Chromatin binding of Gcn5 in *Drosophila* is largely mediated by CP190

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ABSTRACT

Centrosomal 190 kDa protein (CP190) is a promoter binding factor, mediates long-range interactions in the context of enhancer-promoter contacts and in chromosomal domain formation. All *Drosophila* insulator proteins bind CP190 suggesting a crucial role in insulator function. CP190 has major effects on chromatin, such as depletion of nucleosomes, high nucleosomal turnover and prevention of heterochromatin expansion. Here, we searched for enzymes, which might be involved in CP190 mediated chromatin changes. Eighty percent of the genomic binding sites of the histone acetyltransferase Gcn5 are colocalizing with CP190 binding. Depletion of CP190 reduces Gcn5 binding to chromatin. Binding dependency was further supported by Gcn5 mediated co-precipitation of CP190. Gcn5 is known to activate transcription by histone acetylation. We used the dCas9 system to target CP190 or Gcn5 to a Polycomb repressed and H3K27me3 marked gene locus. Both, CP190 as well as Gcn5, activate this locus, thus supporting the model that CP190 recruits Gcn5 and thereby activates chromatin.

INTRODUCTION

Insulators are DNA sequences bound by specific proteins that are required to insulate active topologically associated domains (TADs) from adjacent inactive ones or to block enhancers from activating promoters (1). In *Drosophila*, several insulator binding proteins (IBPs), such as dCTCF, Su(Hw), BEAF-32, GAF, ZW5, Ibf1 and 2, Pita and ZIPIC, have been identified and characterized (2–12). These proteins form different complexes by recruiting additional factors such as CP190. It has been shown that CP190 is the essential constituent of the *gypsy* insulator complex together with Su(Hw) and Mdo(mdg4)2.2. Among all these DNA-binding insulator factors CP190 binding is shared. Insulator strength is determined by the number of aligned DNA-bound factors (13). It has been shown that two separated domains of CP190 interact with specific IBPs (3), suggesting a bridging role of CP190 allowing clustered IBPs to target CP190 more efficiently. Indeed, synergistic recruitment of CP190 by IBP clusters has been shown (3). Furthermore, insulator function and TAD border strength correlate with IBP protein occupancy (14), suggesting that the efficiency of CP190 binding is an important regulator of CP190 function. In addition to insulator binding CP190 function has been shown to bind active promoters (15). A unified chromatin feature for most of the CP190 bound TADs is an increase of histone acetylation at these sites (16).

The histone acetyltransferase Gcn5 is highly conserved, found in yeast as well as in man and is a component of the SAGA complex. A recent *in vitro* study proposed that SAGA binding at active promoters is mediated by the sgf29 Tudor domain binding to H3K4me3 and allowing Gcn5 to acetylate histone H3K9 (17). In human cells, genome-wide ChIP-Seq revealed that Gcn5 is localized at gene promoters (18). Recent analysis of Gcn5 binding in ES cells demonstrated that from the 7000 Gcn5 peaks identified about 50% are located close to the transcriptional start site (TSS) (19). In *Drosophila*, Gcn5 was shown to be dependent on some Su(Hw) insulator sites, which are aligned with origin of replication complexes (20).

Here, we reveal that the recruitment of Gcn5 to chromatin of *Drosophila* is dependent on CP190. Depletion of Gcn5 deregulates a subset of genes that strongly overlap with those deregulated by CP190. Targeted recruitment of Gcn5 or CP190 to silent chromatin resulted in gene activation.
MATERIALS AND METHODS

Data access

All data sets reported in this study have been submitted to the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE83409.

DNA plasmids and primers

CRISPR/Cas9 plasmid (all in one) was purchased from Addgene (#49330). Wild-type Cas9 was deactivated by site-directed mutagenesis kit (Agilent) using the following primers:

- D10A:
  - RP: 5’-gtacagctcggcctggtcagccgcaactgtc-3’
  - FP: 5’-cagagttggtccgatatcagcccgtacgctgta-3’
- H840A:
  - RP: 5’-gtccacatagtggaacagcatgtcgctagcttctc-3’
  - FP: 5’-gaaagctctgaggcacgatggcgtccacatcgtagtcggac-3’

dCas9 (D10A and H840A) sequence was validated by sequencing. CP190 and Gcn5 full length cDNA sequences were purchased from Addgene (Addgene #49330). Wild-type Cas9 was deactivated by site-directed mutagenesis kit (Agilent) using the following primers:

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  - FP: 5’-gaaagctctgaggcacgatggcgtccacatcgtagtcggac-3’

Cell culture

Drosophila S2 and Kc cells were maintained in Schneider’s media supplemented with 10% FBS and kept at 25°C. Cells were transfected with 2 μg plasmid DNA using FuGENE® HD Transfection Reagent. RNAi treatment and dsRNA synthesis was performed as previously described (21). The knockdown efficiency was further confirmed by western blot.

ChIP-seq assay

Chromatin immunoprecipitation was carried out as previously described (22). Briefly, 10⁶ cells were used for each IP. Cells were cross-linked in 1% formaldehyde at room temperature for 10 min then the formaldehyde was quenched by addition of Glycine to a final concentration of 125 mM. Nuclei were isolated by adding 1 ml ice-cold IP buffer (150 mM NaCl, 50 mM Tris–HCl (pH 7.5), 5 mM EDTA, NP-40 (0.5%, vol/vol), Triton X-100 (1.0% vol/vol) supplemented with EDTA-free protease inhibitor cocktail (Roche) and 5 μl/ml PMSF (100 mM)). Nuclei were washed in IP buffer then resuspended in 1ml IP buffer. Nuclei were sonicated using bioruptor for 15 cycles (30 s on and 30 s off). Cleared chromatin was incubated with the antibodies overnight at 4°C on rotating wheel. Aliquot 40 μl protein A/G slurry were added to each IP. After 1 h, the beads were washed five times and the DNA was extracted using Chelex-100 resin (Bio-Rad). Sequencing libraries were prepared from 10 ng of immunoprecipitated DNA with the Illumina ChIP-seq DNA Sample Prep Kit according to Illumina’s instructions. Cluster generation was performed using the Illumina cluster station, sequencing on the HiSeq 2500 followed a standard protocol. The fluorescent images were processed to sequences using the Genome Analyzer Pipeline Analysis software 1.8 (Illumina). The reads were then mapped to dm3 with Bowtie (23) and peaks were called using MACS1.4 (24), while differential peak binding was calculated using two alternative strategies. On the one hand HOMER software (25) was used with the command getDifferentialPeaks (default settings with log2 fold reduction set to −F 2). This approach uses read count normalization based on total number of reads per library and is summarized in Figure 2B. In an independent approach extraction of read counts per Gcn5 peak was done using BioConductor packages (26) GenomicRanges (27) and Rsamtools (http://bioconductor.org/packages/release/bioc/html/Rsamtools.html) (28). We calculated the union of Gcn5 peaks called in independent experiments and extracted corresponding read counts. Normalization of the resulting count matrix and identification of differentially bound peaks and corresponding confidence intervals was done using DESeq2 with default settings (29) (Figure 2C and D). Heat maps and average plots were generated using seqMINER (30) and NGSplot (31), using default settings, respectively. All other plots were generated using R (http://www.R-project.org) (32). Genome browsers snapshots were generated using Gviz Bioconductor package (33).

Co-immunoprecipitation and mass spectrometry

Wildtype S2 cells (120 × 10⁶ cells) were harvested. Nuclei were isolated and the immunoprecipitation was carried out as previously described (34). The immunoprecipitated proteins were separated on SDS-PAGE (4–12%) then the peptides were extracted using In-Gel trypsinization method as previously described (35). The amount of peptides was measured using label-free quantification method (LFQ), and then the ratio between Gcn5 IP/IgG was calculated.

RNA-seq

RNA extraction was carried out using TRIzol extraction method. For each experimental condition we prepared two biological replicates from 1 μg total RNA. RNA was depleted of rRNA using RiboMinus technology. Libraries were prepaered from purified RNA using ScriptSeq™ v2 and were sequenced on an Illumina HiSeq 2500. Read mapping was done with Bowtie2 called from TopHat (36) with default settings. For known transcript models we used dm3 RefSeq (UCSC) annotations downloaded from Illumina’s iGenome repository. The aligned reads were analysed with custom R scripts in order to obtain gene expression measures. For normalization of read counts and identification of differentially expressed genes we used DESeq2 (29) with default settings. Overlap between gene lists obtained from different treatments was analysed by the GeneOverlap BioConductor package (http://shenlab-sinai.github.io/shenlab-sinai/) (37).

Antibodies

Rabbit polyclonal anti-dCTCF (15): for each IP 5 μl were used. Mouse monoclonal anti-CP190 (gift from Harald
Figure 1. Gcn5 ChIP-Seq. (A) Density heatmaps of Gcn5 reads over insulator binding proteins in Kc cells. K-means clustering of normalized Gcn5-specific reads with CP190 (red) sites, dCTCF (blue) sites that overlap with CP190 (+) and those that don't overlap with CP190 (−), BEAF-32 (green) and Su(Hw) (purple) sites (±3 kb). (B) Binary heat map (black = overlapping binding) indicates overlap of 5054 Gcn5 binding sites detected in Kc cells with several insulator factors (publicly available data from GEO: GSM762842, GSM762836, GSM762839 and GSM762845). (C) Barplot shows total numbers of binding sites for the analysed factors, where dark colours indicate the fraction of sites overlapping with Gcn5. The highest fraction of sites overlapping with Gcn5 shows CP190. (D) Barplot as in (C), but only binding sites devoid of CP190 are depicted. Only minor fractions of insulator sites devoid of CP190 are overlapping with Gcn5. (E) Scatter plots with linear regression between Gcn5 and CP190 read counts. The correlation shows a strong correlation coefficient (0.6). (F) Genome-browser snapshots of Gcn5 and CP190 binding. Gcn5 and CP190 ChIP-Seq profiles at BX-C (200 kb) showing co-occurrence at the insulator regions between segment-specific homeotic genes. (G) Gcn5 standalone peaks (*) that do not overlap with CP190 and/or with other insulator factors (CG17337, LIMK1, Eip78C and Su(z)12).
Figure 2. Gcn5 ChIP-Seq analysis in Kc cells after CP190 depletion: (A) Venn diagram showing Gcn5 peaks that overlap with CP190 after CP190 kd. (B) Differential Gcn5 peaks which still overlap with CP190 after CP190 kd. 62% of the 2667 Gcn5 peaks with a Poisson $P$-value < 0.0001 underwent at least a two-fold reduction after CP190 kd ($F = 2$) (green). (C and D) Binding dependency of Gcn5 on CP190, but not on CTCF. 2D density representation of scatterplots show differential Gcn5 binding as a function of CP190 (C) or CTCF (D) read counts across all Gcn5 peaks. Scale bar in (D) indicates colour coding of densities. Linear models were fitted (dotted lines) and indicate a negative linear relationship between CP190 binding and differential Gcn5 binding (C, $P < 2.2e^{-16}$), which is not the case for CTCF (D, $P = 0.967$). (E) Average profile of Gcn5 loss across genomic regions revealing that many of the Gcn5 reduced peaks after CP190 depletion are in the promoter region. The semi-transparent grey shade around the average signal indicates the interval of the average change of signal ± the standard error of the mean.
CP190 recruits Gcn5 to chromatin at CP190 binding sites

Based on the correlation of CP190 and Gcn5 sites we wanted to determine, whether CP190 may be a recruiter for Gcn5. Therefore, we depleted CP190 in Kc cells by dsRNA (Supplementary Figure S2A) and tested for genome-wide binding of Gcn5 by ChIP-Seq. The number of 5095 Gcn5 peaks after control knockdown (dsRNA against GFP) was reduced to 4227 peaks after CP190 knockdown. However, 2667 Gcn5 peaks remained at CP190 sites (Figure 2A). To identify any change in binding strength of the remaining Gcn5 sites after CP190 depletion, we applied differential binding analysis on these 2667 peaks. We found that 62% of the peaks underwent a reduction of at least two-fold (Figure 2B), while 11% increased and 27% remained unchanged. Furthermore, depletion of CP190 from strong binding sites should cause stronger binding changes of Gcn5 as compared to weak CP190 sites. This is indeed the case (Figure 2C). A significant correlation between Gcn5 binding changes upon CP190 depletion and CP190 binding strength is detected. In contrast, when analysing the Gcn5 binding changes with respect to dCTCF binding strength, there is no correlation detected (Figure 2D). ChIP-qPCR of CP190 binding sites grouped in respect to binding strength showed a similar result with a strong dependency of Gcn5 binding on CP190 at strong CP190 binding sites (Supplementary Figure S3). Thus, we can conclude that CP190 recruits Gcn5 to CP190 bound sites. To test for a preferential location of CP190 dependent Gcn5 binding we determined the average profile of Gcn5 binding changes across the genome (Figure 2E). A specific location is the region just upstream of the TSS. In this region Gcn5 binding is highly dependent on CP190.

In order to validate the ChIP-Seq results, the CP190 RNAi experiment was carried out in three biological replicates and ChIP-qPCR reactions were performed (Figure 3). From the ChIP-Seq results we chose twenty sites, representing four different groups, sites positive for dCTCF, CP190 and Gcn5, sites positive for CP190 and Gcn5, Gcn5-standalone sites and sites negative for any of these three factors. As a knockdown control we used GFP-dsRNA (GFP kkd) and for specific knockdown we used a double knockdown (dkd) against CP190 and against dCTCF (CP190/Ctcf dkd) to achieve a better depletion of CP190 at dCTCF binding sites. For unspecific precipitation we used IgG. Negative sites and the IgG precipitation show a background PCR signal only. All dCTCF/CP190 and all CP190 sites show the expected specific signals, which are markedly reduced after CP190/Ctcf dkd. Furthermore, the CP190/Ctcf dkd causes a strong reduction in Gcn5 precipitation at sites positive for CP190 irrespective of the presence of dCTCF. Thus, the knockdown effect can be attributed to CP190 depletion. Gcn5-standalone sites remain unchanged (Figure 3). The reverse experiment to deplete Gcn5 and determine CP190 binding was done as well (Supplementary Figure S3). The binding of CP190 is only slightly effected by Gcn5 depletion, whereas the binding of Gcn5 is highly dependent on CP190.
Gcn5 is physically interacting with CP190

Endogenous Gcn5 was immunoprecipitated from embryonic S2 cells in two independent biological replicates. The immunocomplex was separated on gradient SDS-PAGE and peptides were extracted after in-gel trypsinization. Mass-spectrometry identified, as expected, most of the SAGA and ATAC complex components (Supplementary Table S1) Atac1, Atac2, Ada2b, Sgf29, Taf module subunits (Taf4, Taf5, Taf6 and Sgf6) and Spt module subunits (Spt1, Spt3, Spt4, Spt5 and Spt20). For both complexes Gcn5 is the central component (38). In addition to these, CP190 was found and ranked within the highest top mascot scores in both biological replicates under stringent washing conditions (Figure 4A). Furthermore, factors known to interact with CP190 were found, such as Dref. This factor, similarly to CP190, is found at active promoters and at insulators (21,39,40). The factors Pzg and Chro, both of which are associated with the NURF complex and are colocalized genome-wide with CP190 (21,41), are detected in the Gcn5 immunocomplex (Figure 4A). In order to further confirm the Gcn5/CP190 interaction, we carried out immunoprecipitations of Gcn5. The precipitate was clearly positive for CP190 (Figure 4B).

Overlapping function of Gcn5 and CP190

Based on the striking overlap of Gcn5 and CP190 binding sites we predicted that an overlapping set of genes should be affected upon depletion of either factor. Depletion of CP190 deregulated 383 genes, when compared to the unspecific GFP knockdown. This number is in the range reported in other publications (42,43). These included 152 up-regulated and 231 down-regulated genes. Gcn5 depletion deregulates 1100 genes (468 up-regulated genes and 632 down-regulated genes) when compared to GFP kd (similar numbers were reported by (44)).

If CP190 is the recruiter for Gcn5, one would predict a significant number of genes being co-regulated. Therefore, we sorted the genes according to the log2 fold expression change after CP190 kd into genes up-regulated or down-regulated (Figure 5A). In most cases, the expression change after Gcn5 kd follows the change seen after CP190 depletion. In focusing on genes showing an expression change with an adjusted \( P \)-value of 0.05 or less, we find 52% of the CP190 deregulated genes to be deregulated by Gcn5 depletion as well (Figure 5B). As tested by qPCR, the knockdown of CP190 and Gcn5 resulted in significant reduction of the respective factor, whereas CP190 seemed to be up-regulated after Gcn5 kd (Figure 5C). On the protein level, this up-regulation was only marginal (Supplementary Figure S2B). In order to verify the finding of CP190 and Gcn5 co-regulated genes, we applied more stringent filtering parameters (\( P \)-value of 0.05 and log2 fold change of 0.7), resulting in a lower number of deregulated genes (Supplementary Figure S4). Yet, again, genes deregulated by either factor are significantly overlapping (Supplementary Figures S4 and S5). Furthermore, these deregulated genes show a...
Figure 4. Gcn5 immunoprecipitation from S2 cells. (A) Mass spectrometry top hits in two biological replicates reveal that CP190 as well as most of SAGA subunits are co-eluted with Gcn5. (B) Co-IP validates the interaction between Gcn5 and CP190.

significant positive correlation ($P$-value = 2.6e–6) (Supplementary Figure S6).

In summary, we can conclude that a co-regulation of a significant number of genes is found with a majority of these being activated by the presence of both factors. This suggests a model involving DNA binding factors like IBPs, binding CP190, which in turn recruits Gcn5. Such an arrangement would be predicted to cause gene activation based on the activation function of Gcn5. As a crucial test for such a function we wanted to target either CP190 or Gcn5 to a silent gene locus to test the effect of either factor on this locus. We chose the even-skipped (eve) gene, which is expressed in the first 6 hours after fertilization. In the late embryonic stage, polycomb proteins shut down eve gene expression completely (13). In Kc as well as in S2 cells, the eve gene is embedded in an H3K27me3 domain and insulated from the surrounding highly expressed genes by Homie/NHomie insulators (Figure 6B) (13,45–47). For specific targeting we used the CRISPR/Cas9 system with a catalytically inactive protein dCas9 (48). We generated fusion constructs of Flag-dCas9 with Gcn5 or with CP190 (Figure 6A). In addition, this construct expresses a guide RNA specific for the eve locus, which is required to target the dCas9 fusion proteins to this locus. We generated two S2 cell clones for each of the fusion constructs and found both of them similarly expressed (Supplementary Figure S2C). Although these fusion proteins are quite large, proteins of the expected full-length molecular weight were detected. As a negative control we also generated cell clones expressing the original dCas9 vector with the same eve-specific guide RNA, but without a Gcn5 or CP190 fusion. The binding of dCas9 in the negative control clone (dCas9) and in two selected clones (dCas9CP190 clone 2 and dCas9Gcn5 clone 6) to the site of the target sequence was confirmed by FLAG ChIP-qPCR using primers flanking the guide RNA sequence (green arrow in Figure 6B) and primers for a negative site about 3 kb downstream within the same domain (yellow arrow Figure 6B). The binding of dCas9 was clearly detected at the target site when compared to the negative control site (Figure 6C). Since the eve gene is embedded within a facultative H3K27me3 domain (Figure 6B), we wanted to know whether this modification may have been changed in case of expression and targeting of either dCas9 fusion proteins. Indeed, when we carry out chromatin IP with an antibody against H3K27me3, we find that the two cell clones C2 and C6 show reduced H3K27me3 levels at the integration site, when compared to the dCas9 control cell clone. In contrast, the downstream control site is not changed in the level of H3K27me3 (Figure 6D, Supplementary Figure S7). When testing eve expression in the negative control clone and in both of the dCas9 fusion clones, for each of the fusions we found that both clones expressing either dCas9 fusion induced eve expression (Figure 6E).

This result confirms that both CP190 and Gcn5 are similarly acting as transcriptional activators.

**DISCUSSION**

*Drosophila* insulator binding proteins (IBPs) are characterized by a multitude of different factors, with only one of which, CTCF, being highly conserved from insects to mammals. CP190, not found in vertebrates, seems to be the unifying factor associated with all of the known *Drosophila* IBPs. Analysis of genome-wide binding revealed clusters of IBPs bound to CP190. Therefore, it is not surprising to find CP190 enriched at chromatin-domain boundaries (14). In addition, CP190 has been found at promoters of active genes (15). Both features, binding to promoters and to domain boundaries, require some kind of bridging, either with enhancers or between paired domain boundaries. In fact, CP190 has been demonstrated to contact and bridge several IBPs (3,49,50). In addition to looping and bridging, CP190 has been implicated in modifying chro-
Figure 5. RNA-Seq in S2 cells. (A) Boxplot of log2 fold change after CP190 kd (blue) and Gcn5 depletion with dsRNA specific for two different Gcn5 sequences (red) shown for all genes (left), genes that are significantly up-regulated after CP190 depletion (center) and genes that are significantly down-regulated (adjusted $P < 0.05$) after CP190 depletion (right). Within the class of up-regulated genes the log2 fold change after Gcn5 depletion is significantly higher than for the average gene (Wilcoxon signed rank test: $P < 2.2e^{-16}$). Conversely, within the class of down-regulated genes the log2 fold change after Gcn5 depletion is lower than average ($P < 2.2e^{-16}$). (B) Venn diagrams showing significant overlap between significantly (adjusted $P < 0.05$) de-regulated, down-regulated and up-regulated genes in CP190 and Gcn5 RNAi relative to GFP. In all cases, the observed overlap was significant (hypergeometric $P < 2.2e^{-16}$, odds ratios were for generally de-regulated, down-regulated and up-regulated genes 7.72, 12.43 and 9.8, respectively). In contrast there was no such strong association between conversely regulated genes (Supplementary Figure S4B). (C) CP190 and Gcn5 RNA levels as determined by rt-PCR after knockdown of CP190 (green) and of Gcn5 (red). The data are represented as $M \pm SD$ using two biological replicates. Significance (unpaired t-test) is indicated (*).

matin. dCTCF/CP190 binding sites show reduced nucleosomal occupancy, whereas dCTCF sites devoid or depleted of CP190 are loaded with nucleosomes (15). Furthermore, CP190 binding sites have been found to be enriched for active histone marks (16). Therefore, we searched for a factor potentially involved in modifying chromatin and being co-localized with chromatin bound by CP190.

Here, we find CP190 chromatin binding to be strongly correlated with Gcn5 genome-wide. Gcn5 is a highly conserved acetyltransferase, which is a component of the SAGA chromatin-modifying complex (for reviews see (38,51)). Deficiencies of SAGA components cause severe developmental defects, suggesting that SAGA and Gcn5 contribute to the transcriptional regulation of developmental genes.

The correlation between Gcn5 and CP190 binding is found for 75% of the Gcn5 sites and for 60% of the CP190 sites. The overlap between CP190 and Gcn5 is stronger than that between Gcn5 and Su(Hw), dCTCF and BEAF-32 standalone sites (Figure 1). Moreover, Gcn5 co-occurs with CP190 at CP190 standalone sites (Figure 3). Previously, the occurrence of SAGA components ENY2, Ada2b or of Gcn5 has been detected at selected dCTCF or Su(Hw) sites (20,52,53), which now may be explained in part due to CP190 binding at these sites in addition to direct contacts with dCTCF or Su(Hw).
Figure 6. Cas9-directed binding of CP190 or of Gcn5 activates the H3K27me3 repressed eve locus. (A) dCas9 fusions targeting eve locus. The dCas9 vector codes for the sgRNA scaffold and for dCas9 fused to CP190 or to Gcn5. (B) The genome browser snapshot of the eve locus indicates the dCas9 crRNA target sequence (green arrow). A non-targeting site is pointed out (yellow arrow), which was used as negative control. (C) Flag ChIP-qPCR validates the specific binding of dCas9 or of dCas9 fusions at the eve-locus crRNA target site (green bars) compared to a non-targeting negative site (yellow bars). (D) H3K27me3 ChIP at dCas9 positive and negative sites normalized to H3 levels. Both, dCas9CP190 and dCas9Gcn5 reduce H3K27me3 at the binding site (green bars) in contrast to the non-targeting site (yellow bars). (E) The normalized expression of the eve gene in dCas9, dCas9CP190 and dCas9Gcn5 expressing S2 cell clones. For each construct two clones are shown. The recruitment of CP190 and Gen5 increases eve gene expression significantly. The data are represented as M±SD using two biological replicates.
The data presented here suggest that Gcn5 and CP190 binding not only show a strong correlation, but rather that Gcn5 chromatin binding is even dependent on CP190. This is further supported by the co-purification of Gcn5 and of CP190. In addition to binding of Gcn5 to CP190, the SAGA component ENY2 has been shown to interact with the zinc finger domains of Su(Hw) and of dCTCF (52,53). Despite the fact that depletion of Gcn5, or of other SAGA components or of CP190 affects a limited number of genes (13,15,44), we were able to detect a significant overlap of genes with changed expression after depletion of Gcn5 and of CP190. In fact, the number of genes being impaired by either depletion is higher as compared with the genes being activated. This suggests, that both, CP190 and Gcn5, in many cases function as activators. This confirms previous data showing the co-activator function of Gcn5 or of CP190 (13,15,44,54).

Several mechanisms of Gcn5 action have been reported. One of the aspects is the connection to DNA replication and cell cycle. The insulator protein Su(Hw) has been shown to recruit Gcn5 to the origin recognition complex (ORC) (20). Furthermore, binding sites for Drosophila IBPs, especially dCTCF and CP190, correlate strongly with replication origins that are enriched at gene promoters (55,56). Interestingly, CP190 recruits Dref (replication-related element-binding factor), which regulates cell cycle progression and replication (40).

Dref is also found to bind to CP190 and to be required for the Fab8 insulator to mediate enhancer-blocking (21). Another acetyltransferase, Myst5, has been shown to colocalize with Dref and the factors Chro (chromator) and Pzg (Putzig) together with other insulator proteins at active gene promoters (41). Here, we show that all three factors purify with Gcn5 and all have been isolated with CP190 (21). This suggests that acetyltransferases, such as Gcn5 or Myst5 may play a functional role in insulation. In fact, the SAGA component ENY2 is recruited to the zinc-finger domain of dCTCF and is required for the barrier activity of dCTCF and of Su(Hw)-dependent insulators (52,53). Furthermore, the NURF complex and Gcn5 have been shown to functionally interact (44). Again, the connection to insulator function has been demonstrated by purifying NURF components with CP190 and showing the requirement for NURF in enhancer blocking (21).

The acetylation activity of Gcn5 has been shown with multiple lysines within histone H3 as substrate. Depletion of Gcn5 results in a reduction of acetylation of lysines 9, 14 and 27 (57) indicating the overall activation function of Gcn5. Interestingly, CP190 binding not only show a strong correlation, but rather that Gcn5 chromatin binding is even dependent on CP190. This is further supported by the co-purification of Gcn5 and of CP190. In addition to binding of Gcn5 to CP190, the SAGA component ENY2 has been shown to interact with the zinc finger domains of Su(Hw) and of dCTCF (52,53). Despite the fact that depletion of Gcn5, or of other SAGA components or of CP190 affects a limited number of genes (13,15,44), we were able to detect a significant overlap of genes with changed expression after depletion of Gcn5 and of CP190. In fact, the number of genes being impaired by either depletion is higher as compared with the genes being activated. This suggests, that both, CP190 and Gcn5, in many cases function as activators. This confirms previous data showing the co-activator function of Gcn5 or of CP190 (13,15,44,54).

In summary, for all of the factors involved and recruited by CP190, such as NURF (21,62), DREF (62,63) and Gcn5 (60), nucleosome depletion has been demonstrated. Here, the concerted action of CP190 recruiting these factors may result in efficient nucleosome removal.

**SUPPLEMENTARY DATA**

Supplementary Data are available at NAR Online.

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