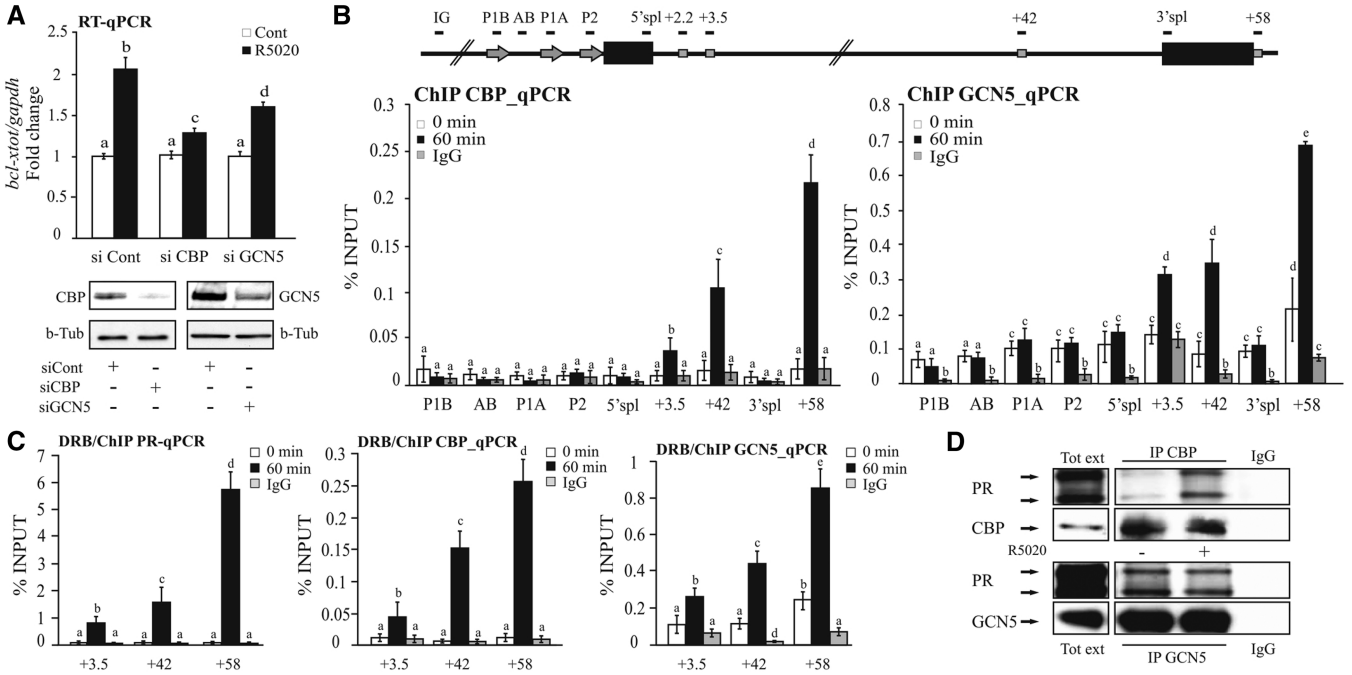

Figure 2. Role of CBP and GCN5 on progestin-dependent *bcl-x* induction. (**A**) T47D cells treated or not treated with R5020 were transfected with control, CBP or GCN5 siRNAs, then total RNA and proteins were extracted. The relative levels of *bcl-x* expression were determined by RT–PCR and normalized to *gapdh* expression. The degree of depletion of CBP and GCN5 was monitored by western blot using specific antibodies. For each condition, β-tubulin was used as loading control. The histograms show the mean ± SD of three experiments performed in duplicate. The different superscript letters are significantly different from each other ($P \leq 0.05$). (**B**) T47D cells were treated with R5020 for 60 min and subjected to ChIP assays with α-CBP- and α-GCN5-specific antibodies. Recruitment of CBP (left) and GCN5 (right) to PRbs, promoter regions and TSS upstream regions of the *bcl-x* gene is shown. Immunoprecipitation with normal rabbit IgG (IgG) was used as control. The graphics show the mean ± SD of three experiments performed in duplicate. The different superscript letters are significantly different from each other ($P \leq 0.05$). (**C**) Effect of transcription elongation on PR (left), CBP (middle) and GCN5 (right) recruitment to the +3.5, +42 and +58 PRbs. T47D cells pre-incubated with 50 μM DRB were treated with R5020 for 60 min and subjected to ChIP assays with α-PR, α-CBP and α-GCN5 antibodies. Immunoprecipitation with normal rabbit IgG (IgG) was used as control. The graphics show the mean ± SD of three experiments performed in duplicate. The different superscript letters are significantly different from each other ($P \leq 0.05$). (**D**) T47D cells treated or not treated with R5020 for 1 h were lysed and immunoprecipitated with α-CBP- and α-GCN5-specific antibodies or with normal rabbit IgG (IgG). The immunoprecipitates were analyzed by western blotting with α-PR, α-CBP and α-GCN5 as indicated.

modification that it cannot be produced by neither by GCN5 nor CBP (45), indicating that other HATs, as hKAT1/(HAT1), hKAT5/(TIP60) or hKAT7/HBO1/MYST2 could also be involved in the histone acetylation process occurring in the *bcl-x* intragenic PRbs. Furthermore, according to ChIP assays performed against H3K14ac, H4panac and H4K8ac analyzed along *bcl-x*, histone acetylation also seems to be mainly increased at the 3′-region of the gene (Figure 3F).

As shown previously, some chromatin remodelers, such as SWI/SNF (BAF and P/BAF complexes) and NURF, contain subunits that recognized specific histone acetylation marks and, therefore, cooperate in their binding to target chromatin (9,46). ChIP experiments showed a significant hormone-dependent recruitment of BAF57, a subunit of the BAF complex to the +3.5, +42 and +58 PRbs, to a level correlated to the extent of PR and HAT recruitment (Figure 3G).

As the recruitment of the SWI/SNF chromatin remodeling enzymes is one of the mechanisms likely to disrupt chromatin at many DHS (47–49), we next asked whether specific binding of PR along with chromatin remodelers to the intragenic PRbs changes the DHS pattern after hormone induction. In this sense, we searched for DHS at the *bcl-x* gene by analyzing a DNase I hypersensitive sites sequencing (DNase I-seq) assay performed in T47D cells treated or not treated for 60 min with R5020 (22). Results showed an increase in DHS at the intragenic PRbs at +42 and +58 on hormone addition (Figure 4, top and bottom). The regions over the intragenic PRbs at +2.5 and +3.5 were hypersensitive to DNase I before hormone treatment and became less sensitive to nucleases in the presence of R5020 (Figure 4). Thus, the promoter distal PRbs of *bcl-x* showed a clear chromatin remodeling after hormone treatment in correlation with their higher binding of PR, HATs and BAF. This different behavior of PRbs located closer to the 3′ of the gene compared with those sited more proximal to promoter regions together with the nucleosomes displacement detected earlier in the text suggests that the more accessible chromatin conformation induced by PR at these sites would favor the passage of the elongating RNA Pol II along *bcl-x*, mainly at the 3′-end region.

To test this hypothesis, we then directly monitored the hormone-dependent Pol II distribution along *bcl-x* gene. Analysis of the read profile obtained from a ChIP-seq showed that the Pol II is stalled in the 5′-end of the gene before hormone addition (Figure 5A, top). Hormone induction promoted accumulation of Pol II toward the

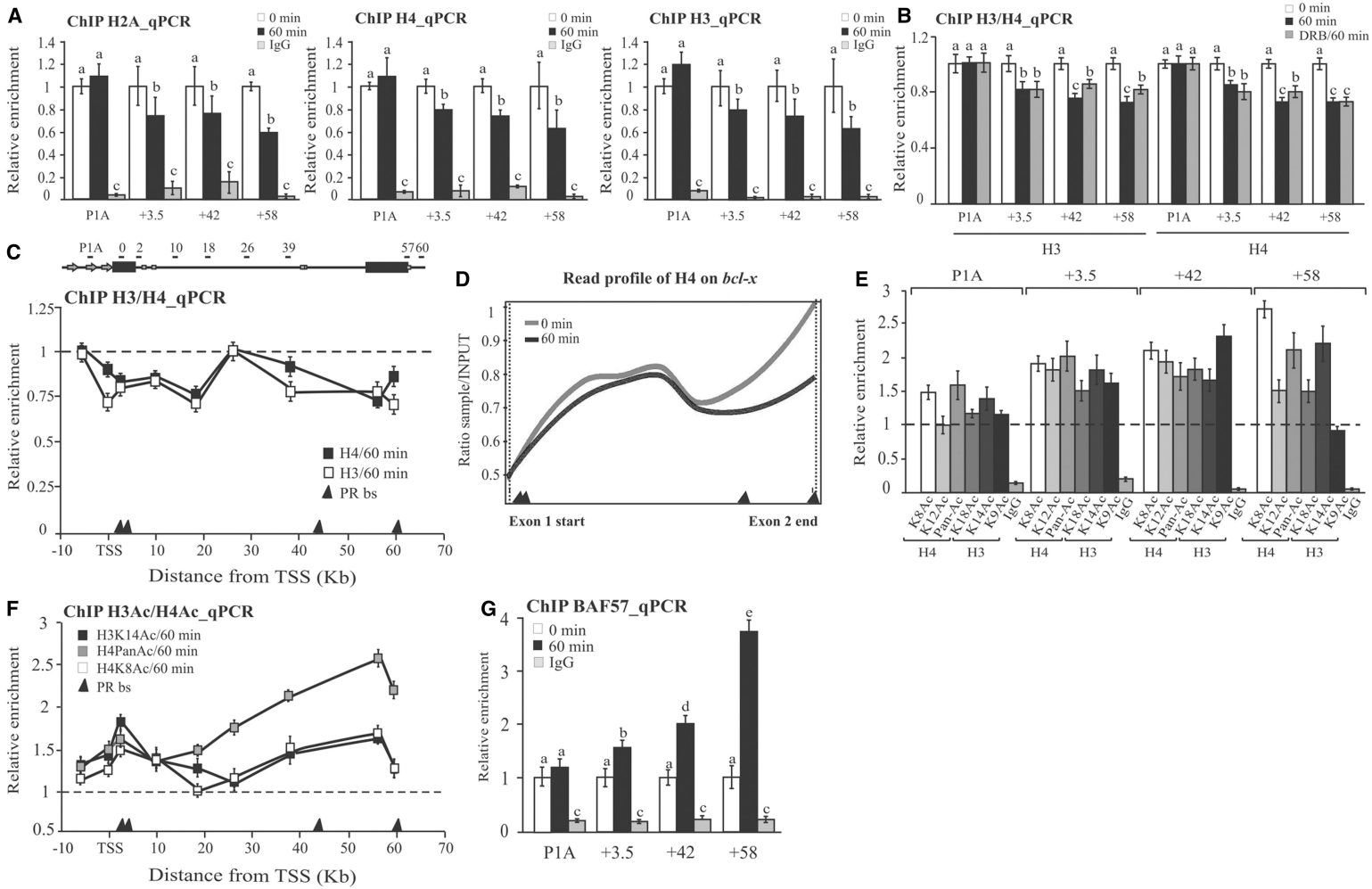

Figure 3. Histone acetylation and chromatin remodeling. (**A**) T47D cells were treated with R5020 for 60 min and subjected to ChIP assays with α-H2A, H4 and H3 antibodies. The precipitated DNA fragments were subjected to PCR analysis to test for the presence of sequences corresponding to +3.5, +42 and +58 PRbs and the promoter P1A. Immunoprecipitation with normal rabbit IgG (IgG) was used as control. The histograms show the mean ± SD of three experiments. Bars with different superscript letters are significantly different from each other ($P \leq 0.05$). (**B**) Effect of transcription elongation on histone H3 and H4 levels at P1A and the +3.5, +42 and +58 PRbs. T47D cells pre-incubated with 50 μM DRB were treated with R5020 for 60 min and subjected to ChIP assays with H4 and H3 antibodies. Immunoprecipitation with normal rabbit IgG (IgG) was used as control. The graphics show the mean ± SD of three experiments performed in duplicate. The different superscript letters are significantly different from each other ($P \leq 0.05$). (**C**) T47D cells were treated with R5020 for 60 min and subjected to ChIP assays with H4 and H3 antibodies. The precipitated DNA fragments were subjected to PCR analysis along *bcl-x*. Primers positions are indicated in the scheme above the figure. The histograms show the mean ± SD of three experiments. The dashed line indicates the relative levels of histones H3 and H4 in the absence of hormone. Black triangles at the absice indicates PRbs location. (**D**) T47D cells were treated with R5020 for 60 min and subjected to ChIP-seq analysis using α-H4 and H3 antibodies (22). The read profiles obtained in the presence and in the absence of hormone in the *bcl-x* gene, are shown. Black triangles at the absice indicates PRbs location. (**E**) T47D cells were treated with R5020 for 60 min and subjected to ChIP assays with antibodies against specific acetylated marks: H4K8ac, H4K12ac, pan-acetylated H4 (Pan-Ac), H3K18ac, H3K14ac and H3K9ac. The precipitated DNA fragments were subjected to PCR analysis to test for the presence of sequences corresponding to +3.5, +42 and +58 PRbs and the promoter P1A. The histograms show the mean ± SD of three experiments performed in duplicate. (**F**) T47D cells were treated with R5020 for 60 min and subjected to ChIP assays with H3K14ac; pan-acetylated H4 (Pan-Ac) and H4K8ac antibodies. The precipitated DNA fragments were subjected to PCR analysis along *bcl-x*. Primers positions are indicated in C. The histograms show the mean ± SD of two experiments. The dashed line indicates the basal levels of the different modifications obtained in the absence of hormone. Black triangles at the absice indicates PRbs location. (**G**) T47D cells were treated with R5020 for 60 min and subjected to ChIP assays with α-BAF57 (a member of the SWI/SNF complex). Recruitment of BAF57 to the +3.5, +42 and +58 PRbs and the promoter P1A was evaluated by RT–PCR. Immunoprecipitation with normal rabbit IgG (IgG) was used as control. The histograms show the mean ± SD of three experiments performed in duplicate. Bars with different superscript letters are significantly different from each other ($P \leq 0.05$).

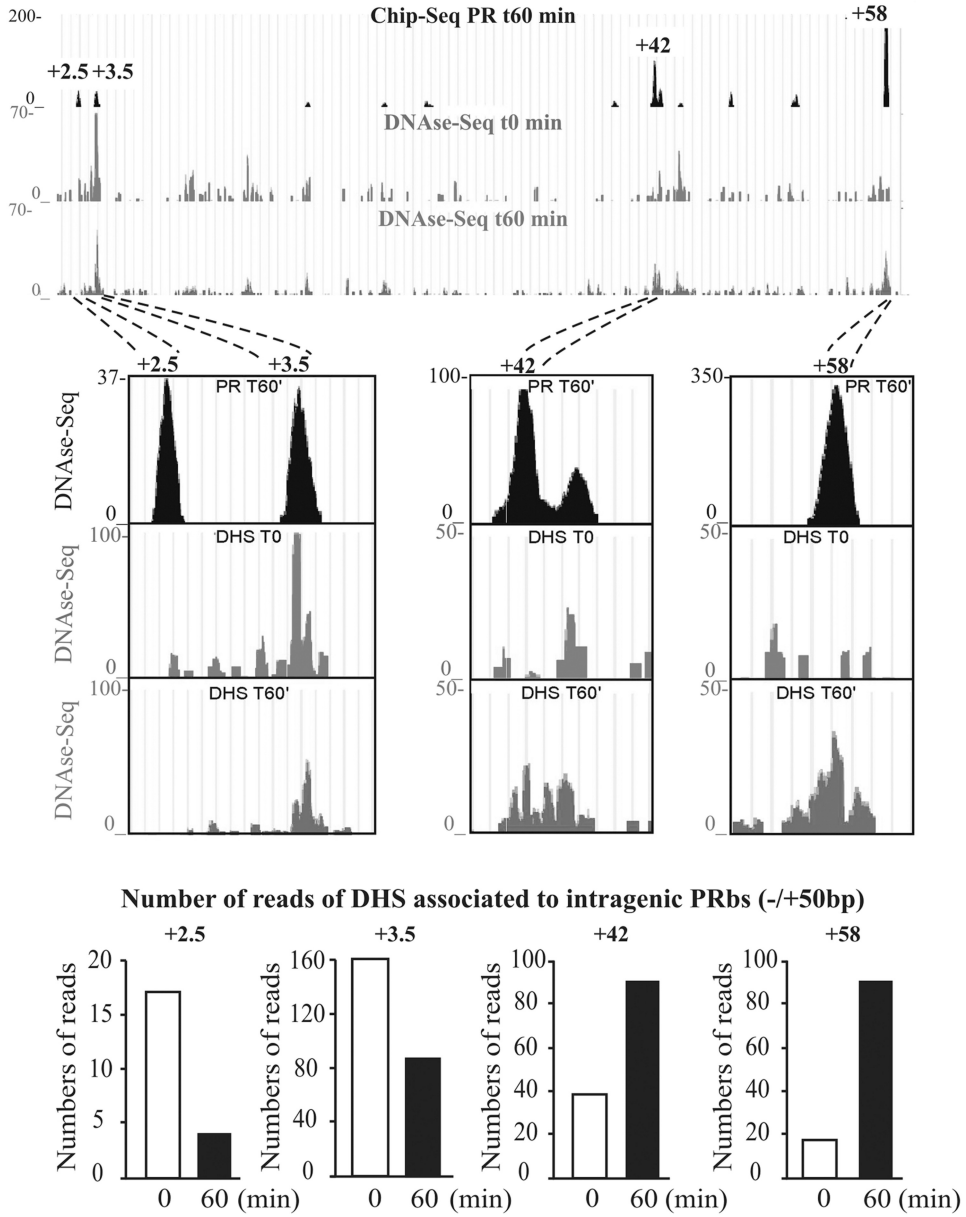

Figure 4. Increase in DHS signal at the intragenic PRbs after hormone induction. T47D cells were treated with R5020 for 60 min and subjected to DNAse-seq analysis (35). Top and middle: the profiles of DHS signals obtained in the presence and in the absence of R5020, as well as the PRbs obtained by ChIP-seq (22) in the *bcl-x* gene are shown. Bottom: Quantification of the DHS reads associated with the +2.5, +3.5, +42 and +58 PRbs. The window used in each PRbs was ± 50 bp.

3′-end of the gene [(22) and Figure 5A, top]. Box plots representing the density of Pol II at 5′ (exon1) and 3′ (exon2) *bcl-x* regions confirmed the statistical significance of this finding (Figure 5A, bottom). Similar results were obtained by using quantitative ChIP–qPCR. The magnitude of Pol II increase was ∼2-fold at the 3′-region of the gene (Figure 5B). As expected, the increase in Pol II levels was abolished by the inhibitor DRB (Figure 5C), ruling out the possibility of an internal Pol II recruitment as a consequence of the activated PR binding to the intragenic PRbs. Thus, the binding of the activated PR would affect the internal Pol II distribution toward the 3′-end rather than the recruitment of new Pol II molecules to the promoter region.

Taking into account that the phosphorylation of the carboxy-terminal domain (CTD) of the largest Pol II subunit in the Ser2 residues is a good marker of active elongation, we performed ChIP experiments against the Pol II-Ser2P to cofirm that hormone treatment affects the elongating polymerase. Figure 5D shows that on progestin addition, the levels of the Pol II-Ser2P increased toward the 3′-end of the gene. Moreover, a time-course curve of the Pol II-Ser2P at P1A and the +3.5, +42 and +58 PRbs showed a similar profile to the time course

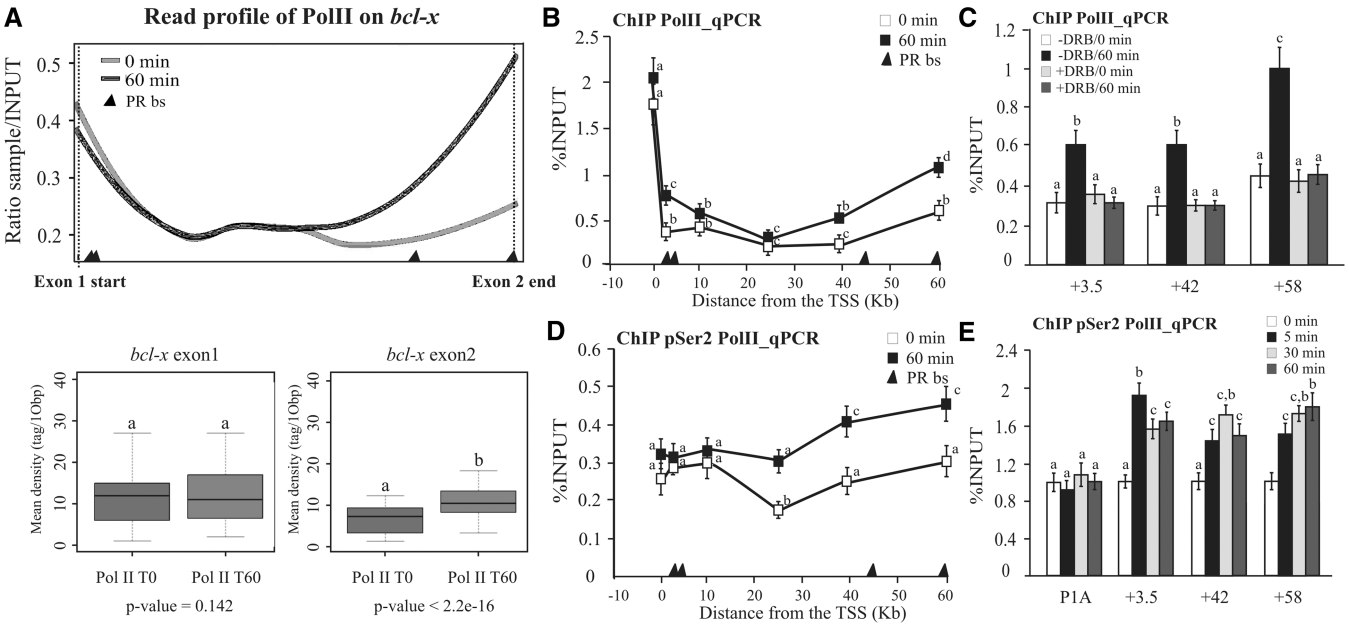

Figure 5. Occupancy of intragenic PRbs after hormone induction increased RNA pol II loading to the 3′-end of the *bcl-x* gene. (**A**) T47D cells were treated with R5020 for 60 min and subjected to ChIP-seq analysis using α-RNA pol II antibody (22). Top: the read profiles obtained in the presence and in the absence of hormone in the *bcl-x* gene are shown. Black triangles at the absice indicate PRbs location. Bottom: after hormone induction, increased loading of RNA pol II polymerase is detected at the 3′-end of the *bcl-x* gene. Quantification of the ChIP-seq reads associated with the *bcl-x* exon 1 (right) and 2 (left) in the presence and in the absence of hormone is shown. The *P*-value is indicated at the bottom of each box plot. (**B**) T47D cells were treated with R5020 for 60 min and subjected to ChIP assays with α-RNA Pol II antibody. The precipitated DNA fragments were subjected to PCR analysis along *bcl-x*. The histograms show the mean ± SD of three experiments. Different superscript letters are significantly different from each other ($P \leq 0.05$). Black triangles at the absice indicates PRbs location. (**C**) Effect of transcription elongation on Pol II distribution at the +3.5, +42 and +58 PRbs. T47D cells pre-incubated with 50 μM DRB were treated with R5020 for 60 min and subjected to ChIP assays with α-RNA Pol II antibody. Immunoprecipitation with normal rabbit IgG (IgG) was used as control. The graphics show the mean ± SD of three experiments performed in duplicate. The different superscript letters are significantly different from each other ($P \leq 0.05$). (**D**) T47D cells were treated with R5020 for 60 min and subjected to ChIP assays with α-RNA Pol II-Ser2P antibody. The precipitated DNA fragments were subjected to PCR analysis along *bcl-x*. The histograms show the mean ± SD of three experiments. Different superscript letters are significantly different from each other ($P \leq 0.05$). Black triangles at the absice indicate PRbs location. (**E**) Time course of RNA Pol II-Ser2P recruitment: T47D cells were treated with R5020 for 0, 5, 30 and 60 min and subjected to ChIP assays using α-RNA Pol II-Ser2P PR antibody. The precipitated DNA fragments were subjected to PCR analysis to test for the presence of sequences corresponding to P1A and the three PRbs: +3.5, +42 and +58 PRbs. The histograms show the mean ± SD of three experiments performed in duplicate. Different superscript letters are significantly different from each other ($P \leq 0.05$).

previously observed for the PR recruitment to these internal PRbs (compare Figure 5E with Supplementary Figure S1B). These results highlight the role of progestins affecting the elongation rate.

P-TEFb is recruited to the intragenic PRbs on hormone addition

As phosphorylation of Pol II-Ser2 is carried out by the positive elongation factor P-TEFb, which is known to be recruited by the SRs to target genes (16,19,20), and given that *bcl-x* hormone-dependent induction is affected by the inhibition of the P-TEFb subunit CDK9 (Supplementary Figure S3), we then tested whether this elongation factor is recruited to the *bcl-x* PRbs. We performed ChIP assays with a specific antibody recognizing CDK9 in T47D cells treated with R5020 for 60 min. Consistently, we found hormone-dependent recruitment of the CDK9 kinase to the intragenic PRbs with a similar pattern to that observed previously for PR and the two HATs (Figure 6A). Moreover, CDK9 was recruited even when its activity was impaired by the presence of DRB (Figure 6B), supporting a PR-mediated recruitment. Indeed, the P-TEFb subunits, CDK9 and cyclinT1, co-precipitate with PR in response to the hormone in this cell line (Figure 6C). As P-TEFb favors the elongation process by phosphorylation of Pol II Ser2 and by displacing negative elongation factors, intragenic PR-mediated recruitment of this factor would favor progesterone enhancement of transcription elongation.

Involvement of intragenic PRbs on the transcription elongation and alternative splicing of *bcl-x*

Changes in the Pol II rate or processivity on endogenous genes in response to a particular stimulus is technically difficult to determine. However, there is a way to asses Pol II processivity by evaluating with specific primers the relative abundance of a pre-mRNAs in a proximal versus a distal region by RT–qPCR, assuming that most pre-mRNAs are co-transcriptional intermediates (50) (Figure 7A). We used this approach to analyze Pol II processivity on *bcl-x* in T47D cells treated with R5020 for 15, 30 and 60 min (Figure 7B, left). Results show that the proximal/distal ratio for *bcl-x* gene decreases after hormone addition, indicating a hormone effect on

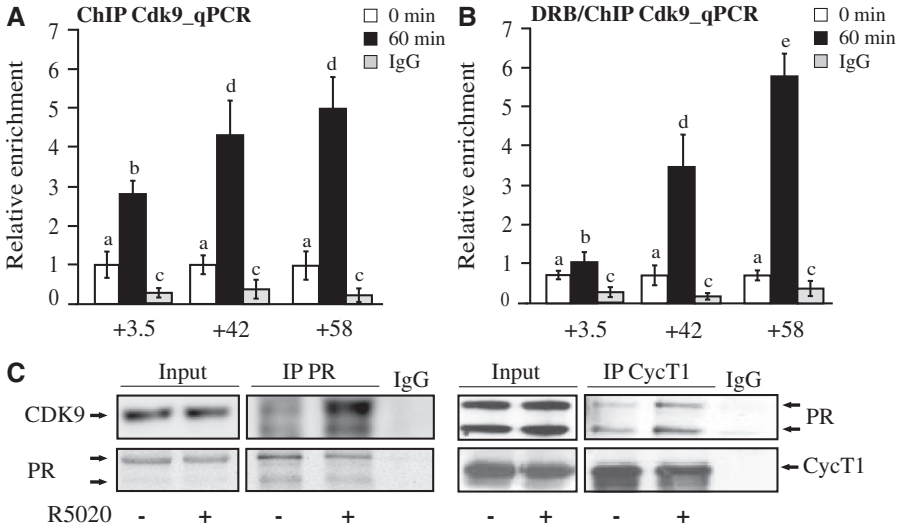

Figure 6. The elongation complex pTEFb is recruited along with PR to the *bcl-x* intragenic PRbs. (**A**) T47D cells were treated with R5020 for 60 min and subjected to ChIP assays with α-Cdk9 antibody. The precipitated DNA fragments were subjected to PCR analysis to test for the presence of sequences corresponding to +3.5, +42 and +58 PRbs. Immunoprecipitation with normal rabbit IgG (IgG) was used as control. The histograms show the mean ± SD of three experiments. Different superscript letters are significantly different from each other ($P \leq 0.05$). (**B**) T47D cells pre-incubated with 50 μM DRB were treated with R5020 for 60 min and subjected to ChIP assays with α-Cdk9 antibody. The precipitated DNA fragments were subjected to PCR analysis to test for the presence of sequences corresponding to +3.5, +42 and +58 PRbs. Immunoprecipitation with normal rabbit IgG (IgG) was used as control. The histograms show the mean ± SD of three experiments. Different superscript letters are significantly different from each other ($P \leq 0.05$). (**C**) T47D cells treated or not treated with R5020 for 1 h were lysed and immunoprecipitated with α-PR or with normal rabbit IgG (IgG). The immunoprecipitates were analyzed by western blotting with α-Cdk9, α-CyclinT1 and α-PR as indicated. Blots correspond to one representative experiment ($n = 3$).

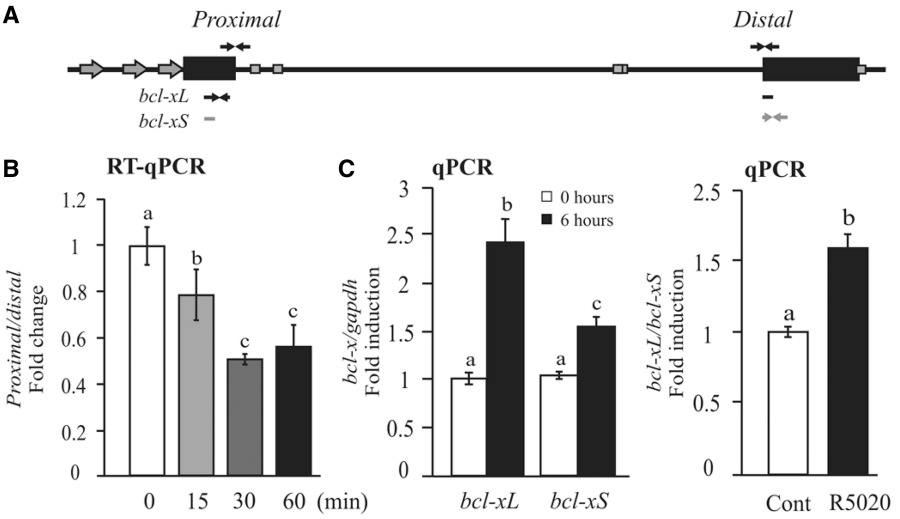

Figure 7. PR-dependent Pol II processivity and *bcl-x* splicing. (**A**) Scheme of the human *bcl-x* gene structure: arrows indicate the sequences that hibridize with the different primers used in these assays. (**B**) T47D cells were treated or not treated with R5020 for 15, 30 and 60 min, and the proximal/distal ratio of mRNA *bcl-x* gene was evaluated. The histograms show the mean ± SD of three experiments performed in duplicate. The different superscript letters are significantly different from each other ($P \leq 0.05$). (**C**) T47D cells were treated or not treated with R5020 for 6 h, and total RNA was prepared. cDNA was generated and used as template for real-time PCR with *bcl-xL* and *bcl-xS*-specific primers (left). Right: the ratio between *bcl-xL* and *bcl-xS* was calcultated. The values were normalized with *gapdh* expression. The histograms show the mean ± SD of three experiments performed in triplicate. Different superscript letters are significantly different from each other ($P \leq 0.05$).

Pol II elongation activity. The observed change in the proximal/distal ratio was detected already after 15 min of hormone treatment and reached the maximum after 30 min.

Taking into account that changes in Pol II processivity influence RNA processing (24,25), and that *bcl-x* alternative splicing is affected by changes in Pol II elongation efficiency (51), we analyzed the hormone-dependent regulation of *bcl-x* alternative spliced isoforms. The results showed that both *bcl-xL* and *bcl-xS* transcript levels significantly increased on R5020 addition (Figure 7B, middle); however, the

relative abundance between each other favors *bcl-xL* accumulation (right). These results add more evidences supporting the hypothesis of a progestin-dependent regulation of Pol II elongation.

A progesterone effect on Pol II elongation activity was also observed in the endoplasmic reticulum to nucleus signaling factor 1 (*ern1*) gene (Supplementary Figure S4D). The *ern1* gene like *bcl-x* contains intragenic PRbs and is also upregulated in the presence of hormone (Supplementary Figure S4A). On the other hand, other induced hormone-dependent genes, such as transforming growth factor α (*tgfα*) (Supplementary Figure S4B) and the insulin-like growth factor 1 receptor (*igf1r*) (Supplementary Figure S4C), show a proximal/distal pre-mRNA ratio that either does not change or even increases after hormone, respectively (Supplementary Figure S4E and F, respectively).

The recent progress in DNA sequencing technology allowed us to increase the coverage 10-fold and to identify 28.474 intragenic PRbs on 60 min of hormone treatment ($P < 10^{-5}$). Combining the results of the ChIP-seq of PR with the gene expression data obtained by RNA-seq, we found that the internal PRbs are significantly enriched in upregulated genes compared with genes that do not respond to hormone (3.2 versus 1 PRbs/gene, respectively) (Supplementary Table S1). Interestingly, intragenic PRbs also showed a preference for downregulated genes with 2 PRbs/gene. Thus, we conclude that internal PRbs are accumulated in hormone-regulated genes, suggesting a more general mechanism than originally expected.

DISCUSSION

The study of how NRs control transcription has been largely focused on the regulation of PIC formation and transcription initiation. However, the putative role of NRs on the regulation of subsequent transcriptional steps has recently been enabled by using new generation sequencing techniques (16). Particularly, ChIP-seq analysis showed that NRs bind to sites located outside and sometimes at great distances of promoters, mainly at intragenic regions (14,16,52). However, the functional relevance and the mechanism of action of these internal NRbs have not been conclusively clarified.

Induction of *bcl-x* transcription correlates with the binding of PR to intragenic sites and the recruitment of active CBP and GCN5

The classical model of SRs-mediated gene activation proposes that Pol II recruitment and PIC formation to SRs target promoters is favored by an open chromatin structure, as demonstrated by high sensitivity to DNase cleavage observed on hormone treatment (53,54). It is assumed that this localized relaxed chromatin conformation is generated by SR-mediated recruitment of chromatin-modifying complexes that are common SR-co-factors during gene activation or induction (8,55–58).

In this work, we found that progestin-dependent *bcl-x* transcriptional induction in the breast cancer cell line T47D is controlled by binding of PR to several intragenic binding sites instead of the classicaly described promoter PRbs. In response to hormone treatment, the activated PR recruits CBP and GCN5 HATs to these sites, as was already demostrated at target promoters. Interestingly, the recruitment of these CBP and GCN5 is independent of transcription elongation, as it cannot be inhibited by DRB. CBP and GCN5 binding was acompanied by a local increase in histones H3 and H4 acetylation and was necessary for the hormone induction of *bcl-x*, as interfering either CBP or GCN5 expression precluded activation of the gene. These results, together with the co-immunoprecipitation of PR with both CBP and GCN5, support the hypothesis of a PR-dependent recruitment of chromatin modifiers to these intragenic regions. In fact, simultaneously with the recruitment of these HATs, accumulation of the ATP-dependent chromatin remodeler SWI/SNF and reduction in nuclesome occupancy in the *bcl-x* intragenic PRbs were observed, suggesting a change in the chromatin structure toward a more open state. Histone displacement was not exclusively restricted to the internal PRbs but also to surrounding regions. This effect correlates with the presence of other PRbs with lower levels of PR recruited than those observed at +2.5, +3.5, +42 and +58 described here (22). These weaker PRbs are mainly localized in the 3′-region of *bcl-x* intron and may contribute to the PR-dependent effect on Pol II elongation mainly at this region of the gene. PR-dependent remodeling of chromatin organization was previously demonstrated at promoter regions as a complex and necessary prerequisite for hormonal induction in a diversity of biological models (59–62). For example, two consecutive cycles of chromatin remodeling involving activated protein kinases, HATs and ATP-dependent chromatin modifiers have been described on hormonal induction of the mouse mammary tumor virus (MMTV) promoter (63).

Supporting our observations, local recruitment of chromatin remodelers and a decrease in histone density were found at four AR intronic sites located ≥90 kb downstream from the TSS of the *fkbp51* locus in VCaP and LNCaP prostate cancer cells, in response to the comercial androgen R1881 (64). Thus, SRs-associated histone acetylation and chromatin remodeling do not seem to occur only at promoter regions but also at distal downstream enhancers.

It has also been described that the glucocorticoid receptor exerts long-range regulation of *fkbp51* transcription through distal sites located ~100 kb from the TSS of the gene (65). Distal SRbs would facilitate the formation of transcriptional chromatin loops in certain genes, favoring the occurrence of protein–protein interactions among TFs bound to widely separated regulatory elements. The concept that the pre-assembled higher-order structures of chromatin can create boundaries for long-range interactions was also reported by Cullen *et al.* (66), who described the juxtaposition of distal ER-binding sites and proximal promoter regions of the prolactin gene in response to estrogens. Although we cannot discard the PR-dependent formation of chromatin loops in the *bcl-x* locus and their role on Pol II activity, ChIPs-seq assays

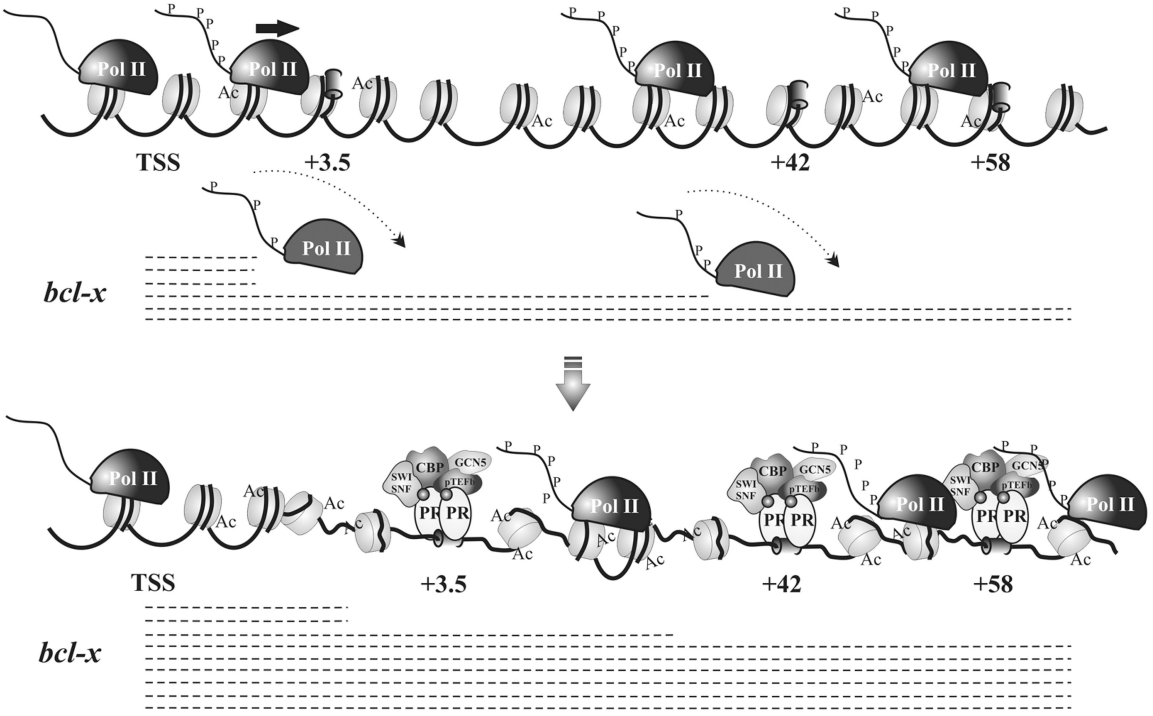

Figure 8. Schematic model of PR-dependent *bcl-x* transcription regulation. In the absence of hormone, the *bcl-x* gene presents a basal transcriptional activity characterized by the presence of some RNA Pol II molecules transcribing the entire gene, whereas they are commonly from the DNA template before reaching the 3′-end. On R5020 addition, PR is recruited to the intragenic PRbs along with the HATs CBP y GCN5 and the positive transcription elongation factor P-TEFb, favoring Pol II elongation. A higher number of Pol II molecules now are able to complete full-length transcription.

did not reveal PR or Pol II enrichment around the promoter and 5′-end regions of the gene, both events expected if a promoter loop is formed. Furthermore, the observed Pol II accumulation toward the 3′-end of the gene in response to hormone treatment suggests a local effect of PR rather than a promoter-directed regulation.

Recent genome-wide studies have shown that nucleosomes downstream of paused polymerases act as physical barriers to polymerase elongation. Therefore, we propose that this local chromatin relaxation triggered by the activated PR bound to the intragenic PRbs would be involved in controling Pol II elongation along *bcl-x* gene.

Chromatin remodeling complexes and P-TEFb recruited to intragenic sites could facilitate transcription elongation

A connection between histone acetylation and transcription elongation was also suggested by the observation that acetylated histones regulate the recruitment of the positive elongator factor P-TEFb (67,68). Binding of P-TEFb would not only induce Pol II activation through the CDK9-dependent specific phosphorylation of the CTD but also counteract negatively acting elongation factors that keep the Pol II stalled near promoters (69–71).

As far as the SR mechanism of action is concerned, the regulation of pre-loaded Pol II activity has been described as the predominant genomic outcome of ER (16). In the absence of estrogens, a large number of ER target promoters are Pol II pre-loaded with minimal occupancy over the coding body, a phenomenon referred as pausing or promoter proximal enrichment of Pol II (PPEP). On hormone addition, ER binding to those promoters triggers the recruitment of P-TEFb favoring Pol II dispausing toward productive elongation (15). Even more, in a recent work, it was demonstrated that recruitment of the ER/P-TEFb complex to a well-know transcriptional pausing site located at ~1.7 kb downstream of the TSS of c-*myb* gene (72) enables Pol II to resume transcriptional elongation (17). These results reinforce the idea that control of elongation in addition to transcription initiation are relevant mechanisms by which SRs control gene expression. In this sense, we propose that PR-dependent recruitment of P-TEFb to the intragenic *bcl-x* PRbs would mantain an elongating Pol II state by regulating the enzyme activity *per se* and/or by displacing negative elongation factors bound to DNA. Accordingly, activated PR not only induces *bcl-x* expression but also causes an increase in distal over proximal transcription.

Taking into account that elongation rate and processivity are functionally distinct and at least partially separable phenomena *in vivo*, and that most so-called elongation factors do not affect the rate at which Pol II travels down the gene (73), the combined recruitment of PR with CBP, GCN5 and P-TEFb to the different intragenic PRbs could explain, at least partially, that the increase in the Pol II abundance to the 3′ gene region and in the generation of full-length *bcl-x* transcripts would be consequence of combinatorial and complementary effects

on the Pol II elongation steps. Thus, modulation of the productive elongation could be a mechanism by which PR induces transcription of target genes containing intragenic PRbs. In a first attempt to extend our findings, we searched for genes that had similar characteristics to *bcl-x* gene, comparable length and encompassing intragenic instead of classical promoter/enhancer PRbs. Out of three genes found, one of them, the *ern1* gene showed hormone-dependent regulation similar to *bcl-x*; thus, taking into account the limitation in the number of genes analyzed, further studies are required to clarify the generality of this mechanism. However, results from the ChIP-seq of PR combined with the data from RNA-seq assays allow us to propose that internal PRbs are accumulated in up- and downregulated genes. Thus, more genes would follow the same trend as *bcl-x*, making the mechanism of gene activation mediated by intragenic PRbs a more general mechanism than originally expected. Second, these sites would also be relevant in hormone-dependent downregulation. How the specificity is acquired is a question that requires more investigation, but the existence of different pools of PR in the cell or the chromatin context of the PRbs in up- and downregulated genes are two interesting hypothesis that would explain, in part, our observations.

Finally, several questions arise from this work: what is the real outcome of P-TEFb recruitment to these intragenic sites? What is the relevance of these intragenic PRbs on other transcriptional steps? Are these sites able to directly recruit factors involved in pre-RNA processing? Here, we have also demonstrated that progestins are able to regulate the relative abundance between two *bcl-x* isoforms that are products of two alternative 5′-splice sites. This effect could be consequence of the increase in Pol II elongation efficiency triggered by the activated PR. In this sense, it was demonstrated that the decrease of Pol II elongation efficiency by ultraviolet irradiation correlates with an increase in the *bcl-xS*/*bcl-xL* ratio favoring apoptosis (51), suggesting that Pol II elongation activity would be directly associated with the alternative splicing of this gene. On the other hand, PR could also locally stimulate the recruitment of splicing factors on triggering chromatin remodelling as was found for the SRs co-activators CAPER, which act as splicing factors associated to hormone receptors (74,75).

In summary, intragenic PRbs distributed along *bcl-x* intron would act as 'filling stations' needed to refill the elongating polymerase with factors required for its proper travel along the gene and affecting the processing of *bcl-x* pre-RNAs (Figure 8).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Table 1 and Supplementary Figures 1–4.

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