Association of RFP with Enhancer of Polycomb

RFp itself functions as a strong transcriptional repressor when targeted to reporter genes. This interaction is mediated by the binding between the coiled-coil domain of RFP and the conserved EPCa domain or the carboxyl-terminal region of EPC. Immunofluorescence study revealed that RFP mostly colocalizes with EPC in the nuclei of human cells, suggesting cooperative roles of RFP and EPC in gene silencing.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—The RING finger B box region (amino acids 2 to 135), the coiled-domain region (amino acids 135 to 217), and the RFP gene region (amino acids 318 to 514) (Fig. 2A) were amplified using specific primers with a flanking EcoRI site on the 5′ primer and a BamHI site on the 3′ primer, using the full-length human RFP cDNA as a template. The resulting PCR products were subcloned into the pGEM-T vector (Promega) and sequenced to ensure that there were no mutations due to PCR errors. Each product was subcloned into the EcoRI/BamHI sites of pAS2-1 vector (CLONTECH).

The cDNA fragment obtained by yeast two-hybrid screening encoded amino acids 1 to 252 of human EPC. To obtain the full-length EPC cDNA, we performed 5′ and 3′ rapid amplification of cDNA ends from human testis cDNA library by the specific nested primers using the Marathon cDNA amplification kit (CLONTECH). Both 5′ and 3′ rapid amplification of cDNA ends products were subcloned into the pGEM-T vectors. Sequence analysis resulted in the identification of an additional 249-bp 5′ cDNA fragment and an additional 1887-bp 3′ cDNA fragment that included a termination codon. To generate the full-length EPC cDNA, an NcoI/XhoI fragment of pACT2-EPC (amino acids 1–252) vector that was derived from the first yeast two-hybrid screening was ligated into the NcoI/XhoI sites of 3′ rapid amplification of cDNA ends product cloned into the pGEM-T vector.

EPCa domain region (A region, amino acids 2 to 285), EPcB domain region (B region, amino acids 280 to 496), EPC domain and glutamine-rich region (C region, amino acids 493 to 820), and CQ and carboxy-terminal region (CQCT region, amino acids 493 to 836) (Fig. 2B) were amplified by the specific primers with a flanking EcoRI site on the 5′ primer and an XhoI site on the 3′ primer, using the full-length EPC cDNA as a template. The PCR products were subcloned into the pGEM-T vector (Promega) and sequenced to ensure that there were no mutations. These products were cloned into the EcoRI/XhoI sites of pACT2 vector (CLONTECH).

The pV5-His-C-GAL4BD plasmid was generated by two steps. A HindIII-EcoRI fragment of the pAS2-1 vector that contains a GAL4BD site was subcloned into the pcDNA3.1/V5-HisC vector (Invitrogen) and then the multicloning site between SfiI and EcoRI was replaced by that of the pACT2 vector. The full-length EPC cDNA or its fragments (A region, B region, CQ region, and CQCT region) were cloned in-frame into the pV5-His-C-GAL4BD plasmid. To obtain pV5-His-C-GAL4BD-RFP plasmids, a HindIII-BamHI fragment of pAS2-1 full-length RFP or pAS2-1 RFP domains was cloned into the same site of the pDGR1 vector (CLONTECH).

Yeast Two-hybrid Screening of RFP Interacting Proteins—The full-length RFP cDNA was cloned into the pAS2-1 vector (CLONTECH) and used as bait to screen for its interacting proteins in a two-hybrid screen. The pAS2-1 RFP plasmid was co-transformed with human testis cDNA library (CLONTECH) into the Y190 strain of Saccharomyces cerevisiae. The transformants were plated first on selective medium lacking tryptophan and leucine, and then on selective medium lacking histidine, tryptophan, and leucine with 40 μM 3-amino-1,2,4-triazole (3-AT) (histidine “jump-start” method). From approximately 5 × 10^6 independent clones, 50 colonies were found to grow on a minimal medium lacking leucine, tryptophan, and histidine, and positive for β-galactosidase expression. β-Galactosidase activity was assayed by the filter assay method according to the manufacturer’s instructions (CLONTECH). After DNA isolation, these clones were further characterized by sequencing and analyzed for gene homology by the BLAST database.

RESULTS

Cloning and Chromosomal Mapping of Human EPC—To identify the interacting proteins with RFP, we performed a yeast two-hybrid screening. The full-length coding region of RFP was cloned into the pAS2-1 vector and co-transformed with a human testis Matchmaker two-hybrid library (CLONTECH) into the yeast Y190 strain. The transformants were plated first on selective medium lacking tryptophan and leucine, and then on selective medium lacking histidine, tryptophan, and leucine with 40 μM 3-AT (histidine jump-start method). From approximately 5 × 10^6 independent clones, 50 colonies were His+ and β-galactosidase positive. Four isolated clones were identical and their inserts were 777 bp in length. The nucleotide sequence was shown to be highly homologous to the EPCa domain of Drosophila Enhancer of Polycomb (E(Pc)) gene by searching the BLAST data base. E(Pc) was identified in a number of Drosophila genomic libraries.
many species and especially the EPcA domain was the longest conserved region (29).

To obtain the full coding sequence of human EPC, we performed the 3' and 5' rapid amplification of cDNA ends method. By using the human testis cDNA library as a template, we identified a 249-bp 5' cDNA. A total 270-bp untranslated sequence includes a stop codon 57-bp upstream of the first ATG codon with rough conformation to a Kozak consensus sequence. A 1887-bp 3' cDNA product included conserved EPcB and EPcC domains and a glutamine-rich (Qx) region, followed by a termination codon. The predicted amino acid sequence of EPC (836 amino acids) is shown in Fig. 1A. The calculated molecular mass of EPC was approximately 92 kDa. EPcA, EPcB, and EPcC domains are highly conserved in many species, including Drosophila, C. elegans, yeast, mouse, and human (29). There appear to be two E(Pc) paralogues in mammals, and mouse paralogues are named Epc1 and Epc2 (29). Although mouse Epc1 and Epc2 are about one-third of the length of the Drosophila E(Pc), all three domains are conserved (Fig. 1B). Mouse Epc1 and Epc2 have longer (Epc1-L and Epc2-L) and shorter variants (Epc1-S and Epc2-S), and the shorter variants contain the EPcA domain only (29) (Fig. 1B).

Alignment of the deduced amino acid sequences from human EPC and mouse Epc1 clearly shows a high degree of conservation (81% amino acid identity) (Fig. 1B).

There seem to be at least two human EPC genes named EPC1 and EPC2 that were mapped to 19p11.12 and 22q13.3, respectively (29). By the radiation hybrid mapping method (Research Genetics, Inc.), the EPC gene that we cloned was mapped on 10p11, corresponding to the location of human EPC1 (data not shown).

Identification of Interacting Domains between RFP and

**FIG. 1.** Amino acid sequence of human EPC and comparison of EPC homologues in eukaryotes. A, the deduced amino acid sequence of human EPC. EPcA, EPcB, and EPcC domains and a glutamine-rich (Qx) region that are conserved in Drosophila, mice, and human are underlined by thin, thick, double, and dotted lines, respectively. B, diagram of the protein structure of EPC homologues. The diagram is oriented with the amino-terminus on the left and the conserved EPcA, EPcB, EPcC domains, and glutamine-rich (Qx) and alanine-rich (Ax) regions are designated. % indicates the amino acid identity of each region.
In the original two-hybrid screening, the full-length RFP was shown to interact with the EPcA domain of human EPC. To further clarify their interacting domains, we cloned the different regions of RFP and EPC in-frame into pAS2-1 and pACT2 vectors, respectively (Fig. 2, A and B). Each vector was transformed into yeast Y190, and interactions were assayed by growth on selective medium lacking histidine, tryptophan, and leucine with 40 mM 3-AT and the β-galactosidase activity. Each experiment was done by using six independent colonies and the results are shown in Fig. 2C. Strong interaction was detected between the RFP coiled-coil domain region (CC region) and the EPcA domain region (A region) or the CQCT region that includes the EPcC domain, glutamine-rich (Qx) region (CC region) and carboxyl-terminal region. Although the CQCT region of EPC interacted with RFP, the CQ region (C domain and Qx region) of EPC had no interacting activity (Fig. 2C), suggesting that the carboxyl-terminal region of EPC is important for EPC-RFP binding. In contrast, the RING finger B box region (RB region) or the RFP domain (RD region) of RFP showed no or very weak interacting activity in two-hybrid assays. In addition, the association is considered positive when the two criteria of the growth in the presence of 40 mM 3-AT and the β-galactosidase activity were met. The positive association is graded by strong (+ +), moderate (+), and weak (+). Absence of the cell growth or detectable color is considered negative (−). Measurements were performed using six independent colonies.

**Fig. 2.** Mapping of binding domains for RFP-EPC interaction in yeast two-hybrid system. A, schematic representation of RFP plasmids used to determine the binding domains in yeast. Plasmids containing GAL4-DNA binding domain (GAL4BD)-RFP fusion genes were constructed by cloning the various regions of RFP in-frame into pAS2-1 vector. Each protein motif of RFP is indicated. B, schematic representation of EPC plasmids used to determine the binding domains in yeast. Plasmids containing GAL4-DNA activating domain (GAL4AD)-EPC fusion genes were constructed by cloning the various regions of EPC in-frame into pACT2 vector. Each protein motif of EPC is indicated. C, the result of binding assays by yeast two-hybrid system.

**Fig. 3.** Coimmunoprecipitation assay of RFP-EPC association. A, the whole cell lysates of 293 human embryo kidney cells and SW480 human colorectal adenocarcinoma cells were analyzed by Western blotting with anti-RFP antibody or anti-EPC antibody. The lysate from 293 cells transfected with the FLAG-tagged EPC expression vector was also analyzed. 58-kDa RFP and 92-kDa EPC proteins are indicated. An additional 88-kDa band was detected in the lysate from 293 cells transfected with the FLAG-tagged EPC expression vector. B, coimmunoprecipitation of RFP and EPC. The plasmid encoding EPC tagged with FLAG was transfected into 293 cells. The whole cell lysates were immunoprecipitated with anti-FLAG mouse monoclonal antibody (FLAG IP) or normal mouse IgG (Mock IP). The precipitated proteins and the whole cell lysate (Input) were analyzed by Western blotting with anti-RFP antibody. Although the same sample was analyzed with anti-RFP antibody, RFP could not be detected because of superimposition of rabbit polyclonal antibodies used for immunoprecipitation (data not shown).
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RFP and EPC Colocalize in the Nuclei of SW480 Cells—Double immunostaining of RFP and EPC was performed. The cells were stained with anti-EPC antibody followed by incubation with FITC-conjugated anti-rabbit IgG antibody, and then stained with Alexa 546-labeled anti-RFP antibody. The staining patterns for RFP (A, D, and G) and EPC (B, E, and H) are shown, corresponding to a single optical section of the representative cells. The two images were merged digitally and colocalization of the two staining is indicated by yellow color (C, F, and I).

In order to test whether the interaction between RFP and EPC in two-hybrid assays signifies an in vivo interaction, we performed immunoprecipitation experiments. However, the detection of RFP with EPC in coprecipitation was impossible because the band of RFP (58 kDa) that should be recognized by the secondary anti-rabbit immunoglobulin antibody was superimposed by that of anti-EPC rabbit polyclonal antibody used for immunoprecipitation (data not shown). Thus, to overcome this problem, we transiently transfected 293 cells with the FLAG-tagged form of EPC expression vector (Fig. 3A) so that EPC can be immunoprecipitated by the anti-FLAG mouse monoclonal antibody. As shown in Fig. 3B, we found coprecipitation of RFP with FLAG-EPC by using the anti-FLAG monoclonal antibody. Reciprocally, EPC was immunoprecipitated with RFP by the anti-RFP polyclonal antibody (Fig. 3C). These interactions were not detected when normal mouse or rabbit immunoglobulins were used for immunoprecipitation (Fig. 3, B and C). From these results, we concluded that EPC and RFP can form complexes in mammalian cells.

RFP and EPC Colocalize in the Nucleus—Having confirmed the association of RFP and EPC in vitro and in vivo, we next analyzed the subcellular localization of EPC in relation to that of RFP by immunofluorescence experiments. In order to determine the subcellular localization of endogenous EPC, SW480 human colorectal adenocarcinoma cells were stained with the anti-EPC polyclonal antibody by indirect immunofluorescence. As shown in Fig. 4, B, E, and H, EPC proteins were found in the nuclei of SW480 cells throughout the nucleoplasm. In addition, the brightly labeled domains and the nuclear membrane distribution were apparent in some nuclei. The size and number of nuclear domains stained with the antibody were different depending on each nuclei and several domains seemed to be attached to the nuclear membrane. Endogenous RFP was also stained by using the anti-RFP polyclonal antibody conjugated with Alexa 546 and showed the similar distribution pattern in the nuclei (Fig. 4, A, D, and G). Superimposition of the two confocal images revealed that EPC and RFP colocalized in brightly labeled nuclear domains and nuclear membrane as visualized by the yellow color (Fig. 4, C, F, and I). Colocalization was also detected in the less-condensed area in the nuclei. The fine granular pattern was too complex to allow analysis of any systematic colocalization (Fig. 4, C, F, and I). Both the homogenous and concentrated distribution patterns in the nuclei were similar to those of other Polycomb group protein complexes (16). These results suggest that RFP and EPC colocalize in the nuclei and form the nuclear structure as observed for other Polycomb group proteins.

It was previously reported that a portion of RFP colocalizes with PML within PML nuclear bodies (9). To further characterize the subcellular localization of RFP and EPC and their association with PML nuclear bodies, the double staining of PML and RFP or EPC was carried out. Fig. 5 shows that the distribution pattern of PML detected as multiple concentrated nuclear bodies was distinct from that of RFP or EPC. Superimposition of the confocal images showed that colocalization of PML and RFP or PML and EPC seemed to be limited and most PML bodies did not colocalize with RFP or EPC in the nuclei of SW480 cells.

RFP and EPC Repress Transcription—Because of the structural similarity of RFP to RING1 which can act as a transcription repressor (16) and the association of RFP with Polycomb group protein EPC, we tested the possibility that both RFP and EPC work as transcriptional repressors. Polycomb group proteins have been found to mediate the transcriptional repression of reporter genes in mammalian cells and Drosophila embryos when they are targeted as GAL4 or LexA fusion proteins (16, 26, 36, 37). Thus, we analyzed the ability of the GAL4-EPC or GAL4-RFP fusion protein to repress the gene activity, using different luciferase reporter constructs (Fig. 6A).

We made four different reporter constructs containing five tandem repeats of GAL4, binding sites for transcriptional en-
hancers (SRE or CRE), and SV40 promoter or herpes simplex virus-thymidine kinase minimal promoter, immediately up-stream of the luciferase gene (Fig. 6A). After transfection into 293 cells, CRE was enhanced by 100 μM forskolin for 2 h. We found that GAL4-RFP and GAL4-EPC constructs repressed the luciferase expression by approximately 75 and 30–40%, respectively, compared with the construct with the GAL4-binding domain alone (Fig. 6B). In addition, repressive activities of both RFP and EPC were independent of the differences of enhancers or promoters used (Fig. 6B), suggesting that their activities could be epigenetic.

We further evaluated the activities of different RFP and EPC domains on the gene transcription. The expression vectors that include the fusion genes encoding the GAL4-binding domain and various regions of RFP or EPC (Fig. 2, A and B) were co-transfected with the luciferase reporter vector containing SRE as an enhancer and SV40 promoter. As shown in Fig. 7A, the coiled-coil and RFP domain regions repressed the luciferase expression by about 80 and 50%, respectively. The repressive activity of the RING finger B-box region was rather weak (~30%). In the case of EPC, the CQCT and B regions repressed the luciferase expression by about 55 and 25%, respectively. On the other hand, the A region strongly activated the transcription (Fig. 7B). The transactivating capacity of the EPcA domain is unique among the Polycomb group proteins and this may provide the reason why the repression by GAL4-full EPC was much weaker than that by GAL4-RFP (Fig. 6).

DISCUSSION

Interaction and Colocalization of RFP and EPC in Nuclear Domains—The Polycomb group genes were first identified in Drosophila as the genes that maintain the homeotic gene repression through a possible chromatin regulatory mechanism. All Polycomb genes cloned from Drosophila have mammalian homologues, suggesting the strong conservation of this system in different species (27, 28, 38). EPC (Enhancer of Polycomb) is known to be a unique member of the Polycomb group genes (29). Although mutations in E(Pc), Drosophila homolog of EPC, exhibit no homeotic transformations, they enhance homeotic mutations by other Polycomb group genes (30, 31). Mutations in EPC also function as the strong suppressors of PEV that is associated with the heterochromatin formation (33). Polycomb group proteins form multiprotein complexes and their interacting proteins except for Pho and E(Pc) have been identified (28). In the present study, we identified RFP as an interacting protein of EPC and showed their nuclear colocalization by immunofluorescence experiments. EPC and RFP were detected in discrete nuclear domains and largely colocalized, suggesting the functional importance of their interaction. This kind of nuclear distribution is similar to those of other Polycomb group proteins, such as HPH1, BMI1, hPc2, and RING1 (16). RFP was reported to associate with PML and partially colocalize in the PML nuclear bodies (9). PML body is the nuclear matrix-associated structure that is 250–500 nm in diameter and is present in the nuclei of most cell lines (39–42). Although the PML nuclear body suspected to be involved in oncogenesis and viral infection (43, 44), its exact function remains unknown. These structures may be the sites of storage of transcription factors, the sites of transcription or the sites of RNA accumulation. La Morte et al. (45) reported that these structures correspond to the sites of incorporation of fluorescein-conjugated uridine triphosphate in nascent RNA polymerase II transcripts. It was also shown that CBP, transcription co-activator/histone acetyltransferase, is present in these nuclear structures (45). On the contrary, Boisvert et al. (46) reported...
that PML nuclear body itself does not accumulate RNA and proposed that these structures may contribute to the formation of the nuclear environment for the expression of specific genes. Our study showed that RFP and EPC associate with only a limited subset of PML nuclear bodies, consistent with the previous observation (9). Thus it seems likely that RFP is involved in the gene silencing mechanism that is different from the speculated functions of the PML nuclear body. Further study will help to determine the roles of RFP-EPC and RFP-PML association in these nuclear complexes.

RFP Acts as a Transcriptional Repressor—RFP was first identified as a gene which becomes oncogenic by rearrangement with RET proto-oncogene (1, 22, 47). RFP is a member of the B-box RING finger family that possesses a tripartite motif consisting of a RING finger, a B-box zinc finger, and a coiled-coil domain. RFP also has a characteristic carboxyl-terminal domain called the RFP domain or B30.2-like domain (6). The RING finger has been found in a wide variety of proteins, such as PML, BMI1/Mel18 and RING1. Among them, BMI1/Mel18 is classified as a Polycomb group protein. RING1 interacts with Polycomb group proteins (HPH, BMI1, and HPC2) and is involved in the formation of Polycomb group protein complex distinct from EED (embryonic ectoderm development)/EZH (enhancer of zeste) (17, 28, 48). In addition, it was shown that RING1 acts as a transcriptional repressor (16).

This study identified, for the first time, the strong transcriptional repression activity of RFP that was dependent on its coiled-coil and RFP domains. Since the coiled-coil domain and RFP domain are thought to be involved in protein-protein interactions (9, 10), it is reasonable to speculate that the function of RFP could depend on the interaction with other nuclear proteins. In fact, consistent with this view, we found that the coiled-coil domain is strongly associated with EPC. Thus, this association may be crucial for the function of RFP as the transcriptional repressor, although it is possible that nuclear proteins other than EPC are also involved in the RFP-mediated

![Figure 6](http://www.jbc.org/)

**FIG. 6.** RFP and EPC repress transcription independent of enhancer or promoter differences. A, physical map of each effector and reporter constructs. The effector plasmids were constructed by ligating GAL4-binding domain (GAL4BD) in-frame with full-length RFP or EPC cDNA. The reporter plasmids contain five tandem repeats of GAL4-binding sites (5 × GAL4), followed by SRE or CRE as an enhancer and SV40 promoter or herpes simplex virus-thymidine kinase minimal promoter (Thm). B, repressive activity of RFP and EPC. Different reporter genes were co-transfected with the GAL4-RFP or GAL4-EPC effector constructs into 293 cells. After transfection, CRE was enhanced by 100 μM forskolin for 2 h. Luciferase activities in cells transfected with the plasmid containing the GAL4-binding domain alone were set at 100% and luciferase activities of cells transfected with the designated effector plasmids were expressed as percentages of control value ± S.E. Each value represents a result of at least three experiments.
gene silencing by forming multiprotein complex. Furthermore, it is important to point out that RFP represses the gene transcription independent of the enhancer or promoter differences, suggesting that the RFP-mediated gene silencing may be epigenetic, cooperating with the Polycomb group proteins.

_Dual Functions of EPC in Transcription—_EPC is classified as a Polycomb group gene and supposed to function as a transcriptional repressor. However, this study indicated that the repressive activity of EPC is relatively weak and surprisingly, the conserved EPcA domain functions as a strong transcriptional activator. EPC was identified in many species and especially the EPcA domain was known to be as the largest conserved domain of EPC (29).

Another Polycomb group protein, E(z), *Drosophila* homologue of human EZH, is also suspected to have a dual function. E(z) cannot only act in gene silencing but also be involved in the maintenance of transcriptional activity by trithorax group proteins (34). Although E(z) associates with Esc, the *Drosophila* homologue of human EED, and works as the repressor (32, 49, 50), this association seems to be restricted in blastoderm and early gastulation stage embryos in *Drosophila* (48, 51). The double heterozygous combination of recessive loss of function mutation of E(z) and trithorax group gene *ash1* alleles expresses homeotic transformation phenotypes similar to those expressed by double heterozygous combination of recessive loss of function trithorax and *ash1* alleles in *Drosophila* (52). E(z) encodes a SET domain that is also present in trithorax group genes (53). These evidences suggested that E(z) may be involved in the complex formation of both the Polycomb group proteins and the trithorax group proteins, probably depending on the developmental stages. E(Pc) and E(z) are different from other polycomb group proteins because their mutations also function as _Su(var)s_ (the suppressor of PEV) that is associated with the heterochromatin formation (29, 32, 33). These findings suggest that E(Pc)/EPC and E(z)/EZH have an additional function different from the function of other Polycomb group proteins.

It is not clarified how the EPcA domain acts as a transactivator. It is possible to speculate that the EPcA domain itself has the transcription activating function or this domain binds
another transactivator such as trithorax group proteins. In this respect, it is interesting to note that mouse homologues, Epc1 and Epc2, have longer (Epc1-L and Epc2-L) and shorter (Epc1-S and Epc2-S) forms (29). Epc1-L and Epc2-L contain the EpcA, EpcB, and EpcC domains and the glutamine-rich region, whereas Epc1-S and Epc2-S have the EpcA domain and the alanine-rich region only (Fig. 1B). Thus, we speculate that Epc1-S and Epc2-S could function primarily as the transcription activator like trithrax group proteins, whereas Epc1-L and Epc2-L can function as the repressor like Polycomb group proteins rather than the transactivator. In addition, weak repressive function of human EPC that we observed may be due to the presence of the EpcA domain.

In summary, we found that RFP could function as the strong transcription repressor in mammalian cells. The fact that RFP and EPC associate and mostly colocalize in the nucleus suggests that EPC may be involved in the epigenetic gene silencing mediated by RFP. In addition, EPC appears to have quite different functions depending on each domain, and especially the conserved EpcA domain acts as the transcriptional activator. Further study will provide new insights into possible unique roles of EPC and RFP in the gene silencing as well as the transactivation that are important for cell growth and/or differentiation.

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REFERENCES
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