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Complex functions of *Gcn5* and *Pcaf* in development and disease

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Abstract

A wealth of biochemical and cellular data, accumulated over several years by multiple groups, has provided a great degree of insight into the molecular mechanisms of actions of GCN5 and PCAF in gene activation. Studies of these lysine acetyltransferases (KATs) *in vitro*, in cultured cells, have revealed general mechanisms for their recruitment by sequence-specific binding factors and their molecular functions as transcriptional co-activators. Genetic studies indicate that GCN5 and PCAF are involved in multiple developmental processes in vertebrates, yet our understanding of their molecular functions in these contexts remains somewhat rudimentary. Understanding the functions of GCN5/PCAF in developmental processes provides clues to the roles of these KATs in disease states. Here we will review what is currently known about the developmental roles of GCN5 and PCAF, as well as emerging role of these KATs in oncogenesis.

Keywords

Gcn5; Pcaf; chromatin; development; cancer

1. Introduction

1.1. GCN5 and PCAF in vertebrates

The *Gcn5* locus was duplicated in vertebrates, giving rise to *Gcn5* (*GCN5L2*, *KAT2a*) and *Pcaf* (p300/CBP Associated Factor; *KAT2b*). Mammalian GCN5 and PCAF exhibit high sequence similarities (75% amino acid identity), and they have similar if not identical

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Author statement

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biochemical specificities. Both acetylate multiple lysines in histones and other proteins, such as transcription factors and cytoskeletal components, and they appear to be the primary KATs for acetylation of H3K9, at least in mouse embryonic fibroblasts (MEFs) [1]. GCN5 and PCAF are both incorporated into STAGA/TFTC complexes [2, 3]; hereafter referred to as SAGA) and ATAC complexes, but in a mutually exclusive way. However, *in vivo*, these KATs are not interchangeable, as indicated by different phenotypes upon mutation of *Gcn5* or *Pcaf* in mice. Loss of *Gcn5* causes early embryonic lethality whereas deletion of *Pcaf* causes no abnormalities, although double knockouts of both KATs results in an earlier and more severe phenotype than the loss of *Gcn5* alone, indicating some redundancy early during mouse embryogenesis [4, 5]. Despite numerous studies over the last 25 years, the unique and shared functions of these KATs in both gene regulation and development are still not clearly defined.

1.2 Conserved functional domains of GCN5/PCAF

The histone acetyltransferase (HAT) domain and the bromodomain in the carboxy-terminal of both mammalian GCN5 and PCAF are highly conserved with their yeast, protozoa and fly counterparts, highlighting the critical functions of these domains throughout evolution [2, 6] (see also reviews by Brownell & Allis and Berger, Grant & Winston in this special issue). However, mammalian GCN5 and PCAF have extended an amino-terminal domain not found in lower organisms that is important for binding to nucleosomes in vitro [2]. and that houses ubiquitin ligase activity [7, 8] (Fig. 1, top panels).

Both the HAT and the bromodomains of GCN5 and PCAF bridge interactions with both histones and transcription factors. For example, the PCAF HAT and bromodomains directly interact with Twist in *vitro* and this interaction inhibits PCAF acetyltransferase activity [9]. Despite the critical role of Twist in the inhibition of differentiation in multiple cell lineages, including muscle, bone and neuronal cells, the potential genetic and functional interactions of Twist and PCAF have not been further addressed, perhaps due to the lack of embryonic phenotypes in *Pcaf* null mice. In another example, the bromodomains of PCAF and GCN5 interact with two transcription factors involved in insulin response, IRF-1 and IRF-2, *in vitro* [10]. PCAF and GCN5 both bind acetylated tails of H3 and H4 [11] through their bromodomains, making them both “writers” and readers” of histone acetylation marks (see also review by Strahl & Briggs in this special issue).

The functional importance of the GCN5 bromodomain was first demonstrated in yeast where phenotypes of a GCN5-deleted strain were only partially complemented by a Gcn5 expression plasmid missing the bromodomain [12]. The bromodomain was later shown to facilitate nucleosome remodeling by the SWI/SNF complex [13] by anchoring the SAGA complex to nucleosomes. The bromodomain also promotes cooperative and cross-tail acetylation of H3 at K9/14/18/23 [14] (see also review by Strahl & Briggs in this special issue). However, the bromodomain of GCN5 is dispensable for HAT activity and gene activation. It remains to be seen whether the molecular functions of the bromodomain in vertebrates hold true to those in yeast. $Gcn5^{-/-}$ mice have a more severe phenotype than $Gcn5^{hat/hat}$ mice [15], indicating that GCN5 has functions outside of its HAT activity, and at least some of those functions may be mediated by the bromodomain. The GCN5/PCAF

bromodomain could provide a targetable domain in disease states, following the precedent of BET domain inhibitors used to combat specific human cancers.

2. *Gcn5 and Pcaf* functions during development

Multiple reports have defined GCN5 and PCAF functions *in vivo* through genetic studies in mice and other vertebrates. Animal development offers an excellent platform to define HAT functions in gene transcription. The dynamic nature of tissue and organ formation requires the integration of multiple spatial, temporal, and quantitative inputs to progressively define and adapt the properties of cells to their final destinations and functional status. Transcriptional regulation in concert with chromatin modulations presents a critical hub for signal integration necessary for fate decisions to be made.

2.1. Gcn5 is required for mesoderm specification and survival

The analysis of mouse knockout phenotypes clearly indicates that Gcn5 plays a critical role during mouse embryogenesis, whereas PCAF does not [4, 5]. These phenotypes are consistent with expression patterns of these HATs, as Gcn5 is strongly and widely expressed early during embryogenesis, but PCAF is expressed at very low levels, indicating PCAF likely has few or limited functions at this time [4, 5].

Gcn5 null embryos are defective in somitogenesis and neurulation. Histological analyses and characterization of lineage markers showed that although the primitive streak formed, downstream formation of paraxial mesoderm and the notochord was disrupted in the mutant embryos. Analysis of the affected mesodermal tissues revealed elevated levels of apoptosis that could be responsible for the disappearance of these lineages [4]. Subsequent studies support this idea, as elimination of p53 in *Gcn5* null mice prevented apoptosis and allowed embryos to survive until mid-gestation [15].

A role for *Gcn5* in the regulation of genes and pathways important for mesoderm specification is further indicated by morphological and molecular analyses of embryoid bodies derived from mouse embryonic stem cells (mESCs) devoid of *Gcn5*. These studies revealed deregulation of FGF signaling activity in the absence of Gcn5 [16]. FGF and downstream Erk1/2 and PI3K pathways are crucial for mouse ESC differentiation towards mesodermal and neural lineages [17]. Genome-wide analyses of $Gcn5^{-/-}$ embryoid bodies did not show a global change in H3K9ac levels, however, localized decreases in H3K9ac levels were observed near the promoters of multiple genes whose expression was affected by *Gcn5* loss [16]. *Gcn5* (but not *Pcaf* or *p300*) has also been identified as an interacting partner of linc1405, WDR5, and the transcription factor Eomes in mesoderm and cardiomyocytes in differentiating embryoid bodies. These factors are co-recruited to the well-characterized enhancer of Mesp1, which regulates a downstream gene transcriptional network responsible for cardiac lineage commitment [18]. This study offers mechanistic insights into how *Gcn5* is potentially recruited to key mesodermal genes though a combinatorial action of transcription factors and long non-coding RNAs (Fig. 1A). However, increased myogenic and cardiogenic potential was observed in embryoid bodies devoid of *Gcn5* [19], highlighting the need for additional studies of *Gcn5* functions in these lineages *in vivo*. Intriguingly, Pcaf was found to interact with the lncRNA *Myoparr* and the transcriptional co-

activator Ddx17 and regulate the transcription of *myogenin* in myogenic differentiation (Fig. 1D) [20]. The recruitment of PCAF to *myogenin* promoter probably involves interactions with MyoD and p300 [21, 22], but more experiments are required for the detailed characterization of myogenin promoter regulation.

Gcn5 involvement in the specification of mesodermal tissues is conserved in other vertebrates. Elegant experiments performed in developing *Xenopus* embryos to define the functions of TBP family members in actively transcribed genes discovered increased recruitment of *Gcn5* to a subset of TBP-family insensitive genes upon ablation of TBP and two related factors, TLF and TBP2. These findings indicate that Gcn5 is important for non-canonical, TBP-independent transcriptional regulation of a densely connected network of developmental genes in the *Xenopus* mesoderm [23].

2.2. Hypomorphic mutations in SAGA components reveal functions in Hox gene regulation

While the early embryonic lethality of *Gcn5* null embryos [5, 24] precludes the study of Gcn5 functions in later developmental processes, hypomorphic mutations that substantially lower Gcn5 expression or KAT function result in axial skeleton defects in lower thoracic regions, including rib fusions and homeotic transformations, along with spina bifida and exencephaly [15, 25, 26]. These rib fusions and homeotic transformations of lumbar L1 to thoracic T14 vertebrae are preceded by posterior shifts in the anterior boundary of Hoxc8 and Hoxc9 expression [25]. Interestingly, hypomorphic mutations in another SAGA component, Supt20 (Suppressor of Ty 20 Homolog), cause similar defects in rostral-caudal somite patterning [27]. Supt20 mutations affect the integrity of the SAGA complex, highlighting the importance of this Gcn5-containing complex - rather than ATAC - in somitic segmentation and Hox gene regulation. However, global *Gcn5* loss of function results in a more severe phenotype in mesoderm specification than does the loss of *Supt20* [28], suggesting that Gcn5 may also act outside of SAGA, either as part of a small ADA complex [29] or the ATAC complex in the developing mesoderm.

2.3 PCAF and Gcn5 interactions with p300/CBP

PCAF is so named due to its physical association in mammalian cell lines with CBP/p300 [30]. Mammalian PCAF was predicted early on to play a pivotal role in the control of cell growth and differentiation by integrating diverse signals for gene expression through direct interactions with the transcription machinery and targeted modifications of chromatin structure. Initial experiments functionally linked PCAF, together with CBP/p300, to transcriptional activators like MyoD, retinoic acid receptor, and CREB in the execution of developmental programs *in vitro* [31–33]. These results suggested that CBP/p300 and PCAF affect transcriptional regulation in part through histone acetylation and that PCAF is potentially recruited to a wide range of promoters via multiple protein-protein interactions (Fig. 1D, E, F). The complete lack of developmental phenotypes in *Pcaf* null mice suggests that *Gcn5* likely provides redundancy to these functions *in vivo*.

The first diversification of CBP/p300 and PCAF functions was suggested by the identification of different histone acetylation patterns within nucleosomal substrates [34]. A

direct comparison of the genome-wide distribution of CBP, p300, and PCAF in human T cells provided further evidence for their functional diversification, as CBP and p300 were associated both with enhancers and promoters, whereas PCAF was elevated along regions of actively transcribed genes beyond promoters [35]. Interestingly though, a high percentage of genes with PCAF bound at their promoters were also occupied by CBP and p300. PCAF and p300 (but not CBP) were found to associate with KLF8 at the Cyclin D1 promoter in MEFs. Their recruitment to the gene promoter increased the acetylation on H3 (Fig. 1F), offering an example of transcriptional activation mediated by both co-activators [36].

Gcn5 likely also interacts with p300/CBP, physically and functionally. Although mice heterozygous for either *Gcn5* or *p300* null alleles are viable, a significant fraction (25%) of *Gcn5*$^{+/-}$ *p300*$^{+/-}$ mice die before birth. In contrast, no abnormalities were observed in *Pcaf* $^{-/-}$ *p300*$^{+/-}$ mice, again suggesting Gcn5 is more important that Pcaf in executing early developmental programs [37]. Of course, Gcn5 and p300 also have distinct functions. For example, p300, together with BMP signaling, is important in establishing ‘pre-patterns’ of histone acetylation at liver and pancreas-specific genes in embryonic endoderm progenitors, but Gcn5 is dispensable [38].

2.4. Gcn5 KAT activity is important for neuronal development

A mouse model bearing point mutations in the catalytic acetyltransferase domain of Gcn5 provided more clues as to the importance of its KAT activity during development [15]. These *Gcn5*$^{hat/hat}$ mice exhibited defects in cranial neural tube closure with exencephaly. The neural folds normally lift and fuse at the hindbrain, the midbrain, and finally at the rostral end of the neural tube [39]. Neural folds of the mutant *Gcn5*$^{hat/hat}$ embryos remained open and dramatic exencephaly was observed from E11.5 onwards. Even though these embryos survived longer than *Gcn5* null mutants, they still died at E13.5 to E16.5 [15]. A detailed study of the *Gcn5*$^{hat/hat}$ mutant phenotype revealed a significant diencephalic expansion and telencephalic compression, indicating a functional requirement for Gcn5 catalytic activity in the developing diencephalon between E9.5 and E10.5 [40]. The expansion of the diencephalic tissue was linked to the deregulation of WNT signaling repression in the *Gcn5*$^{hat/hat}$ embryos, which acts upon Gli3 to limit Shh expression. Gcn5 was found in a complex with the transcription factor TACC1 and RARα in neuroepithelial cells isolated from E9.0 mouse forebrains. Recruitment of this complex to retinoic acid-responsive elements (RARE) of *Foxa1* and *Rarb* genes (Fig. 1B) was dependent on TACC1 acetylation mediated by Gcn5 acetyltransferase activity [40]. The Gcn5-mediated acetylation of TACC1 causes the dissociation of the transcription factor from the promoter and allows the transcription of the downstream targets. In the absence of Gcn5, the RA target genes remain repressed, the WNT signaling is not inhibited and Shh is derepressed causing an expansion of the zona limitans intrathalamica (ZLI) [40]. PCAF is also among the co-activators identified to interact with nuclear receptors and activate transcription of downstream targets [32]. Although there are implications of PCAF function in neuronal cells through its interaction with the RARβ receptor [41, 42], PCAF’s recruitment to gene promoters through RAR interactions is better characterized during the proliferation of preadipocytes (Fig.1E) [43].

Gcn5$^{hat/hat}$ mutant embryos also showed defects in the neural crest cell-derived craniofacial cartilage and bone. Cartilage and bone differentiation factors like *sox9a*, *cola1,* and *runx2a/2b* were reduced in the E12.5 mutant embryos. Interestingly the craniofacial abnormalities observed in *Gcn5*$^{hat/hat}$ mutant mouse embryos were also present in zebrafish devoid of either *Gcn5* or *Pcaf*, with a more severe phenotype in double *Gcn5/Pcaf* mutants [44]. Lack of Gcn5 was also found to cause decreased osteogenic differentiation in human periodontal ligament stem cells, again linked to aberrant activation of the WNT pathway, through the transcriptional deregulation of the WNT pathway inhibitor DKK1 [45].

2.5. Gcn5/PCAF functions in stem cell pluripotency and differentiation

mESCs have the capacity to self-renew indefinitely and the potential to give rise to all the somatic lineages and germ cells of a multicellular organism [46]. Both their pluripotency and differentiation potential *in vitro* is supported by a network of signaling, transcriptional, and epigenetic regulatory interactions [47]. Gcn5 has been identified as a critical component of a feedforward loop that controls Myc-dependent circuits in stem cells. Chromatin immunoprecipitation for the analysis of Gcn5 occupancy in undifferentiated ES cells revealed preferred recruitment to Myc and E2F1 binding motifs, and genes affected by *Gcn5* loss were enriched in Myc and E2F1 targets (Fig.1C). However, *Gcn5* loss of function in mouse embryos did not hinder the development of the blastocyst, and embryonic stem cells that lack *Gcn5* can be derived and cultured *in vitro* [19, 48], perhaps reflecting redundancies with *Pcaf* in ESCs. Connections with Myc functions are also indicated by overlapping gene expression programs observed in neurospheres devoid of Gcn5 or N-myc in *vitro*. Many of the genes affected by both *N-Myc* and *Gcn5* loss encoded components of signaling cascades, including the Ras and Wnt pathways. Downregulation of these genes in the absence of *Gcn5* or *N-myc* was associated with reduced levels of H3K9ac at their promoters, further highlighting a key role for Gcn5 in the regulation of these genes [49].

Myc is one of the four canonical “Yamanaka” factors that drive reprogramming of differentiated cells towards pluripotency. c-Myc was later found to be dispensable for inducing pluripotency in somatic cells [50], but it likely enhances reprogramming by augmenting transcription of the other pluripotency inducing factors, Sox2, Oct4, or KLF4, via direct promoter binding. A siRNA screen identified Gcn5 as required for reprogramming of fibroblasts to induced pluripotent stem cells (iPSCs) upon induction of expression of the Yamanaka factors [48]. Overexpression of GCN5 alone did not induce reprogramming in the absence of MYC, consistent with a need for MYC-dependent recruitment of GCN5 to downstream target genes. Interestingly, multiple other SAGA components were also identified as essential for somatic cell reprogramming in this screen (e.g. Taf12, Sgf29, and Trrap), indicating the importance of SAGA in iPSC formation. These findings are consistent with previous studies *in vitro* that indicated the TRRAP subunit of SAGA interacts directly with Myc [51, 52], although these interactions have not yet been confirmed in a developmental context. Interestingly, genome-wide analysis of TRRAP occupancy in mESCs shows significant overlap with Oct4 binding sites, supporting a functional role of TRRAP in the maintenance of self-renewal [53] that may reflect TRRAP functions in the Tip60 HAT complex [54].

3. Functional connections with other modifiers during development

Epigenetic writers and readers often work combinatorially to achieve specific developmental outcomes [55], but at present, we do not have a clear understanding of how GCN5 and PCAF might cooperate with other histone modifiers during embryogenesis. GCN5 and PCAF were identified as key writers for H3K9 and K14 acetylation [1] in mouse fibroblasts. H3K9/14ac and H3K4me3 are enriched along with RNA Pol II at the promoters of most active genes in human embryonic stem cells, suggesting GCN5 and PCAF might cooperate with MLL family members in this context [56]. Precedence for MLL-KAT interactions comes from observation of synergetic H3K4 methylation and histone acetylation at active genes, facilitated by physical interaction between trithorax and CBP, in drosophila [57]. Interactions between MLL1 and CBP also occur in human cells [58]. Besides, MLL1 physically interacts with another HAT, MOF, and joint recruitment of these factors regulates expression of Hoxa9 in MEFs as well as the closely correlated distribution of H3K4me3 and H4K16ac at other active genes [59].

Global deletion of the Moz KAT in mice showed a profound homeotic transformation in the skeleton and defects in the nervous system [60], somewhat similar to the phenotype of *Gcn5*$^{hat/hat}$ mutants. In the absence of Moz, H3K9ac levels were decreased at Hox gene promoters, even though Moz is generally thought of as an H4-specific HAT. MLL recruitment was also decreased at the Hox genes upon Moz deletion, suggesting functional interplay between MOZ and Mll1. Mll1-mediated methylation is stimulated by the presence of histone acetylation (H3K9ac/H3K14ac) on peptides [61]. Interestingly, despite the obvious deregulation of Hox genes patterning in Moz mutant embryos, the role of MOF acetyltransferase in the regulation of Hox genes remains elusive, as MOF gene deletion causes early embryonic lethality at implantation [62, 63]. How or if Gcn5/PCAF mediated acetylation of H3K9/K14 is connected to Moz functions has not been directly addressed, but the similarities in the homeotic phenotypes caused by loss or diminution of MLL1, MOZ and GCN5 are consistent with overlapping or synergistic functions. One major obstacle in dissecting the genome-wide functions of GCN5 and PCAF in different developmental contexts remains the low reproducibility of the available antibodies against Gcn5 and Pcaf in chromatin immunoprecipitation experiments in mammals. Genome-wide studies of GCN5 and PCAF, in conjunction with H3K9ac and H3K4me3 or H3K4me1 and H3K27ac in different developmental contexts, will shed light on their critical functions in promoters and enhancers and will help define developmental gene regulatory networks and/or their global impact on different steps of transcriptional progression.

4. Gcn5 and Pcaf functions in adult tissues and disease

In addition to their functions in developing embryos, both Gcn5 and Pcaf have important functions in adult cells. The use of conditional alleles has revealed functions for Gcn5 in specific tissues, such as immune cells, and molecular analyses have uncovered additional, non-histone substrates for Gcn5 and Pcaf. The importance of these KATs is also reflected by emerging studies implicating their importance in cancer.

4.1. Gcn5/Pcaf functions in hematopoietic cell development and immune function

Both *Gcn5* and *Pcaf* are implicated in the development and function of immune cells. Deletion of *Pcaf* in Foxp3$^+$ inducible T regulatory cells (iTregs) reduces the production of CD4$^+$ Foxp3$^+$ Tregs [64]. *Gcn5* deletion in iTregs does not appear to reduce the production of absolute numbers of Treg *in vivo*, however, loss of GCN5 and/or PCAF does affect their function as evidenced by allograft rejection and loss of IL-2 production [64, 65]. *Gcn5* deletion also impairs T cell development at other distinct stages. While mice with *Gcn5* conditional knockout in CD4 CD8 double-negative (DN) T cells do not show significant alteration to development or survival of this population [66], there is a decrease in single-positive CD4$^+$ and CD8$^+$ cells as well as a reduction of Treg cells in the spleen [65]. There is also an accumulation of DN3 stage cells and a reduction of DN4 stage cells suggesting that GCN5 regulates this transition. GCN5 is also required for the maturation of these cells into invariant natural killer T (iNKT) cells via acetylation of early response growth protein 2 (EGR2), a key transcription factor in the development of iNKT cells [66]. GCN5 enhances the interaction between steroid receptor coactivator 1 (SRC1) and retinoic acid receptor-related orphan receptor gamma (RORγt) in T helper 17 (T_H17) cells [67]. Loss of *Gcn5* in T_H17 cells impairs differentiation by downregulating genes essential to T_H17 cells. In most of these studies, GCN5/PCAF appears to facilitate the function of T cells subsets by regulating the transcription of key factors such as interleukin- 2 (IL-2) and interferon-gamma (IFN-γ). Double knockout of *Gcn5* and *Pcaf* in iTregs leads to smaller body size and thymic atrophy, as well as autoimmunity and premature death in mice. These effects seem to be due to the loss of peripheral Tregs and activation of T effector cells [64]. These findings illustrate that proper regulation of T cell development and function is dependent on expression of *Gcn5*/*Pcaf*.

Gcn5 is also associated with the function of other myeloid cells. Acetylation of the transcription factor CCAAT Enhancer Binding Protein Alpha (C/EBPα) by GCN5 inhibits its ability to bind to DNA and transcribe target genes leading to impairment of granulocyte differentiation [68]. GCN5 interacts and colocalizes with transcription factor Zinc Finger DNA-Binding Protein 89 (Zbp-89) in human erythroid cells at genes that are necessary for globin regulation and terminal maturation of erythroid cells [69]. *Gcn5* expression also increases during the “differentiation phase” of an erythroid differentiation time course of human CD34 cells further indicating a potential role for GCN5 in erythroid maturation [69]. The SAGA complex protein SUPT20 (also known as P38-Interacting protein (P38IP)) regulates monocyte and macrophage differentiation in part by regulating the stability of GCN5 in the SAGA complex [70]. GCN5/PCAF regulates innate immune cell signaling by targeting Tank-binding kinase 1 (TBK1), a kinase that regulates the production of the cytokine IFN-β, in MEFs [71]. Regulation of interferons are tightly controlled as overproduction may lead to the development of autoimmune diseases such as lupus erythematosus and multiple sclerosis. These findings highlight that *Gcn5*/*Pcaf* plays significant roles in different lineages of hematopoietic cells and that early deletion of these KATs has a significant impact on the development of many types of blood cells.

Taken together, these studies indicate that *Gcn5* and *Pcaf* play important roles in both the innate and adaptive immune systems. In most studies, these KATs appear to play an essential

role in the differentiation of hematopoietic cells into mature cells. The contrasting effects of deletion of *Gcn5* in the different subsets of T cells reveal the dynamic manner by which expression of and gene regulation by GCN5 can affect immune cell development in vertebrates. The results of these studies also highlight the differences in the functions of *Gcn5* and *Pcaf* in development and how loss of one KAT may compensate for the other in some contexts. In summary, *Gcn5* and *Pcaf* regulate the development of immune cells and function through the transcription of genes that are essential for immune system homeostasis. Furthermore, the studies implicate both *Gcn5* and *Pcaf* in the development of autoimmune diseases and suggest that they may be attractive therapeutic targets in the treatment of these maladies.

4.2. Gcn5/Pcaf acetylate non-histone proteins

Although GCN5 and PCAF are primarily known as histone acetyltransferases, both proteins also acetylate non-histone proteins (see also review by Michael Downey in this special issue). Fournier and colleagues identified 398 proteins that are acetylated by GCN5 and PCAF using shotgun proteomic analysis comparing control versus Gcn5 and Pcaf knockdown cells [72]. The targets identified are involved in multiple cellular processes beyond transcription including mitotic cell cycle, DNA replication and repair, and cell death. Their study revealed that acetylation of PLK4 by GCN5/PCAF prevents centrosome amplification. GCN5 also acetylates α-tubulin, a reaction that is enhanced by its interaction with MYC, increasing the stability of the protein and subsequently the microtubules formed that are necessary for spindle formation and cell cycle progression [73, 74]. PCAF acetylates PTEN on lysines 125 and 128, which inhibits its ability to regulate AKT activation and to regulate G1 arrest [75]. GCN5 acetylates c-MYC, stabilizing this oncogenic transcription factor involved in cell division, growth, and survival [76].

Transcriptional regulation by GCN5/PCAF is also facilitated by acetylation of transcription factors. Acetylation of β-Catenin by PCAF improves its stability by preventing its ubiquitination and degradation through the proteasomal pathway and regulating its transcriptional activity [77]. Autoacetylation by PCAF enables its translocation to the nucleus where it facilitates transcription of target genes [78]. PCAF acetylates E2F1 in its DNA-binding domain enabling its ability to interact with the DNA and subsequently increases its transcriptional function [79]. GCN5/PCAF acetylate C/EBPβ in 3T3 L1 preadipocyte cells leading to transcription of C/EBPα, another GCN5 target [80, 81]. This modification was necessary for the differentiation of the cells into mature adipocytes when stimulated. Acetylation of the SAGA HAT module structural protein Alteration in activation 3 (ADA3) by GCN5, PCAF, and P300 is necessary to maintain global histone acetylation levels thus impacting transcription and cell proliferation [82]. *Gcn5*/*Pcaf* expression is necessary for the proper regulation of cell cycle progression and cell division via both the transcription of essential genes and acetylation and stabilization of proteins necessary for these processes.

4.3. Emerging Roles of Gcn5 and PCAF in Cancer

Unsurprisingly, many of the genes regulated by GCN5/PCAF in development have roles in cancer. Knockdown of PCAF in glioma reduced proliferation through transcriptional

regulation of Hedgehog-Gli dependent genes [83]. GCN5 regulates the expression of E2F1, Cyclin D1, and Cyclin E1 in lung cancer [84]. GCN5 also regulates the expression of genes related to epithelial to mesenchymal transition (MMP-9 and E2F1) induced by TGFβ signaling in breast cancer cells [85]. Interestingly, ADA3 is overexpressed in breast cancer cells which also correlates to c-MYC overexpression [86]. Most genes regulated by GCN5 in cancer are associated with cell cycle progression, which MYC is a master regulator of. MYC is known to be the most overexpressed gene in cancer and GCN5 acting as a transcriptional coactivator as well as mediating acetylation of MYC most certainly enhances the role of MYC in cancer. The partnership between MYC and GCN5 in cancer has been shown so far in colon cancer [87], Burkitt lymphoma [88], and lung cancer [84, 89–91]. The association of GCN5 with c-MYC provides a direct link to the transcription of genes associated with cancer development and progression.

Consistent with its role in the development of blood cell types, GCN5 functions are critical in leukemias and lymphomas. Inhibition of GCN5 in Burkitt lymphoma reduced viability and induced apoptosis through regulation of the PI3K pathway [88]. A CRISPR dropout screen identified GCN5 as an essential gene in the survival of acute myeloid leukemia (AML) cells [92]. GCN5 expression is necessary for the expression of genes related to leukemogenesis ensuring proliferation and cell survival. Kahl and colleagues also showed that GCN5 is overexpressed in AML cells from patients [93]. Several studies showed that inhibition of Gcn5 caused differentiation and eventual cell death of AML cells [89, 92, 93]. Chemical inhibition of GCN5 combined with all-trans retinoic acid (ATRA) induces apoptosis in AML cells. Interestingly, acute promyelocytic leukemia (APL) cells treated with ATRA induced PCAF expression and differentiation of the APL cells into granulocytic cells. Knockdown of PCAF prevented this ATRA induced differentiation. Also knockdown of GCN5, but not PCAF, in some cancer cell types, reduces proliferation of the cells [84, 94]. This further demonstrates the differences in the roles of *GCN5* and *PCAF* in developmental as well as cancer phenotypes.

PCAF has been identified as a potential tumor suppressor in some settings. PCAF is downregulated in gastric cancer (GC) tissues and its loss is associated with poor survival [95]. PCAF overexpression in GC cells promotes expression of the tumor suppressor p16 as well as impairing the interaction between CDK4 and Cyclin D1 inhibiting proliferation and colony formation. It will be important to determine in which particular cancers GCN5 and PCAF play roles as tumor suppressors versus oncogenes and whether those roles are dependent on their inclusion in either the SAGA or ATAC complexes.

Bondey-Chorney and colleagues also profiled the non-histone protein targets of GCN5/PCAF that are implicated in the progression of cancer, thus we will highlight a few key interactions [96]. PCAF acetylates EZH2 which prevents it from regulating its target genes leading to the progression of lung adenocarcinoma [97]. PCAF-mediated acetylation of EZH2 was also associated with poor patient survival. PCAF acetylates both p53 and hypoxia inducible-1α (HIF-1α) influencing transcriptional activity of both and regulating proapoptotic pathways under hypoxic conditions [98, 99]. E2F1 acetylation at lysines 117, 120, and 125 is partially mediated by PCAF as well as by CBP/p300 and TIP60 stabilizes the protein and is necessary for efficient binding to DNA [79, 100]. In MEFs these

acetylation sites act as docking points for the bromodomains of p300 and CBP at double-strand breaks, however, are not transcriptionally linked to its role in DNA damage response but in the transcription of genes involved in nervous system development and differentiation [101]. The acetylation of E2F1 by PCAF, in addition to the co-transcriptional role played by GCN5 and PCAF, certainly plays a role in its oncogenic function. It would be interesting to ascertain what the transcriptional effects would be with mutations of these residues in a cancer cell context. Gcn5 also acetylates c-MYC stabilizing the protein which contributes to the transcriptional role in promoting cancer progression [76]. Targeting GCN5 and PCAF in cancer will have multiple repercussions in addition to transcription affects and these processes continue to be an active area of study.

GCN5/PCAF acetyltransferase activity is being increasingly linked with survival of various human cancer cell types. Currently, there are no clinically useful inhibitors of GCN5/PCAF. Inhibitors used in *in vitro* studies such as CPTH6 and MB-3 have IC50 values that are too high to be used in either animal models or humans. The implications of these KATs in many disease models underscore the need to develop compounds that could be used to treat humans for autoimmune disorders or cancer.

5. Future directions

Because Gcn5 and Pcaf are part of multiple, multisubunit complexes, the phenotypes observed upon mutation of these KATs reflect a concurrent loss of these activities. *In vitro* assays that monitor changes in cell proliferation, survival, or differentiation upon loss of Gcn5 or Pcaf must also be interpreted with the complexities of SAGA and ATAC in mind (see also review by Helmlinger et al in this special issue). A key question for the future is the definition of genes that depend specifically on each of these complexes for activation, and the definition of specific domains, partner proteins, and transcription factors that direct them to those genes.

Another unanswered question is how the various activities within SAGA work together to activate gene expression through histone acetylation, deubiquitination of H2B, and delivery of TBP (see also reviews by Goswami et al and Marc Timmers in this special issue). The elegant integration of these functional modules is highlighted by recent high-resolution cryo-EM structures of two different yeast SAGA complexes [102, 103]. The increased complexity of SAGA composition in vertebrates raises the possibility of even more specialized functions for these modules (see also review by Nuno-Cabanes and Rodriguez-Navarro in this special issue). Post-translational modifications of SAGA components in mammalian systems are also understudied at present, yet such modifications might affect both the structure and function of SAGA and its interactions with other factors. Meshing our increased understanding of pathways that require SAGA (Fig. 2), as defined by genetic studies, with an increased understanding of the structural plasticity of the complex, as highlighted by the recent structures, will perhaps allow us to finally determine how SAGA assimilates inputs from multiple developmental signals to deliver properly calibrated transcriptional outputs, and in turn, how co-opting of those functions by oncogenes such as Myc facilitates cancer and other disease states.

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Highlights

- *Gcn5* null embryos are defective in somitogenesis and neurulation, whereas *Pcaf* null mice show a complete lack of developmental phenotypes.
- Hypomorphic mutations that substantially lower *Gcn5* expression or abrogate KAT function result in axial skeleton defects, including rib fusions and homeotic transformations, along with spina bifida and exencephaly.
- Chromatin immunoprecipitation of GCN5 in undifferentiated ES cells reveals preferred recruitment to Myc and E2F1 binding motifs, and genes affected by *Gcn5* loss were enriched in Myc and E2F1 targets.
- *Gcn5* and *Pcaf* play important roles in both the innate and adaptive immune systems.
- GCN5/PCAF KAT activity is being increasingly linked with the survival of various human cancer cell types, however, no clinically useful inhibitors of GCN5/PCAF exist to date.

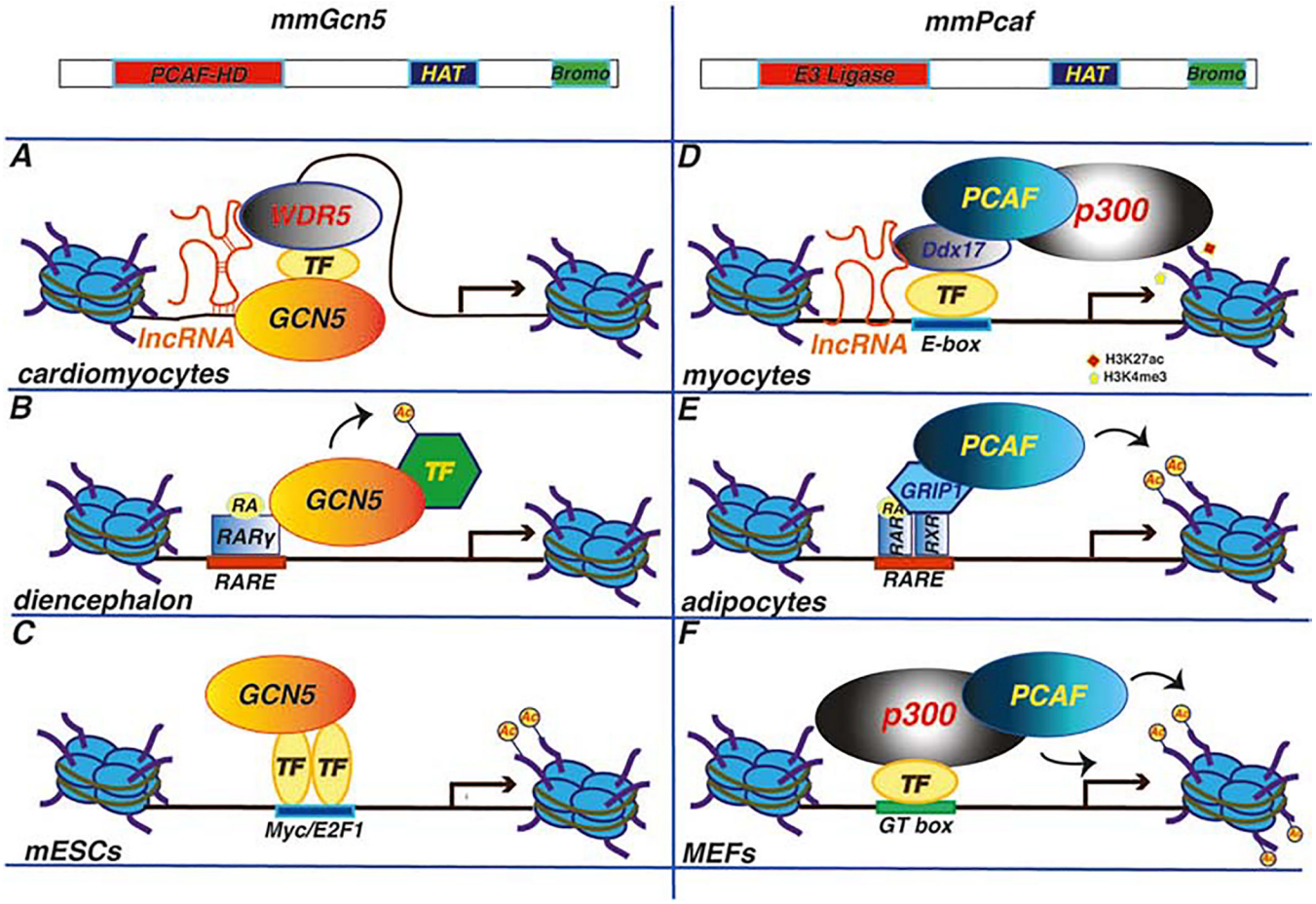

Fig.1: Examples of different mechanisms of GCN5 or PCAF recruitment to promoters or enhancers in embryonic stem cells, tissues, or organs.

Highlighted are GCN5 or PCAF, transcription factors (TFs), co-activators (WDR5, p300, GRIP-1, Ddx17), DNA binding elements (E-box, GT box, RARE) and lncRNAs (linc1405, Myoparr) as well as histone modifications analyzed (H3 acetylation, H3K27ac, H3K4me3) **A.** GCN5 is recruited to multiple gene promoters through its direct or indirect interaction with TFs (like c-Myc and E2F1) in mouse embryonic stem cells [48]. **B.** GCN5-dependent acetylates TACC1 after retinoic acid (RA) induction in the developing mouse diencephalon [40]. **C.** Recruitment of GCN5 to the enhancer of Mesp1 in cardiomyocytes is mediated by linc1405, WDR5 and Eomes [18] **D**. PCAF and p300 acetylate histone H3 when recruited to Cyclin D1 promoter by KLF8 in MEFs [36]. **E.** PCAF-dependent histone H3 acetylation after its RA-dependent recruitment to the promoter of orphan nuclear receptor TR2 [43]. PCAF interaction with RAR/RXR in preadipocytes is mediated by GRIP1. **F.** Long non-coding RNA Myoparr and Ddx17 mediates the recruitment of PCAF and (possibly p300) to gene promoters with a subsequent increase in H3K4me3 and H3K27ac levels [20–22]

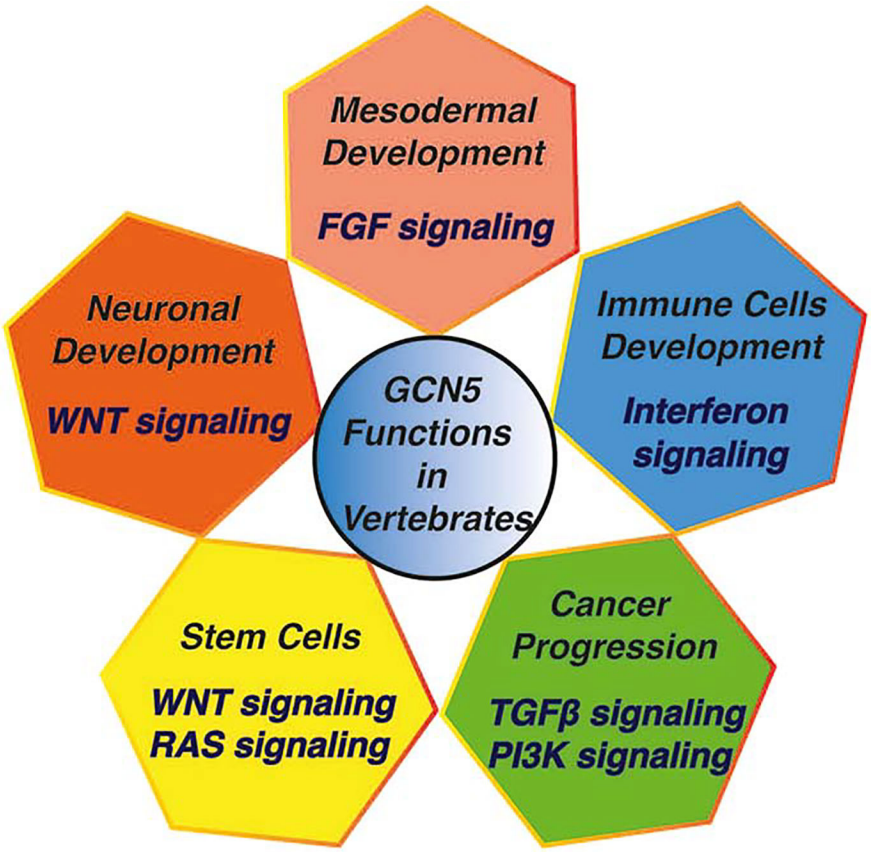

Fig.2: Signaling Pathways Regulated by Gcn5.

Gcn5 influences the development of different cell types and tissues by regulating essential signaling pathways. Regulation of these signaling pathways may also affect the development of diseases in a cell-type specific manner.

Table 1:

Representative examples of mouse models with global or conditional deletions of Gcn5/Kat2a allele and global deletion of Pcaf/Kat2b in different tissues and organs and their observed phenotypes.

Year	Allele Name	Allele Type	Cre	Phenotype	REF
2000	*Gcn5Δ*	loss of function	NA	embryonic lethal E11.0	[4]
2000	*PcafΔ*	loss of function	NA	viable and fertile	[4]
2000	*Gcn5Δ* and *PcafΔ*	loss of function (double knockout)	NA	embryonic lethal E8.0	[4]
2000	*PcafΔ*	loss of function	NA	viable and fertile	[5]
2000	*Gcn5Δ*HAT&Bromo	loss of function	NA	embryonic lethal E11.5	[5]
2007	*Gcn5HAT*	catalytic inactive	NA	neural tube closure defects	[15]
2008	*Gcn5flox(neo)*	hypomorphic	NA	Embryonic lethal E18.5	[26]
2008	*Gcn5flox*	conditional	NA	NA	[26]
2008	*Gcn5Δ3-19*	loss of function	NA	embryonic lethal E11.0	[26]
2008	*Gcn5 flox(neo)*	hypomorphic	NA	abnormal patterning of mouse skeleton	[26]
2008	*Gcn5 flox(neo)/Gcn5Δ3-19*	hypomorphic	NA	abnormal patterning of mouse skeleton	[26]
2011	*Gcn5flox* and *PcafΔ*	loss of function (double knockout)	retroviral Cre	no effect on morphology or growth of MEFs	[1]
2012	*Gcn5flox*	neural progenitor cell specific loss of function	Nestin-Cre	impaired brain growth and microcephaly	[49]
2012	*Gcn5flox*	Purkinje-cell specific loss of function	Pcp2-Cre	impaired brain growth and microcephaly	[104]
2014	*Gcn5flox* and *PcafΔ*	brown fat and skeletal muscle specific loss of function (double knockout)	Myf5-Cre	prevention of adipocyte differentiation	[105]
2014	*Gcn5flox*	adult forebrain specific loss of function	CamKIIα-cre	impaired hippocampal synaptic plasticity and long-term memory consolidation	[106]
2017	*Gcn5flox*	muscle-specific loss of function	MCK-Cre	no effect on metabolic remodeling in mouse skeletal muscle	[107]
2017	*Gcn5flox*	T-Cell-specific loss of function	Lck-Cre (CD4 CD8 DN T cells)	impaired T cell activation	[65]
2017	*Gcn5flox*	tamoxifen-induced T cell specific loss of function	UB/ESR-Cre (CD4+ T cells)	impaired T cell differentiation in vitro	[65]
2018	*Gcn5HAT*	catalytic inactive	NA	craniofacial cartilage and bone defects	[44]
2019	*Gcn5flox*	T-Cell-specific loss of function	Foxp3-YFP Cre	inhibitory effects on Treg function	[64]
2019	*Gcn5flox*	T-Cell-specific loss of function	CD4-Cre	impaired proliferation	[64]
2019	*PcafΔ*	loss of function	NA	impaired Treg peripheral conversion in vitro	[64]
2019	*Gcn5flox* and *PcafΔ*	T-Cell-specific loss of function (double knockout)	Foxp3-YFP Cre	lethal autoimmunity	[64]
2019	*Gcn5flox*	sperm-specific	Stra8-Cre	abnormal sperm formation and male infertility	[108]
2020	*Gcn5flox*	tamoxifen-induced muscle specific loss of function	iHSA-Cre (human a-skeletal actin)	no effect on metabolic remodeling in mouse skeletal muscle with combined overexpression of SIRT1	[109]

Year	Allele Name	Allele Type	Cre	Phenotype	REF
2020	*Gcn5flox*	neural crest cell specific	Wnt1-Cre	severe craniofacial defects and neural tube closure defects	[110]
2020	*Gcn5flox/Gcn5HAT*	neural crest cell specific	Wnt1-Cre	defects in the mandibular portion of the craniofacial skeleton	[110]