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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 holocomplex. 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 species[11] Analysis of conservation performed with Ensembl Compara, release 89. (Aken et al. 2016). Here, we

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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-

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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 is it 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

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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.

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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

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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.

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References

Aken BL et al. (2016) The Ensembl gene annotation system. Database-Oxford doi:10.1093/database/baw093

Ali JY, Bender W (2004) Cross-regulation among the polycomb group genes in Drosophila melanogaster. Mol Cell Biol 24:7737–7747. doi:10.1128/MCB.24.17.7737-7747.2004 [PubMed: 15314179]

Arancio W et al. (2010) The nucleosome remodeling factor ISWI functionally interacts with an evolutionarily conserved network of cellular factors. Genetics 185:129–140. doi:10.1534/genetics.110.114256 [PubMed: 20194965]

Attwooll C et al. (2005) A novel repressive E2F6 complex containing the polycomb group protein, EPC1, that interacts with EZH2 in a proliferation-specific manner. J Biol Chem 280:1199–1208. doi:10.1074/jbc.M412509200 [PubMed: 15536069]

Avvakumov N, Côté J (2007) The MYST family of histone acetyltransferases and their intimate links to cancer. Oncogene 26:5395–5407. doi:10.1038/sj.onc.1210608 [PubMed: 17694081]

Berndsen CE, Selleck W, McBryant SJ, Hansen JC, Tan S, Denu JM (2007) Nucleosome recognition by the Piccolo NuA4 histone acetyltransferase complex. Biochemistry 46:2091–2099. doi:10.1021/bi602366n [PubMed: 17274630]

Biankin AV et al. (2012) Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 491:399–405. doi:10.1038/nature11547 [PubMed: 23103869]

Boudreault AA et al. (2003) Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. Genes Dev 17:1415–1428. doi:10.1101/gad.1056603 [PubMed: 12782659]

Ceol CJ, Horvitz HR (2004) A new class of *C.* elegans synMuv genes implicates a Tip60/NuA4-like HAT complex as a negative regulator of Ras signaling. Dev Cell 6:563–576 [PubMed: 15068795]

Cheng NN, Sinclair DA, Campbell RB, Brock HW (1994) Interactions of polyhomeotic with Polycomb group genes of Drosophila melanogaster. Genetics 138:1151–1162 [PubMed: 7896097]

Chiang S et al. (2011) Frequency of known gene rearrangements in endometrial stromal tumors. Am J Surg Pathol 35:1364–1372. doi:10.1097/PAS.0b013e3182262743 [PubMed: 21836477]

Chittuluru JR et al. (2011) Structure and nucleosome interaction of the yeast NuA4 and Piccolo-NuA4 histone acetyltransferase complexes. Nat Struct Mol Biol 18:1196–1203. doi:10.1038/nsmb.2128 [PubMed: 21984211]

Clegg NJ et al. (1998) Suppressors of position-effect variegation in Drosophila melanogaster affect expression of the heterochromatic gene light in the absence of a chromosome rearrangement. Genome 41:495–503 [PubMed: 9796098]

Dong Y et al. (2017) EPC1/TIP60-mediated histone acetylation facilitates spermiogenesis in mice. Mol Cell Biol doi:10.1128/MCB.00082-17

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Downey M et al. (2015) Acetylome profiling reveals overlap in the regulation of diverse processes by sirtuins, gcn5, and esa1. Mol Cell Proteomics 14:162–176. doi:10.1074/mcp.M114.043141 [PubMed: 25381059]

Doyon Y, Côté J (2004) The highly conserved and multifunctional NuA4 HAT complex. Curr Opin Genet Dev 14:147–154. doi:10.1016/j.gde.2004.02.009 [PubMed: 15196461]

Doyon Y, Selleck W, Lane WS, Tan S, Côté J (2004) Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. Mol Cell Biol 24:1884–1896 [PubMed: 14966270]

Feng L, Shi Z, Chen X (2017) Enhancer of polycomb coordinates multiple signaling pathways to promote both cyst and germline stem cell differentiation in the Drosophila adult testis. PLoS Genet 13:e1006571. doi:10.1371/journal.pgen.1006571 [PubMed: 28196077]

Flegel K, Grushko O, Bolin K, Griggs E, Buttitta L (2016) Roles for the histone modifying and exchange complex NuA4 in cell cycle progression in Drosophila melanogaster. Genetics 203:1265–1281. doi:10.1534/genetics.116.188581 [PubMed: 27184390]

Fullard JF, Baker NE (2015) Signaling by the engulfment receptor draper: a screen in Drosophila melanogaster implicates cytoskeletal regulators, Jun N-terminal Kinase, and Yorkie. Genetics 199:117–134. doi:10.1534/genetics.114.172544 [PubMed: 25395664]

Galarneau L et al. (2000) Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. Mol Cell 5:927–937 [PubMed: 10911987]

Guil S et al. (2012) Intronic RNAs mediate EZH2 regulation of epigenetic targets. Nat Struct Mol Biol 19:664–670. doi:10.1038/nsmb.2315 [PubMed: 22659877]

Hamza A et al. (2015) Complementation of yeast genes with human genes as an experimental platform for functional testing of human genetic variants. Genetics 201:1263–1274. doi:10.1534/genetics.115.181099 [PubMed: 26354769]

Holmes AM, Weedmark KA, Gloor GB (2006) Mutations in the extra sex combs and Enhancer of Polycomb genes increase homologous recombination in somatic cells of Drosophila melanogaster. Genetics 172:2367–2377. doi:10.1534/genetics.105.042473 [PubMed: 16452150]

Huang J, Tan S (2013) Piccolo NuA4-catalyzed acetylation of nucleosomal histones: critical roles of an Esa1 Tudor/chromo barrel loop and an Epl1 Enhancer of Polycomb A (EPcA) basic region. Mol Cell Biol 33:159–169. doi:10.1128/MCB.01131-12 [PubMed: 23109429]

Huang HT et al. (2013) A network of epigenetic regulators guides developmental haematopoiesis in vivo. Nat Cell Biol 15:1516–1525. doi:10.1038/ncb2870 [PubMed: 24240475]

Huang X, Spencer GJ, Lynch JT, Ciceri F, Somerville TD, Somervaille TC (2014) Enhancers of polycomb EPC1 and EPC2 sustain the oncogenic potential of MLL leukemia stem cells. Leukemia 28:1081–1091. doi:10.1038/leu.2013.316 [PubMed: 24166297]

Hughes D (2016) Using the power of genetic suppressors to probe the essential functions of RNase E. Curr Genet 62:53–57. doi:10.1007/s00294-015-0510-1 [PubMed: 26232079]

Jacquet K et al. (2016) The TIP60 complex regulates bivalent chromatin recognition by 53BP1 through direct H4K20me binding and H2AK15 acetylation. Mol Cell 62:409–421. doi:10.1016/j.molcel.2016.03.031 [PubMed: 27153538]

Jarjanazi H et al. (2008) Discovery of genetic profiles impacting response to chemotherapy: application to gemcitabine. Hum Mutat 29:461–467. doi:10.1002/humu.20732 [PubMed: 18330920]

Joanis V, Lloyd VK (2002) Genomic imprinting in *Drosophila* is maintained by the products of Suppressor of variegation and trithorax group, but not Polycomb group, genes. Mol Genet Genomics 268:103–112. doi:10.1007/s00438-002-0731-0 [PubMed: 12242505]

Joung H et al. (2012) Enhancer of polycomb1 lessens neointima formation by potentiation of myocardin-induced smooth muscle differentiation. Atherosclerosis 222:84–91. doi:10.1016/j.atherosclerosis.2012.02.015 [PubMed: 22398275]

Kassis JA, Kennison JA, Tamkun JW (2017) Polycomb and Trithorax Group Genes in *Drosophila*. Genetics 206:1699–1725. doi:10.1534/genetics.115.185116 [PubMed: 28778878]

Kavi H, Lu X, Xu N, Bartholdy BA, Vershilova E, Skoultchi AI, Fyodorov DV (2015) A genetic screen and transcript profiling reveal a shared regulatory program for Drosophila linker histone H1

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and chromatin remodeler CHD1. G3 (Bethesda) 5:677–687. doi:10.1534/g3.115.016709 [PubMed: 25628309]

Kee HJ et al. (2007) Enhancer of polycomb1, a novel homeodomain only protein-binding partner, induces skeletal muscle differentiation. J Biol Chem 282:7700–7709. doi:10.1074/jbc.M611198200 [PubMed: 17192267]

Kee HJ et al. (2012) Ret finger protein inhibits muscle differentiation by modulating serum response factor and enhancer of polycomb1. Cell Death Differ 19:121–131. doi:10.1038/cdd.2011.72 [PubMed: 21637294]

Kim Y, Sun H (2007) Functional genomic approach to identify novel genes involved in the regulation of oxidative stress resistance and animal lifespan. Aging Cell 6:489–503. doi:10.1111/j.1474-9726.2007.00302.x [PubMed: 17608836]

Kim JR et al. (2009) Enhancer of polycomb1 acts on serum response factor to regulate skeletal muscle differentiation. J Biol Chem 284:16308–16316. doi:10.1074/jbc.M807725200 [PubMed: 19359245]

Kuo YM, Henry RA, Tan S, Côté J, Andrews AJ (2015) Site specificity analysis of Piccolo NuA4-mediated acetylation for differ-ent histone complexes. Biochem J 472:239–248. doi:10.1042/BJ20150654 [PubMed: 26420880]

Lalonde ME et al. (2013) Exchange of associated factors directs a switch in HBO1 acetyltransferase histone tail specificity. Genes Dev 27:2009–2024. doi:10.1101/gad.223396.113 [PubMed: 24065767]

Lee KK, Workman JL (2007) Histone acetyltransferase complexes: one size doesn't fit all. Nat Rev Mol Cell Biol 8:284–295. doi:10.1038/nrm2145 [PubMed: 17380162]

Lee N, Maurange C, Ringrose L, Paro R (2005) Suppression of Polycomb group proteins by JNK signalling induces transdetermination in Drosophila imaginal discs. Nature 438:234–237. doi:10.1038/nature04120 [PubMed: 16281037]

Lin YY et al. (2008) A comprehensive synthetic genetic interaction network governing yeast histone acetylation and deacetylation. Genes Dev 22:2062–2074. doi:10.1101/gad.1679508 [PubMed: 18676811]

Lin YY et al. (2009) Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. Cell 136:1073–1084. doi:10.1016/j.cell.2009.01.033 [PubMed: 19303850]

Mazor Y, Kupiec M (2009) Developmentally regulated MAPK pathways modulate heterochromatin in Saccharomyces cerevisiae. Nucleic Acids Res 37:4839–4849. doi:10.1093/nar/gkp512 [PubMed: 19520767]

Micci F, Panagopoulos I, Bjerkehagen B, Heim S (2006) Consistent rearrangement of chromosomal band 6p21 with generation of fusion genes JAZF1/PHF1 and EPC1/PHF1 in endometrial stromal sarcoma. Cancer Res 66:107–112. doi:10.1158/0008-5472.CAN-05-2485 [PubMed: 16397222]

Mitchell L, Lambert JP, Gerdes M, Al-Madhoun AS, Skerjanc IS, Figeys D, Baetz K (2008) Functional dissection of the NuA4 histone acetyltransferase reveals its role as a genetic hub and that Eaf1 is essential for complex integrity. Mol Cell Biol 28:2244–2256. doi:10.1128/MCB.01653-07 [PubMed: 18212056]

Mitchell L et al. (2013) mChIP-KAT-MS, a method to map protein interactions and acetylation sites for lysine acetyltransferases. Proc Natl Acad Sci USA 110:E1641–E1650. doi:10.1073/pnas.1218515110 [PubMed: 23572591]

Nakahata S et al. (2009) Alteration of enhancer of polycomb 1 at 10p11.2 is one of the genetic events leading to development of adult T-cell leukemia/lymphoma. Genes Chromosomes Cancer 48:768–776. doi:10.1002/gcc.20681 [PubMed: 19484761]

Owusu-Ansah E, Banerjee U (2009) Reactive oxygen species prime Drosophila haematopoietic progenitors for differentiation. Nature 461:537–541. doi:10.1038/nature08313 [PubMed: 19727075]

Perry J (2006) The Epc-N domain: a predicted protein-protein interaction domain found in select chromatin associated proteins. BMC Genomics 7:6. doi:10.1186/1471-2164-7-6 [PubMed: 16412250]

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Prasad P et al. (2014) High-throughput transcription profiling identifies putative epigenetic regulators of hematopoiesis. Blood 123:e46–e57. doi:10.1182/blood-2013-02-483537 [PubMed: 24671951]

Prelich G (1999) Suppression mechanisms: themes from variations. Trends Genet 15:261–266 [PubMed: 10390624]

Rossetto D et al. (2014) Eaf5/7/3 form a functionally independent NuA4 submodule linked to RNA polymerase II-coupled nucleosome recycling. EMBO J 33:1397–1415. doi:10.15252/embj.201386433 [PubMed: 24843044]

Sato T, Russell MA, Denell RE (1983) Homoeosis in Drosophila: a new enhancer of polycomb and related homoeotic mutations. Genetics 105:357–370 [PubMed: 17246164]

Searle NE, Torres-Machorro AL, Pillus L (2017) Chromatin regulation by the NuA4 acetyltransferase complex is mediated by essential interactions between Enhancer of Polycomb (Epl1) and Esa1. Genetics 205:1125–1137. doi:10.1534/genetics.116.197830 [PubMed: 28108589]

Selleck W, Fortin I, Sermwittayawong D, Côté J, Tan S (2005) The Saccharomyces cerevisiae Piccolo NuA4 histone acetyltransferase complex requires the Enhancer of Polycomb A domain and chromodomain to acetylate nucleosomes. Mol Cell Biol 25:5535–5542. doi:10.1128/MCB.25.13.5535-5542.2005 [PubMed: 15964809]

Shimono Y, Murakami H, Hasegawa Y, Takahashi M (2000) RET finger protein is a transcriptional repressor and interacts with enhancer of polycomb that has dual transcriptional functions. J Biol Chem 275:39411–39419. doi:10.1074/jbc.M006585200 [PubMed: 10976108]

Sinclair DA et al. (1998) Enhancer of Polycomb is a suppressor of position-effect variegation in *Drosophila melanogaster*. Genetics 148:211–220 [PubMed: 9475733]

Soto MC, Chou TB, Bender W (1995) Comparison of germline mosaics of genes in the Polycomb group of *Drosophila melanogaster*. Genetics 140:231–243 [PubMed: 7635288]

Springer NM, Danilevskaya ON, Hermon P, Helentjaris TG, Phillips RL, Kaeppler HF, Kaeppler SM (2002) Sequence relationships, conserved domains, and expression patterns for maize homologs of the polycomb group genes E(z), esc, and E(Pc). Plant Physiol 128:1332–1345. doi:10.1104/pp.010742 [PubMed: 11950982]

Squatrito M, Gorrini C, Amati B (2006) Tip60 in DNA damage response and growth control: many tricks in one HAT. Trends Cell Biol 16:433–442. doi:10.1016/j.tcb.2006.07.007 [PubMed: 16904321]

Stankunas K, Berger J, Ruse C, Sinclair DA, Randazzo F, Brock HW (1998) The enhancer of polycomb gene of *Drosophila* encodes a chromatin protein conserved in yeast and mammals. Development 125:4055–4066 [PubMed: 9735366]

Tezel G, Shimono Y, Murakumo Y, Kawai K, Fukuda T, Iwahashi N, Takahashi M (2002) Role for *O*-glycosylation of RFP in the interaction with enhancer of polycomb. Biochem Biophys Res Commun 290:409–414. doi:10.1006/bbrc.2001.6161 [PubMed: 11779184]

van Leeuwen J, Pons C, Boone C, Andrews BJ (2017) Mechanisms of suppression: the wiring of genetic resilience. BioEssays doi:10.1002/bies.201700042

Wang Y et al. (2016) Epigenetic factor EPC1 is a master regulator of DNA damage response by interacting with E2F1 to silence death and activate metastasis-related gene signatures. Nucleic Acids Res 44:117–133. doi:10.1093/nar/gkv885 [PubMed: 26350215]

Xu P et al. (2016) The NuA4 core complex acetylates nucleosomal histone H4 through a double recognition mechanism. Mol Cell 63:965–975. doi:10.1016/j.molcel.2016.07.024 [PubMed: 27594449]

Yi C et al. (2012) Function and molecular mechanism of acetylation in autophagy regulation. Science 336:474–477. doi:10.1126/science.1216990 [PubMed: 22539722]

Zeng X et al. (2015) Genome-wide RNAi screen identifies networks involved in intestinal stem cell regulation in *Drosophila*. Cell Rep 10:1226–1238. doi:10.1016/j.celrep.2015.01.051 [PubMed: 25704823]

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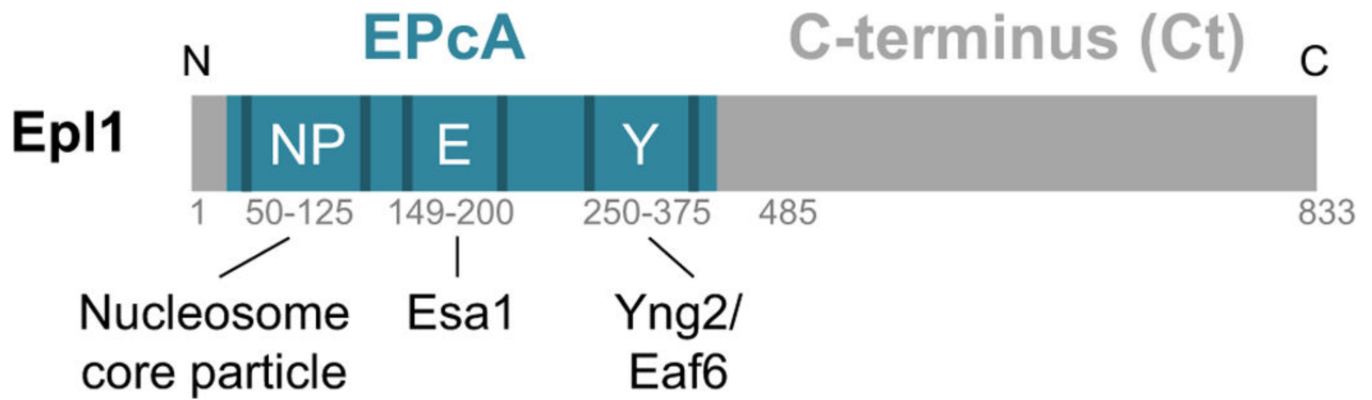

Fig. 1.

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)

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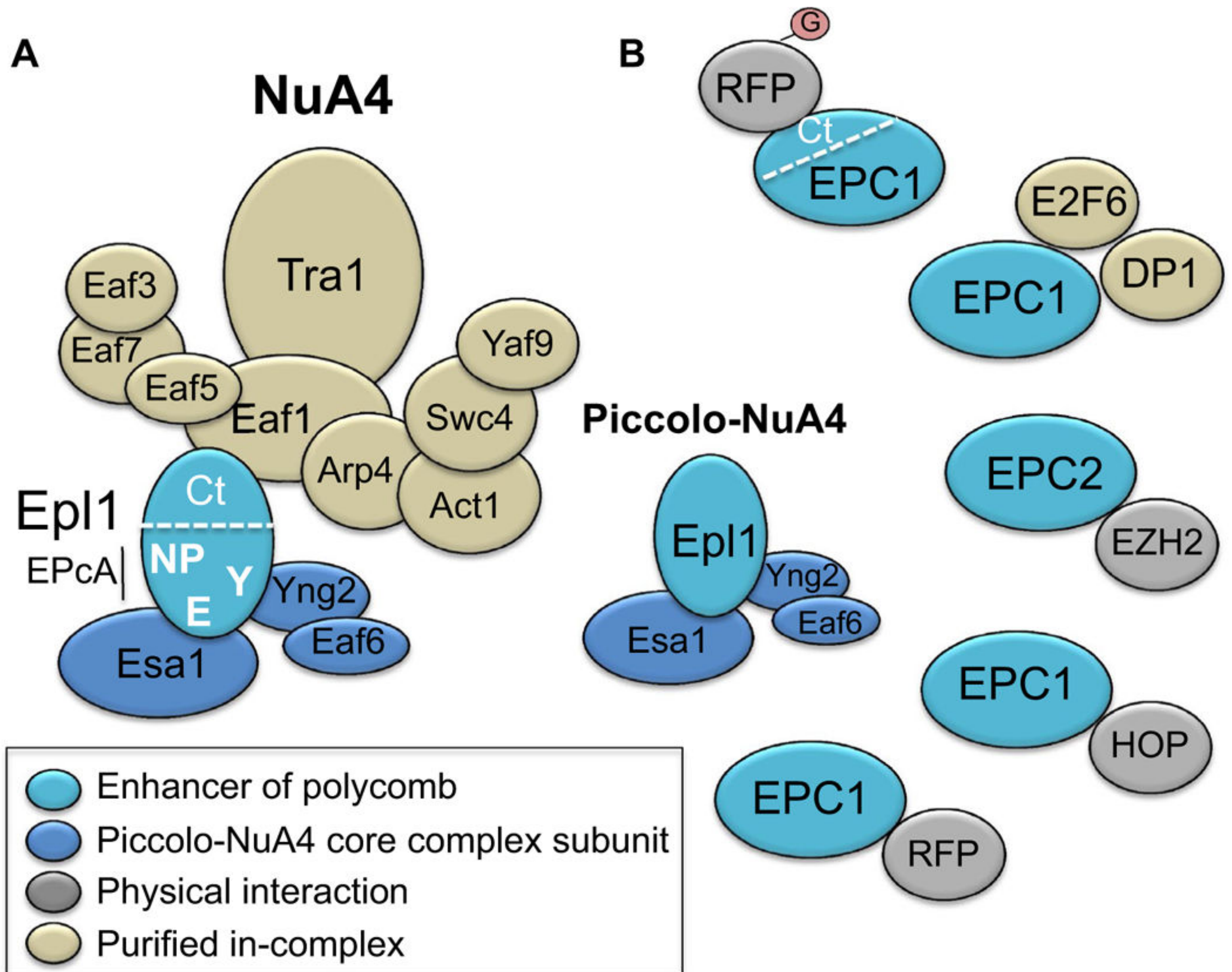

Fig. 2.

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)

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Table 1

Enhancer of Polycomb orthologs

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

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Table 2

EPC interactors and effectors referenced in text

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