Published in final edited form as:

Curr Genet. 2018 February ; 64(1): 147–154. doi:10.1007/s00294-017-0742-3.

Critical genomic regulation mediated by Enhancer of Polycomb

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Abstract

Enhancer of Polycomb (EPC) was first identified for its contributions to development in *Drosophila* and was soon-thereafter purified as a subunit of the NuA4/TIP60 acetyltransferase complex. Since then, EPC has often been left in the shadows as an essential, yet non-catalytic subunit of NuA4/TIP60; however, its deep conservation and disease association make clear that it warrants additional attention. In fact, recent studies in yeast demonstrated that its Enhancer of Polycomb, Epl1, was just as important for gene expression and acetylation as is the catalytic subunit of NuA4. Despite its conservation, studies of EPC have often remained siloed between organisms. Here, our goal is to provide a cohesive view of the current state of the EPC literature as it stands among the major model organisms in which it has been studied. EPC is involved in multiple processes, beginning with its cardinal role in regulating global and targeted histone acetylation. EPC also frequently serves as an important interaction partner in these basic cellular functions, as well as in multicellular development, such as in hematopoiesis and skeletal muscle differentiation, and in human disease. Taken together, a unifying theme from these studies highlights EPC as a critical genomic regulator.

Keywords

Epl1; EPC1; EPC2; E(Pc); NuA4; Chromatin

Enhancer of Polycomb is broadly conserved

The NuA4/TIP60 complex is a multimeric lysine acetyltransferase complex that has been characterized in multiple species (Doyon and Côté 2004) for its role in modification of chromatin and other protein substrates. As is the case with many enzymatic complexes, defining the function of noncatalytic subunits is often challenging, despite the potential for crucial contributions within or beyond the holo-complex. Establishing these functions often requires integrated genetic, genomic, and biochemical analyses. For subunits that are broadly conserved, these efforts can be aided by the compilation of studies from many organisms. Enhancer of Polycomb (EPC) is one such NuA4/TIP60 subunit with no known catalytic activity, though it has been annotated in more than 65 species¹¹ Analysis of conservation performed with Ensembl Compara, release 89. (Aken et al. 2016). Here, we

highlight both key recent and historical studies that together provide a comprehensive overview of EPC function. Specifically, we focus on studies that have contributed to the understanding of EPC as an individual protein rather than detailing the diverse functions of the NuA4 complex as a whole.

Enhancer of Polycomb was first characterized as E(Pc) in *Drosophila melanogaster*, as a “new enhancer of polycomb” (Sato et al. 1983). *E(Pc)* mutants did not have homeotic phenotypes as did the Polycomb group mutants, which have defects in silencing HOX gene expression (Kassis et al. 2017). Instead *E(Pc)* mutants acted as dominant enhancers of Polycomb group mutants in adult flies, indicating unique underlying genetic interactions; *E(Pc)^{-/-}* flies, however, are themselves embryonic lethal (Cheng et al. 1994; Sato et al. 1983; Soto et al. 1995). Early phenotypic characterization of E(Pc) also led to the observation that *E(Pc)* was a suppressor of position-effect variegation, a phenotype generally associated with non-histone chromatin proteins that influence the spread of heterochromatin (Clegg et al. 1998; Sinclair et al. 1998). This was a timely observation, as several months later, orthologs of E(Pc) were identified by sequence homology in yeast (Epl1), mammals (EPC1), and *C. elegans* (Stankunas et al. 1998), with plant species soon to follow (Springer et al. 2002). Within 2 years, yeast Epl1 was identified as an essential subunit in the NuA4 acetyltransferase complex (Galarneau et al. 2000). This early cross-species identification (Table 1) promoted concurrent multi-organism studies of EPC, and overall, led to an enhanced understanding of function.

Comparative studies between model organisms promote functional definition

Whereas the earliest studies of E(Pc) relied on *Drosophila* phenotypic characterization, a deepened molecular understanding of E(Pc) was gained from fundamental genetic and biochemical experiments in *Saccharomyces cerevisiae*. Similar to *Drosophila*, yeast Epl1 was found to be essential for viability (Galarneau et al. 2000), and low-dosage alleles of Epl1 indicated its importance in progression through the cell cycle, response to DNA damage, histone H4 and H2A acetylation, gene silencing, and in autophagy (Boudreault et al. 2003; Yi et al. 2012).

Many of Epl1's functions have been defined based upon domain structure, dividing Epl1 into a non-essential C-terminus and an essential N-terminus (Fig. 1). The C-terminus is quite variable in sequence among species, although it does serve to tether piccolo-NuA4 subunits to the NuA4 holo-complex and targets the acetyltransferase, Esa1, to chromatin (Boudreault et al. 2003; Searle et al. 2017). In contrast, the conserved N-terminus of Epl1, known as the EPcA domain, physically interacts with the NuA4 subunits Yng2, Eaf6, and the acetyltransferase, Esa1, which collectively with Epl1 are known as piccolo-NuA4 (Fig. 2a) (Boudreault et al. 2003; Mitchell et al. 2008; Rossetto et al. 2014). Epl1, through its EPcA region, is critical for Esa1 acetyltransferase activity, especially toward nucleosomes in vitro, and further contributes to target specificity (Berndsen et al. 2007; Chittuluru et al. 2011; Huang and Tan 2013; Kuo et al. 2015; Lalonde et al. 2013; Selleck et al. 2005). Most recently, the structure of the EPcA domain was solved in complex with the other piccolo-

NuA4 subunits, and the first bypass mutant of *EPL1* was identified using genetic suppression analysis that has been so powerful for the study of many critical proteins and biological processes (Hughes 2016; Prelich 1999; van Leeuwen et al. 2017). These studies, building on earlier suppression of non-null alleles (Lin et al. 2008), implicated Epl1 as being a critical Esa1 co-factor, and highlighted the importance of the physical Epl1-Esa1 interactions for acetyltransferase activity (Searle et al. 2017; Xu et al. 2016).

Whereas yeast studies shed light on the basic cellular function of Epl1, and demonstrated its importance in chromatin regulation, studies in multicellular organisms allowed for expansion of these seminal results to understand how Epl1 is involved in other cellular processes. Early studies of E(Pc) in *Drosophila* illustrated its critical role in chromatin regulation and we learned that E(Pc) is also involved in genomic imprint maintenance in *Drosophila*, likely through its role in heterochromatin maintenance (Joanis and Lloyd 2002). E(Pc) has been further highlighted for its important interactions (Table 2) with various genes and proteins involved in apoptosis and chromatin regulation, such as with *ISWI* (Imitation SWI), *His1* (Histone H1), and Polycomb group genes (Ali and Bender 2004; Arancio et al. 2010; Fullard and Baker 2015; Kavi et al. 2015). As in yeast, E(Pc) is important in the cell cycle in *Drosophila*, where it is required during development for mitotic exit during the transition to a post-mitotic state (Flegel et al. 2016). Additionally, there is evidence to suggest that E(Pc) is also important in DNA damage repair, whereby mutation increases the rate of homologous recombination (Holmes et al. 2006).

Developmental work in *Drosophila* revealed E(Pc)'s involvement in differentiation and stem cell-fate determination. E(Pc) is downregulated upon activation of the JNK (Jun amino-terminal kinase) signaling pathway in imaginal disc cells undergoing regeneration. This promotes wound healing, giving rise to most of the major structures in the adult fly (Lee et al. 2005). In multipotent hematopoietic progenitors, E(Pc) again acts downstream of JNK, here in combination with FoxO (Forkhead box protein O transcription factor), to trigger cellular differentiation (Owusu-Ansah and Banerjee 2009). E(Pc) was also identified as a regulator of cell fate and differentiation in intestinal stem cells and germ cells in the testes, respectively (Feng et al. 2017; Zeng et al. 2015). Related MAP-kinase signaling has also been linked to heterochromatin formation in yeast, providing additional relevance to the EPC–JNK relationship (Mazor and Kupiec 2009). Together, these examples highlight the importance of the EPC–JNK regulation axis in fly development.

In addition to pioneering work in *Drosophila* and yeast, recent progress has been made in studies of EPC in additional metazoans (Table 1), including *C. elegans* and *D. rerio*. These studies began to hint at roles for EPC in oncogenesis, perhaps not surprisingly, given its central role in chromatin regulation and in stem cell identity. Knockdown of *epc1* was found to decrease lifespan in a *daf-16*-dependent manner in *C. elegans*, and was found to be a Ras antagonist in the regulation of cell division and cell-fate determination (Ceol and Horvitz 2004; Kim and Sun 2007). Additionally, analogous to *Drosophila* studies, *epc2* was found to regulate hematopoietic development in zebrafish, specifically in the development of primitive erythroid cells. In this case knockdown of *epc2* was consistent with a role in mesodermal precursor differentiation in blood development via upregulation of *scl*, *gata1*, and *βe3-globin* (Huang et al. 2013). These studies add further support for EPC as a critical

regulator of cellular processes, from early development through subsequent aging and development of disease.

Human EPC was first purified in MCF7 and HeLa cell lines as a subunit of the NuA4/TIP60 complex. Both splice variants and paralogs EPC1 and EPC2, were concurrently identified (Doyon et al. 2004). EPC1 was found to tether MBTD1 (Malignant Brain Tumor Domain Containing 1) to the human TIP60 complex, promoting TIP60-driven repair of DNA double stranded breaks by homologous recombination (Jacquet et al. 2016).

Studies of EPC1 outside the NuA4/TIP60 complex in mammals have pointed to roles for it and EPC2 that are independent of their canonical roles as NuA4 subunits. Beyond NuA4, EPC1/2 interacts with other proteins, supporting the presence of additional novel functions in mice and humans (Fig. 2b). For example, a unique interaction between EPC1 and RFP (RET Finger Protein) was identified in mice. Specifically, a glycosylated form of RFP was found to interact with the C-terminus of EPC1 in repressive activities, whereas the EPcA domain of EPC1 was found to have transcriptional activating activities (Shimono et al. 2000; Tezel et al. 2002). EPC1 was also identified as an E2F6 (E2F Transcription Factor 6) binding partner, and furthermore was found to exist in a distinct stable complex in vitro and in vivo with E2F6 and DP1. This complex was found to exist in proliferating normal and transformed human cells and to co-elute with Sin3B to promote repressive activities (Attwooll et al. 2005). Finally, the paralog EPC2 was shown to interact with EZH2 in human colorectal cancer cells, with an involvement in transcriptional regulation (Guil et al. 2012).

The diverse interactions of EPC1 and EPC2 begin to point toward specialized roles for each paralog, hinting at cell-type and developmental stage-specific EPC-containing complexes. This observation may be particularly noteworthy especially considering the translational importance of EPC1 and EPC2 that has begun to be defined in recent years.

From basic function to translational significance

With its well-established, broadly critical genetic roles, it is not surprising that studies in more recent years have also shed light on the clinical importance of EPC1 and EPC2, both in patient samples and in murine models of human disease. EPC1 and EPC2 have been primarily implicated in basic cancer biology and metastasis, and have also been found to function in skeletal muscle differentiation. Many of these examples highlight EPC1 and EPC2 apart from NuA4/TIP60, and underscore the importance of EPC's diverse interacting partners.

EPC1 has been mechanistically implicated in metastasis. For example, it was found that EPC1 activates E2F1 (E2F Transcription Factor 1), leading to the upregulation of anti-apoptotic survival genes. This triggers a metastasis-related gene signature that is prognostic of poor patient outcome. Cisplatin treatment of cancer cell lines, such as SK-Mel-147 melanoma cells, resulted in upregulation of *EPC1*, further pointing towards EPC1 enabling survival of cancer cells. Accordingly, knockdown of *EPC1* led to increased DNA damage sensitivity and apoptosis in an E2F1-dependent mechanism (Wang et al. 2016).

EPC is also genetically altered in several cancers, including both hematological cancers and solid tumors. Basic findings in zebrafish, illustrating a role for EPC in blood development (Huang et al. 2013), may lead to further insights for multiple roles of EPC in leukemia and other hematological conditions. For example, *EPC1* expression is downregulated in leukemia cells as compared to its expression in hematopoietic progenitor cells, and has been found as a breakpoint site in adults with T-cell leukemia (Nakahata et al. 2009; Prasad et al. 2014). Both EPC1 and EPC2 are required for acute myeloid leukemia cell proliferation; knockdown of *EPC1* and/or *EPC2* leads to accumulation of *MYC* in acute myeloid leukemia cells, contributing to selective apoptosis (Huang et al. 2014).

EPC1 is also a reported site of breakpoints in solid tumors, such as in endometrial stromal sarcoma, though these EPC1 translocations account for a minority of reported cases (Chiang et al. 2011; Micci et al. 2006). EPC is also altered in sequence and in copy number, such as in early sporadic pancreatic ductal adenocarcinoma, where *EPC1*, and to a lesser frequency, *EPC2* are mutated and have a loss of heterozygosity (Biankin et al. 2012). Finally, a site of common genetic variation within the second intron of *EPC2* was reported to elicit differential response to gemcitabine, common chemotherapeutic agent (Jarjanazi et al. 2008). This points beyond the demonstrated importance of EPC in cancer biology, to triggering a differential response to cancer therapy.

Discussion above illustrated the importance of EPC in cellular differentiation and development. This is also highlighted in EPC1's role in spermatogenesis in mice (Dong et al. 2017), as well as in applied models of skeletal muscle differentiation. EPC1 regulates skeletal muscle differentiation through interaction with HOP (Homeodomain Only Protein) and also recruits Serum Response Factor (SRF) and p300, in a manner that appears to be independent of NuA4/TIP60. Indeed, TIP60 is undetectable in various muscle cell lines and tissues (Kee et al. 2007; Kim et al. 2009). The positive regulation of skeletal muscle differentiation by the EPC1-HOP interaction is opposed by an interaction between EPC1 and RFP, whereby RFP blocks the skeletal muscle differentiation that is induced by the collaboration of EPC1 and HOP (Kee et al. 2012). Knowledge of this role of EPC1 in muscle differentiation may become directly applicable in a clinical setting. In a model of arterial injury, it was found that local delivery of EPC1 reduced formation of scar tissue in smooth muscle by promoting vascular smooth muscle cell differentiation (Joung et al. 2012). Whereas translational studies of EPC are, to date, more limited than those of TIP60, those discussed here underscore the role of EPC as a critical genomic regulator perhaps ultimately bridging basic cellular functions to clinical significance.

An eye to the future

Since the initial discovery as a developmental mutant in *Drosophila* in the early 1980s, EPC has been characterized as an essential chromatin protein involved in many cellular processes. Our understanding of EPC has benefited from studies in multiple organisms, from the single-celled budding yeast, where it was found to be an essential co-factor to an acetyltransferase, to multi-organ-system models and humans, where it is a key player in a growing number of translational studies. Despite the progress that has been made, there are many questions that remain to be addressed.

Building on earlier work, recent, mutagenesis- and structure-based studies in *S. cerevisiae* have assigned function to many residues of Epl1 (Searle et al. 2017; Xu et al. 2016), promoting a great expansion in understanding the importance of specific residues and their corresponding roles. Similar studies in other organisms might prove fruitful. For example, although the EPcA domain is highly conserved, and thought to generally encompass a protein-binding domain in a selection of chromatin proteins (Perry 2006), the C-terminus is much more variable. Some metazoans, for example, have additional domains termed EPc-B and EPc-C within the C-terminus of their Epl1-orthologs; however, these are not ubiquitous as is EPcA (Fig. 1). Although some studies have assigned domains required for interactions reported here (Table 2), a CRISPR-Cas9-based approach would facilitate a more comprehensive functional assignment for specific residues, and may correspondingly be used to test the significance of cancer-associated mutations. Along these lines, a recent study reported generation of a viable *Epc1*^{-/-} mouse (Dong et al. 2017), whereas previous studies demonstrated that homozygous deletion of *Epc1* resulted in embryonic lethality (Kim et al. 2009). The specific *Epc1* residues involved in the disruption might explain this apparent disparity in the necessity for EPC1 in mice. The viable *Epc1*^{-/-} mice were generated by disrupting exons 3–5 (Dong et al. 2017) thereby leaving the EPcA domain largely intact, whereas the non-viable homozygous null *Epc1* knockout mice involved disruption within the first exon (Kim et al. 2009). These differing results underscore the importance of considering structure–function when assessing EPC.

Another important issue not yet thoroughly addressed is the significance of the duplication of EPC1 to EPC2. Throughout the course of evolution, a paralog of Enhancer of Polycomb, EPC2, arose by duplication in zebrafish, rodents, and humans. Multiple studies have highlighted either EPC1 or EPC2 independently, yet it generally remains unclear under what conditions one paralog may be preferentially critical. Neither EPC1 nor EPC2 can functionally replace yeast Epl1 (Hamza et al. 2015). However, it is possible that coexpression of EPC1 and EPC2 might promote viability in *epl1*⁻, and shed light on the importance of both paralogs.

How many distinct moonlighting roles might EPC have beyond NuA4/TIP60 complexes? As noted, studies have described functions of EPC appearing independent of NuA4/piccolo-NuA4, either as a part of a distinct complex (Attwooll et al. 2005), or as presumed due to expression patterns distinct from NuA4 (Kim et al. 2009). Further, several large-scale proteomic and genomic screens returned EPC as a hit without identifying other NuA4 subunits, such as TIP60 (Huang et al. 2013; Kim and Sun 2007; Zeng et al. 2015). It is possible that EPC exists in additional multimeric complexes, yet to be identified, which for example, may assemble upon specific stimuli or during particular developmental states. EPC function may also be fine-tuned by splice variants, many of which have been identified in metazoans (Table 1).

Finally, although several studies have pointed to EPC1/2 alterations in cancer, few have yet to shed light on the functional significance of EPC alterations in cancer biology. For example, in the case where EPC1 is downregulated in leukemia (Prasad et al. 2014), is this downregulation EPC-specific, or does it have more broad effects on NuA4 complex dynamics and stoichiometry? One could speculate that in the latter case, if NuA4 activity

were altered, acetylation of histone-substrates would be disrupted. Non-histone NuA4 substrates have also been identified in humans [reviewed in (Avvakumov and Côté 2007; Lee and Workman 2007; Squatrito et al. 2006)] and in yeast (Downey et al. 2015; Lin et al. 2009; Mitchell et al. 2013; Yi et al. 2012). In some cases, the effect of diminished catalytic activity has also been evaluated. Pursuing the corresponding effect of EPC alteration on these substrates will be an important direction for future research. Indeed, much remains to be learned about EPC, building on the significant progress that has been made as evident by the substantial body of work from yeast to humans summarized here.

Acknowledgements

We thank those who have made contributions to the EPC literature, including those whose work was beyond the scope of this review. Additionally, we thank Emily Petty for suggestions and critical feedback on this manuscript. This work was supported by the UC-CRCC and support from NIH T32 GM008666, the UCSD Frontiers of Innovation Scholars Program Graduate Fellowship, and the UCSD Section of Molecular Biology Cancer Fellowship (NES).

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Fig. 1.

Epl1 domain structure in *S. cerevisiae*. Epl1 contains the essential and conserved EpCA domain, broken down into three subdomains, named for their initial characterization based on physical interaction [as modified from (Boudreault et al. 2003; Chittuluru et al. 2011; Searle et al. 2017; Selleck et al. 2005)]. The C-terminus is highly variable among species, and accounts for the bulk of the differences in size of orthologs listed in Table 1. Some metazoans do have shorter stretches of conserved residues within the C-terminus, referred to as EPc-B and EPc-C (Stankunas et al. 1998)

Fig. 2.

EPC has widespread functions as evidenced by multimeric complex diversity and physical interactors. **a** EPC is best characterized as a subunit of the NuA4/TIP60 complex and the smaller piccolo-NuA4 complex. **b** However, it is also present in at least one other multimeric complex, and has been verified as critical in several physical interactions with functional implications, as illustrated here that are noted in the text, some of which may be independent of NuA4/TIP60. The EPC1-RFP interaction is dependent on a glycosylated form of RFP. The interactions included here are limited and it is likely that additional proteins or post-translational modifications will be identified potentially as part of these interactions. Domains where characterized interactions occur are noted in accordance with the labeling in Fig. 1. The metazoan NuA4/TIP60 complex contains several additional subunits as recently described (Jacquet et al. 2016)

Table 1

Enhancer of Polycomb orthologs

Organism	Name	Size (aa)	Paralog	
			Name	Size
<i>S. cerevisiae</i>	Epl1	832	–	
<i>D. melanogaster</i>	E(Pc)	2023	–	
<i>C. elegans</i>	epc-1	795	–	
<i>D. rerio</i>	epc1a	796	epc2	751
	epc1b	809		
<i>M. musculus</i>	EPC1	813	EPC2	808
<i>H. sapiens</i>	EPC1	836	EPC2	807

A selection from among the >65 known orthologs highlights those discussed, including their species-specific names, size (in amino acids), and any corresponding paralogs. This table refers to only the major splice variants; however, there are additional splice variants in many species

Table 2

EPC interactors and effectors referenced in text

Interactor	Species	Description
Iswi	<i>D. melanogaster</i>	ATPase member of chromatin remodeling complexes
His1	<i>D. melanogaster</i>	Linker histone H1
JNK	<i>D. melanogaster</i>	Jun amino-terminal kinase, a mitogen activated protein (MAP) kinase
FoxO	<i>D. melanogaster</i>	Forkhead box protein O transcription factor
RAS	<i>C. elegans</i>	Small GTP-ase signaling protein, with established oncogenic properties in mammals
<i>scl</i>	<i>D. rerio</i>	Transcription factor critical for hematopoietic development
<i>gata1</i>	<i>D. rerio</i>	Erythroid-specific transcription factor
<i>βe3-globin</i>	<i>D. rerio</i>	Hemoglobin beta embryonic-3
RFP	<i>H. sapiens/M. musculus</i>	RET finger protein in the large B-box RING finger protein family
E2F6	<i>H. sapiens</i>	E2F transcription factor, critical in cell cycle regulation
DP1	<i>H. sapiens</i>	Transcription factor that heterodimerizes with E2F proteins to stimulate their transcription
Sin3B	<i>H. sapiens</i>	SIN3 transcriptional regulator
EZH2	<i>H. sapiens</i>	Enhancer of Zeste homolog 2, histone methyltransferase activity
HOP	<i>H. sapiens/M. musculus/R. norvegicus</i>	Hsp70–Hsp90 organizing protein, a co-chaperone in the stress-inducible (STI) family of proteins
SRF	<i>H. sapiens/M. musculus</i>	Serum response factor, a master regulator transcription factor required for many processes, including cardiac development
p300	<i>M. musculus/R. norvegicus</i>	Transcriptional co-activator with a histone acetyltransferase domain, bromodomain, and a PHD finger domain

Additional interactions have been reported via genome-wide screens and other methods. This list and review highlight verified interactions of functional relevance, including the species for which the interaction was reported