

## TFIIH Operates through an Expanded Proximal Promoter To Fine-Tune *c-myc* Expression

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Received 15 July 2004/Returned for modification 12 August 2004/Accepted 30 September 2004

**A continuous stream of activating and repressing signals is processed by the transcription complex paused at the promoter of the *c-myc* proto-oncogene. The general transcription factor IIIH (TFIIH) is held at promoters prior to promoter escape and so is well situated to channel the input of activators and repressors to modulate *c-myc* expression. We have compared cells expressing only a mutated p89 (xeroderma pigmentosum complementation group B [XPB]), the largest TFIIH subunit, with the same cells functionally complemented with the wild-type protein (XPB/wt-p89). Here, we show structural, compositional, and functional differences in transcription complexes between XPB and XPB/wt-89 cells at the native *c-myc* promoter. Remarkably, although the mean levels of c-Myc are only modestly elevated in XPB compared to those in XPB/wt-p89 cells, the range of expression and the cell-to-cell variation of c-Myc are markedly increased. Our modeling indicates that the data can be explained if TFIIH integrates inputs from multiple signals, regulating transcription at multiple kinetically equivalent steps between initiation and promoter escape. This helps to suppress the intrinsic noise of transcription and to ensure the steady transcriptional output of *c-myc* necessary for cellular homeostasis.**

The c-Myc transcription factor targets approximately 10% of genes, coordinating many essential cellular processes, including proliferation, growth, differentiation, metabolism, and apoptosis (37, 38). Small changes in c-Myc protein levels, either up or down, modify these processes, and so *c-myc* expression must be held to close tolerances (69). Although many proteins regulate *c-myc* expression (Fig. 1), none impose dominating influence over all the rest; the mechanisms integrating these multiple inputs to provide a controlled output have not been elucidated. Due to the fast turnover and low abundance of both *c-myc* mRNA and protein in most normal tissues, rapid feedback mechanisms must operate to constrain c-Myc levels (8, 22, 54, 61, 69). Otherwise, c-Myc levels would fluctuate because transcription from a single promoter is an intrinsically noisy process; promoter firing is a low-probability event on a molecular time scale (13, 55).

The *c-myc* promoter is regulated by a transcriptionally engaged RNA polymerase, paused at the start site which must escape the promoter to commence elongation (44, 56, 63). Paused polymerases may be poised for rapid response but may also protect promoters from spurious activation, because activators facilitating preinitiation complex formation are thwarted until the start site is again available. To regulate promoters harboring paused polymerases, signals must be delivered to those components controlling early nascent transcript extension (Fig. 1). Although the regulatory mechanisms between initiation and promoter escape have been incompletely described, the general transcription factor IIIH (TFIIH) operates throughout this interval. It is known that TFIIH is a

multifunctional, multisubunit protein complex that plays central roles in transcription and DNA repair (nucleotide excision repair [NER]) (5, 64, 71). In addition, the three-subunit cyclin-dependent kinase (Cdk)-activating kinase (CAK)-kinase subcomplex of TFIIH, Cdk7/cyclin H/ménage à trois 1 (MAT1), separately contributes to cell cycle control. The two largest subunits of TFIIH, p89 (xeroderma pigmentosum complementation group B [XPB]) and p80 (XPD), have 3'-to-5' and 5'-to-3' helicase activities, respectively. TFIIH interacts with a variety of cellular and viral transcription activators and repressors that deliver signals to and receive signals from the transcription machinery. For example, transcription activation by steroid hormone receptors requires phosphorylation by Cdk7, and p53 both regulates and is phosphorylated by Cdk7 (3, 30, 43, 59). The activating events subsequent to Cdk7 action have been incompletely illuminated. Though some activators and repressors target p89/XPB helicase activity in vitro, the role of p89 action on native gene expression is largely unexplored (21, 23, 67, 76).

Joining preinitiation complexes after promoter selection, TFIIH contributes to basal transcription at several early stages of the transcription cycle (4, 11, 25, 26, 35, 50, 62). Generalizing from a limited number of promoters, p89/XPB first facilitates promoter melting and open complex formation; then ATP hydrolysis by the XPB helicase is required throughout the transitions, leading to promoter escape (16). In vitro, this requirement may be bypassed by premelting the start site (using mismatched bases to prop open the double helix) or, for some promoters, by providing supercoiled templates (19, 53). p80/XPD also contributes to nascent transcript growth, although XPD helicase activity is not required to support basal transcription, and so it has been proposed that p80 plays a structural role anchoring the CAK (9). The mutations of XPD that cause trichothiodystrophy impair TFIIH assembly and/or stability and so reduce basal transcription. CAK phosphorylates

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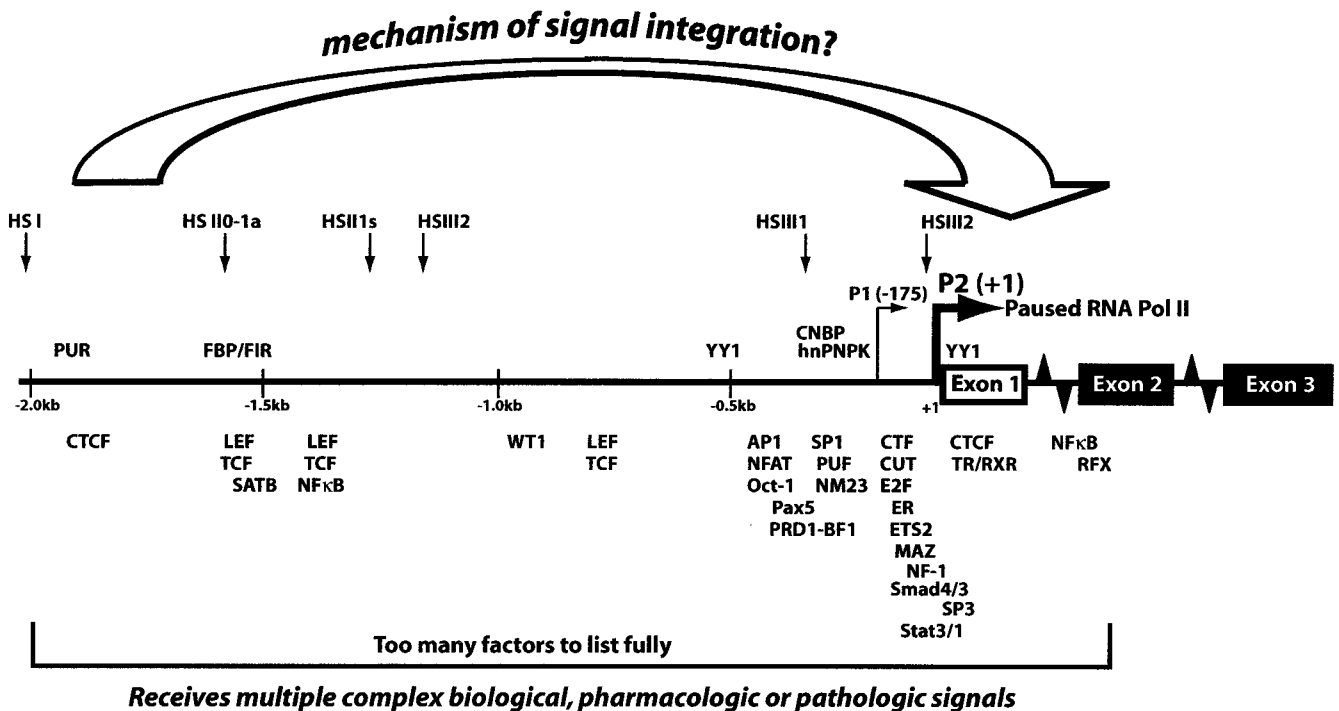


FIG. 1. Schematic representation of transcription factors regulating *c-myc* promoter activity. Selected transcription activators and repressors that directly bind to the *c-myc* promoter are shown at their approximate binding sites. Transcriptionally engaged RNA polymerase II is paused immediately downstream of the major transcription start sites P1 and P2. DNase I hypersensitive sites (HS) indicative of altered chromatin structures are also shown. To maintain appropriate c-Myc levels, some mechanism should exist to integrate this plethora of signals.

the carboxyl-terminal domain (CTD) of the largest subunit of RNA polymerase II. Phosphorylating serine-5 in the heptad repeat comprising the CTD, Cdk7 action enables the recruitment of factors required for mRNA capping; other Cdk7 targets likely contribute to the transcription and processing of RNAs in a gene-specific manner (32, 68).

The sequence-specific single-stranded binding transcription factor, FUSE-binding protein (FBP), and its antagonist, the FBP-interacting repressor (FIR), have been proposed to help impose tight regulation on *c-myc* transcription (10, 24, 40, 41, 46). FBP and FIR are recruited to single-stranded DNA at the FUSE sequence, far upstream of the major P2 promoter of the *c-myc* gene (10, 24, 40, 41, 46). Additionally, the conformation of FUSE DNA is especially sensitive to the torsional strain that is a transient by-product of transcription (24). The action of both FBP and FIR is channeled through TFIID (40, 41). From several lines of experimental evidence, *in vitro* as well as *in vivo*, FBP and FIR modulate transcription; FBP hastens the passage from initiation through postinitiation steps until promoter escape, whereas FIR delays these transits. Recently, *in vitro* systems have shown that transcription activation via FBP and repression via FIR are lost in XPB and are impaired in XPD disease (41). Transfected FBP increases endogenous c-Myc levels in XPB cells only when coexpressed with wild-type p89, suggesting that FBP acts through TFIID to help maintain proper *c-myc* regulation. It is expected that, lacking proper input from TFIID-interacting factors such as FBP, FIR, and E2F, *c-myc* regulation in XPB cells would be disturbed.

This study shows that the tight control, characteristic of the

*c-myc* gene, is compromised in XPB-mutant cells in a surprising manner. XPB cells exhibit striking cell-to-cell heterogeneity in c-Myc levels. Upon restitution of wild-type TFIID, cellular c-Myc levels become much more uniform. Changes at the *c-myc* promoter indicate that the zone of TFIID influence is contracted with mutation of p89/XPB and promoter escape occurs closer to the start site. As shown previously for FBP, FIR is found to depend on functional TFIID to modulate endogenous c-Myc levels. Reasoning from these results, it is shown that by regulating transcription at multiple points between initiation and promoter escape, stochastic fluctuation of gene expression may be suppressed.

#### MATERIALS AND METHODS

**Plasmid constructs.** pGS5 M/XPB (kindly provided by Kenneth Kraemer) was used as a source for human p89 cDNA. For generation of the inducible p89 expression system, a hemagglutinin (HA) tag was added to XPB cDNA by PCR, using *Pfu* polymerase. Clones were checked with direct sequencing. HA-tagged p89 was first cloned into pSP72 and then into pMEP<sub>4</sub> (Invitrogen).

**Cell culture, cell cycle manipulation, and transfection.** XPB-deficient lymphoblasts (GM02252C/XPB11BE from the Coriell Cell Repository, Camden, N.J.) were cultured in RPMI 1640 with 15% serum (Cellgro). Stable cell lines were generated by transfection with 1  $\mu$ g of plasmid with FuGENE 6 (Roche), followed by selection under hygromycin (0.1 mg/ml). Protein expression was induced with cadmium and added directly into the cell culture medium at the indicated concentrations. BJAB cells were grown in RPMI 1640 with 10% serum (Cellgro). Cell number was determined by counting cells excluding trypan blue in a Neubauer chamber. Unless otherwise indicated, all experiments were performed at a cell density of  $5 \times 10^5$  cells per ml to avoid biases due to differential growth kinetics. A previously described simian virus 40-transformed fibroblast system that was optimized for the purification of tagged TFIID was not used to

avoid the influences of chromosomal integration and repeated cycles of UV treatment used during clonal selection (74).

**Cell survival assay.** Survival of UV-treated cells was assayed as previously described, with slight modifications (20). Briefly, cells were washed twice with 1× phosphate-buffered saline (PBS);  $2 \times 10^5$  cells per 35-mm dish were plated, PBS was removed, and cells were treated with different doses of UV (0 to 50 J/m<sup>2</sup>) with a calibrated Stratalinker UV Cross-Linker (model 2400). Medium was added back to cells, and the survival rate was calculated after counting cells 72 h after UV treatment. For the proliferation assay, cells were plated (in triplicate) at the same density and cultured under the same conditions. Cell growth was determined by counting cell numbers daily.

**Immunofluorescence microscopy.** Suspended cells were washed and spun onto coverslips prior to fixation. Cells were washed twice in 1× PBS (5 min) and fixed. For most staining procedures, cells were fixed for 12 min in 2% paraformaldehyde, washed twice in PBS, permeabilized for 5 min in permeabilization buffer (80 mM HEPES [pH 6.8], 5 mM EGTA, 1 mM MgCl<sub>2</sub>, 0.5% Triton X-100), and refixed for 6 min again in 2% paraformaldehyde, followed by four additional PBS washes. After fixation, cells were blocked for 12 min (0.5% Triton X-100 and 2% bovine serum albumin in 1× PBS) and incubated with primary antibody or species-identical nonspecific immunoglobulin G at the same concentration for 1 h.

Primary antibodies were as follows: α-HA (rabbit polyclonal; Roche), 1:50 dilution; α-cyclin H (mouse monoclonal; Austral), 1:50 dilution; and α-c-Myc (rabbit polyclonal; Upstate), 1:100 dilution. For staining c-Myc, incubation was performed overnight.

After incubation with primary antibody, cells were washed three times in PBS and incubated with fluorescein isothiocyanate-labeled secondary antibody (1:50 dilution; Jackson Immunochemicals) or tetramethyl rhodamine isocyanate-labeled secondary antibody (1:200 dilution; Sigma) for 30 min, followed by three additional washes. After DNA was counterstained with propidium iodide or 4',6'-diamidino-2-phenylindole (DAPI), slides were mounted with Vectashield mounting solution (Vector Laboratories).

**Flow cytometry for c-Myc quantification.** Cells were fixed and permeabilized with Intra stain (DAKO). After this, staining for c-Myc was performed as for immunofluorescence. Phycoerythrin-conjugated α-rabbit antibody (Molecular Probes) was used as a secondary antibody, and fluorescence was detected in the FL-2 channel.

**Western blotting.** Cells were washed twice in 1× PBS, resuspended in ice-cold radioimmunoprecipitation assay buffer (1% NP-40; 0.5% Na-deoxycholate, 0.1% sodium dodecyl sulfate [SDS]), swollen on ice for 20 min, homogenized, and disrupted by being passaged five times through a 25-gauge (5/8-in.) needle, incubated on ice for an additional 20 min, and centrifuged in a microcentrifuge at full speed for 30 min to recover total cell lysates. Protein lysates were separated on a 4 to 20% gradient gel (Novex) and blotted onto a nitrocellulose membrane. Primary antibodies were used as follows: α-HA (mouse monoclonal; Roche), 1:2,000 dilution; α-p89 (SC-293, rabbit polyclonal; Santa Cruz) that recognizes wild-type, but not C-terminal truncated p89, 1:500 dilution; α-p89 (Austral Biotech) that recognizes an N-terminal epitope of p89, in both wild-type and truncated p89/XPB; α-p62 (rabbit polyclonal; Santa Cruz), 1:500 dilution; α-c-Myc (N-262, rabbit polyclonal; Santa Cruz), 1:500 dilution; and α-actin (mouse monoclonal; Oncogene), 1:10,000 dilution.

**RNAse protection assay and nuclear run-on assay.** RNase protection assays (RPAs) and nuclear run-on experiments for determining *c-myc* mRNA levels were performed essentially as previously described (6).

**KMnO<sub>4</sub> footprinting.** Cells were harvested, washed once with room temperature PBS, resuspended in buffer A (15 mM Tris-HCl [pH 7.5], 60 mM KCl, 15 mM NaCl, 5 mM MgCl<sub>2</sub>, 0.5 mM EGTA, 300 mM sucrose), and incubated for 2 min at room temperature. Fresh KMnO<sub>4</sub> was added to a final concentration of 25 mM and incubated at room temperature for 45 s. The reaction was stopped with β-mercaptoethanol (343 mM) and SDS (0.5%). Ligation-mediated PCR was performed as previously described and as modified (18, 45, 49). For heat shock response, cells were incubated at 42°C for 30 min and then incubated at 37°C for 30 min for recovery. Genomic DNA was treated with KMnO<sub>4</sub> in vitro at 25°C for 1 min and treated as above (46).

**Chromatin immunoprecipitation (ChIP).** The ChIP method was a variant of that previously described (73). Formaldehyde was added to cells in medium (final concentration of 1.0%). Cells were fixed for 15 min at 37°C, and the reaction was stopped by the addition of glycine to a final concentration of 125 mM. Cells were collected, washed twice with cold PBS, and then resuspended in Tris-EDTA (TE) with protease inhibitor cocktail (Calbiochem). The cell suspension was sonicated on ice with an ultrasonic sonicator for 6 pulses of 30 s each to an average DNA length of ~1,000 bp. After centrifugation, SDS, sodium deoxycholate, Triton X-100, and NaCl were added to the chromatin solution to final

concentrations of 0.1, 0.1, and 1.0% and 300 mM, respectively. The chromatin solutions were cleared with protein A-agarose beads (Roche) for 1 h at 4°C. For immunoprecipitations, chromatin solutions from  $2 \times 10^6$  cells were mixed with the indicated antibodies, protein A-agarose beads preblocked with 0.1 mg of herring sperm DNA/ml, and 2 mg of bovine serum albumin/ml. After incubation at 4°C overnight, beads were washed twice with buffer 1 (1× TE, 0.1% SDS, 0.1% sodium deoxycholate, 1.0% Triton X-100, and 300 mM NaCl), once with buffer 2 (1× TE, 0.1% SDS, 0.1% sodium deoxycholate, 1% Triton X-100, and 150 mM NaCl), twice with buffer 3 (1× TE, 0.25% sodium deoxycholate, 0.5% NP-40, and 250 mM LiCl) and once with TE. Immunoprecipitates were eluted from beads by incubation with elution buffer (50 mM Tris-HCl [pH 8.0], 1% SDS) at 65°C for 15 min. The beads were extracted again with TE, and proteinase K (final concentration, 0.5 mg/ml) was added to the combined elutes and incubated at 65°C overnight to reverse cross-linking. The samples were extracted with phenol and then phenol-chloroform, and DNA was precipitated with ethanol. A total of 10% of precipitated DNA was used for PCR. Antibodies used were p89 (recognizes an N-terminal epitope of p89, in both wild-type and truncated p89/XPB; Austral Biotech), cycH (Austral Biotech), hHSF1 (a kind gift of Carl Wu), and polymerase II (catalogue number 8WG16; Babco). Primers for PCR were as follows: for the *c-myc* promoter, the forward primer was 5'GGA TCG CGC TGA GTA TAA AAG CCG3' and the reverse primer was 5'CTA TTC GCT CCG GAT CTC CCT TC3'; for the *hsp70* promoter, the forward primer was 5'GCG AAA CCC CTG GAA TAT TCC CGA3' and the reverse primer was 5'GAA GCC TTG GGA CAA CGG GAG TC3'. Titration of input DNA into PCR confirmed that amplifications occurred in the linear range.

## RESULTS

**A cell system to study the influence of p89/XPB on *c-myc* expression.** A model system was created to study the FBP/FIR/TFIIH regulatory system in cells with normal or defective TFIIH function. The extensively characterized XPB lymphoblast cell line from patient XP11BE served as the foundation for further investigation (27). Due to a splice mutation in the *p89/xpb* gene leading to a frameshift, the last 41 amino acids of the protein are out of frame. The mutant cells show a severe defect in NER with UV hypersensitivity and an impaired helicase activity, as well as reduced in vitro basal transcription activity. Wild-type p89 was expressed in XPB cells to rescue TFIIH function with an Epstein-Barr virus-derived episomal vector system with a metal-inducible promoter. The use of the episome ensured p89 expression in pools of cells without the confounding complications of clonal selection. Transfecting the empty vector and performing the same selection created a control cell line in addition to the parental mutant cells. The resulting cell lines were designated XPB<sub>0</sub> (the original XPB cells from which the others were derived), XPB (the XPB<sub>0</sub> cells harboring the empty vector), and XPB/wt-p89 (XPB<sub>0</sub> with an episome expressing wild-type p89). Microarray hybridization experiments revealed that the ratio of episomally expressed, wild-type p89 mRNA to endogenous, frameshifted p89 mRNA was about 4:1 under steady-state conditions without induction and was increased to more than 25:1 following treatment with cadmium (H.-J. Chung and D. Levens, unpublished data). p89 protein levels increased in a dose-dependent fashion along with mRNA expression upon the addition of cadmium. p89 protein was even more profoundly induced than the RNA, most likely due to accretion. Wild-type p89 was already detectable in XPB/wt-p89 cells even under uninduced steady-state conditions (Fig. 2A, lane 2), and was at levels comparable to those in the lymphoblast cell line BJAB (Fig. 2B, bottom panel, lanes 1 and 3). Although complemented cells showed modestly increased levels of p89 protein when an antibody recognizing both the truncated and wild-type p89 was

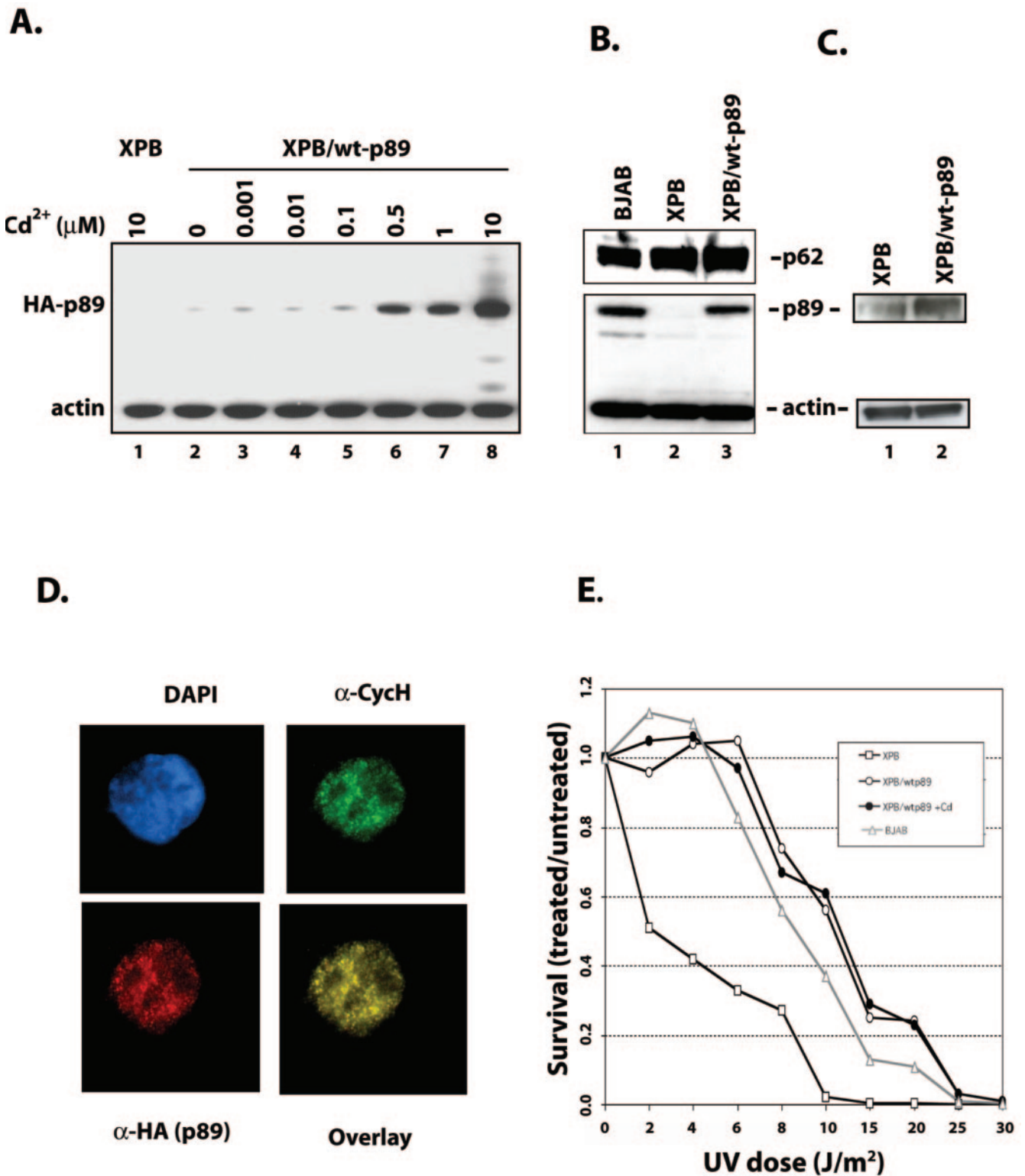


FIG. 2. Inducible expression of wild-type p89 in XPB cells. (A) Expression of wild-type p89 can be induced by cadmium. HA-tagged wild-type p89 is under the control of a metal-inducible promoter. Wild-type p89 is detectable in XPB/wt-p89 cells even under steady-state conditions (lane 2) and can be induced by cadmium (lanes 3 to 8). No wild-type p89 is expressed even under high doses of cadmium in the XPB cell line (lane 1). (B) Quantification of p89 expression. A polyclonal antibody against the C terminus of p89 (SC-293) recognizes wild-type p89 but not truncated p89. Under steady-state conditions, XPB/wt-p89 cells express p89 to a level similar to that with the BJAB reference cell line. Probing for p62, another subunit of core TFIIH, shows similar levels of expression in all three cell lines, indicating that expressing wild-type p89 alters TFIIH quality but not protein turnover. (C) Quantification of p89 protein levels. XPB and XPB/wt-p89 whole-cell lysates were subjected to Western blotting with  $\alpha$ -p89 (Austral), which recognizes both truncated and wild-type p89. (D) Subcellular localization of wild-type p89 in XPB/wt-p89 cells. Wild-type

used (Fig. 2C), restitution of wild-type p89 with uninduced expression did not obviously disturb the expression of other TFIIH components, since immunoblot analysis showed that levels of the p62 subunit were strikingly similar when levels of XPB, XPB/wt-p89, and the BJAB cells were compared (Fig. 2B, top panel). Thus, any difference between XPB cells and XPB/wt-p89 cells could be attributed to a difference in TFIIH quality rather than TFIIH quantity. XPB/wt-p89 B cells were stained with fluorescent antibodies to ascertain TFIIH assembly incorporating episomally encoded wild-type p89 *in vivo* and to visualize proper TFIIH subcellular localization. Wild-type p89 was found in a finely dotted pattern throughout the nucleus with relative sparing of the nucleoli, a pattern previously seen for TFIIH (28). Costaining for cyclin H, another component of TFIIH, revealed extensive colocalization of these two subunits, indicating proper assembly of the holoenzyme complex (Fig. 2D).

To assess the functional significance of the stable expression of wild-type p89, cells were subjected to a standard cell survival assay. Cells were UV irradiated with increasing doses (2 to 50 J/m<sup>2</sup>), and numbers of viable cells were counted 4 days after UV irradiation. Empty vector-transfected XPB lymphoblasts (XPB cells) showed reduced cell survival compared to the reference lymphoblast cell line, BJAB. In contrast, XPB lymphoblasts with wild-type p89 displayed UV sensitivity comparable that of BJAB cells. Thus, by stable transfection of wild-type p89, XPB cells were functionally corrected for survival after UV irradiation (Fig. 2E). Since uninduced levels of p89 expression obviously yielded sufficient TFIIH to sustain proper NER, the all of the following experiments were performed under basal levels of episomal wild-type p89 expression. Full rescue of TFIIH function in NER would be paralleled by the reversal of transcriptional deficits attributable to defective TFIIH. In addition, since XPB and XPB/wt-p89 cells differ only with respect to their p89 status but otherwise share the same genetic background, this model system seems suitable to explore the consequences of the XPB mutation on transcription *in vivo*.

**c-Myc expression is coarse and heterogeneous in XPB versus XPB/wt-p89 cells.** *In vitro*, FBP accelerates and FIR delays promoter escape via TFIIH. The cell system described above was employed to assess the net consequences on *c-myc* expression of the TFIIH mutation in XPB disease. Reasoning that by simultaneously disabling opposing activators and repressors (41), *c-myc* regulation might become more erratic, the variation of endogenous *c-Myc* levels was assessed in individual cells by staining with anti-*c-Myc*, followed by flow cytometry. Comparison of the *c-Myc* distributions between uncorrected and corrected cells revealed two quantitative distinctions. First, the entire population of XPB cells showed ~1.5-fold-higher mean levels of *c-Myc* than did the XPB/wt-p89 cells (375 versus 248 on an arbitrary scale corrected for the immunoglobulin G

control) (Fig. 3A). Concomitant analysis of DNA content indicated that the difference in *c-Myc* was not due to a differential distribution in the cell cycle (data not shown). Second, the mutant cells indeed displayed a much broader distribution of *c-Myc* levels. Following restitution of wild-type p89, the variance of *c-Myc* distribution in the corrected cells was reduced to 31% of that of the mutant cells. The XPB cells had both higher levels of *c-Myc* and a higher coefficient of variation than the wild-type cells (0.53 versus 0.45, respectively); a higher level of expression would be expected to reduce the coefficient of variation.

Analyzing BJAB cells revealed a *c-Myc* distribution similar to that seen with XPB/wt-p89 cells but distinct from the abnormally broad profile observed with XPB cells (Fig. 3B). Thus, even in lymphoma cells, *c-myc* expression is less variable than is seen upon mutation of TFIIH.

**Concordant levels of *c-myc* mRNA and protein in XPB cells and XPB/wt-p89 cells.** The most simple explanation for the 1.5-fold increase in levels of *c-Myc* protein in cells with mutant p89/XPB would be a similar increase in *c-myc* mRNA, since TFIIH is a transcription factor. Therefore, RPAs were performed to ascertain whether the 50% increase in mean *c-Myc* protein measured by flow cytometry of XPB relative to that of XPB/wt-p89 lymphoblasts was paralleled at the *c-myc* mRNA level. In multiple independent RPA experiments, the uncorrected cells consistently contained at least 50% more *c-myc* mRNA than the corrected cells (Fig. 4A, lane 1 versus lane 2). Since *c-myc* mRNA and protein levels are tightly linked in most circumstances, *c-Myc* protein levels were compared to cross-check the differences between the corrected and uncorrected cells. Indeed, immunoblot analysis of XPB whole-cell extracts under steady-state conditions repeatedly revealed an increase of *c-Myc* protein, consistent with the levels determined by flow cytometry (Fig. 3) and commensurate with the increase in mRNA (Fig. 4A, lane 3 versus lane 4). Thus, at the RNA and protein levels, *c-Myc* expression was increased.

To confirm the concordance between *c-myc* mRNA and protein levels in both the XPB and XPB/wt-p89 cells, maneuvers were attempted to alter transcriptional output of the gene. Reasoning that redeployment of TFIIH to sites of DNA damage might exacerbate the difference between them, XPB and XPB/wt-p89 cells were irradiated with UV light, a treatment known to down-regulate *c-myc* (1, 17, 47). RNA was collected 3 h postirradiation, and *c-myc* mRNA levels were measured by RPA. Because *c-myc* mRNA was more effectively down-regulated in XPB/wt-p89 than in XPB cells, the excess of *c-myc* transcripts in the mutant cells relative to the corrected increased to 2.7 fold, from 1.5 fold at steady state (Fig. 4B, lane 1 versus lane 2). *c-myc* shutoff was also delayed in the XPB cells; 3 h post-UV treatment, *c-myc* levels in the mutant cells persisted at 80 to 100% of steady state, whereas the *c-myc* levels in the corrected cells had declined by 50%. Eventually,

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p89 (detected by  $\alpha$ -HA) is distributed in a finely dotted pattern throughout the nucleoplasm with relative sparing of nucleoli. Staining for cyclin H reveals colocalization with p89, indicating proper TFIIH assembly. Nuclei were counterstained with DAPI. (E) XPB/wt-p89 cells are DNA repair proficient. XPB cells, XPB/wt-p89 cells, XPB/wt-p89 cells under cadmium induction, and BJAB cells were UV irradiated with increasing doses, and survival rates (the ratio of treated cells/untreated cells) were assessed 4 days later. Low-dose UV irradiation dramatically reduced XPB cell survival, whereas XPB/wt-p89 cells were less sensitive. Comparison to a reference cell line (BJAB) indicates that the repair capacity is within normal range. Induction of wild-type p89 expression with 500 nM cadmium does not change survival compared to steady-state expression levels.

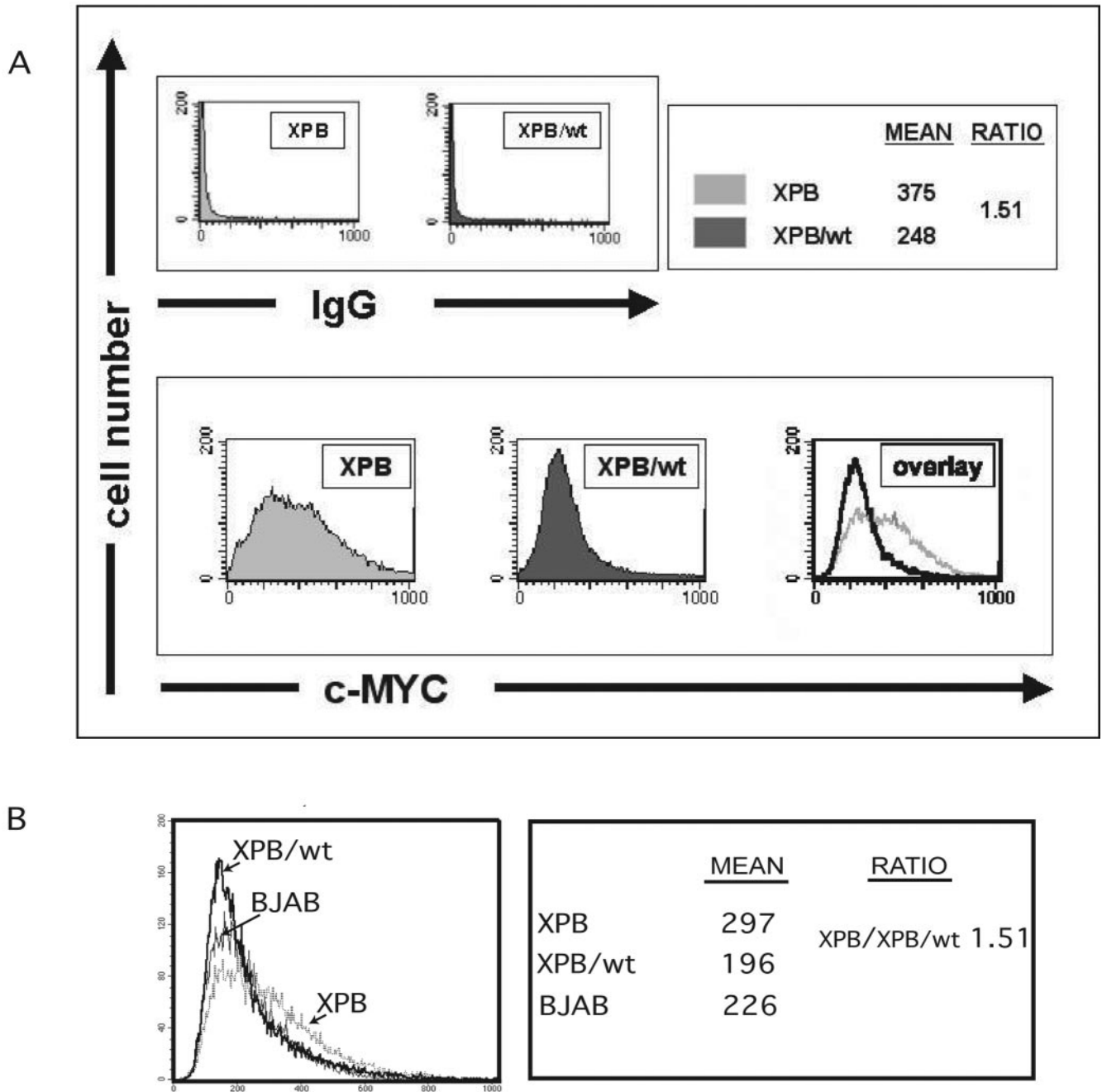


FIG. 3. TFIIH suppresses cell-to-cell variation in c-Myc. (A) To determine expression levels with single-cell resolution, cells were stained for c-Myc and analyzed by flow cytometry. (Top) Background from secondary antibody; (bottom) anti-c-Myc-specific fluorescence. Average c-Myc levels were 1.51-fold higher in XPB than in XPB/wt-p89 cells. Whereas expression levels of c-Myc resembled a Gaussian distribution in XPB/wt-p89 cells, c-Myc levels were more broadly distributed in XPB cells with some cells showing more than four times the mean of XPB/wt-p89 cells. The CV (coefficient of variation) of XPB and XPB/wt-p89 was 0.53 and 0.45, respectively. K-S statistics reveal these distributions to be different with  $P \ll 0.001$ . (B) BJAB (normal TFIIH) cells show a distribution more similar to that of XPB/wt-p89 cells but distinct from the broad profile observed with XPB cells. Note that the absolute, but not relative, levels of staining would be expected to vary between experiments. The CVs for XPB, XPB/wt-p89, and BJAB were 0.52, 0.46, and 0.46, respectively (note that at high levels of c-Myc, the BJAB curve drops below the XPB/wt-p89 curve, allowing both cells to have similar CVs).

6 h after irradiation, *c-myc* mRNA bottomed at similarly low levels in both cell lines (data not shown). These changes in *c-myc* mRNA were reproducibly paralleled by the expected decline in c-Myc protein; following UV irradiation (Fig. 4B,

lane 3 versus lane 4), the excess of c-Myc in XPB relative to XPB/wt-p89 cells became more pronounced.

Is the sluggish down-regulation of *c-myc* transcription in XPB cells secondary to the XP-NER deficit and so peculiar to

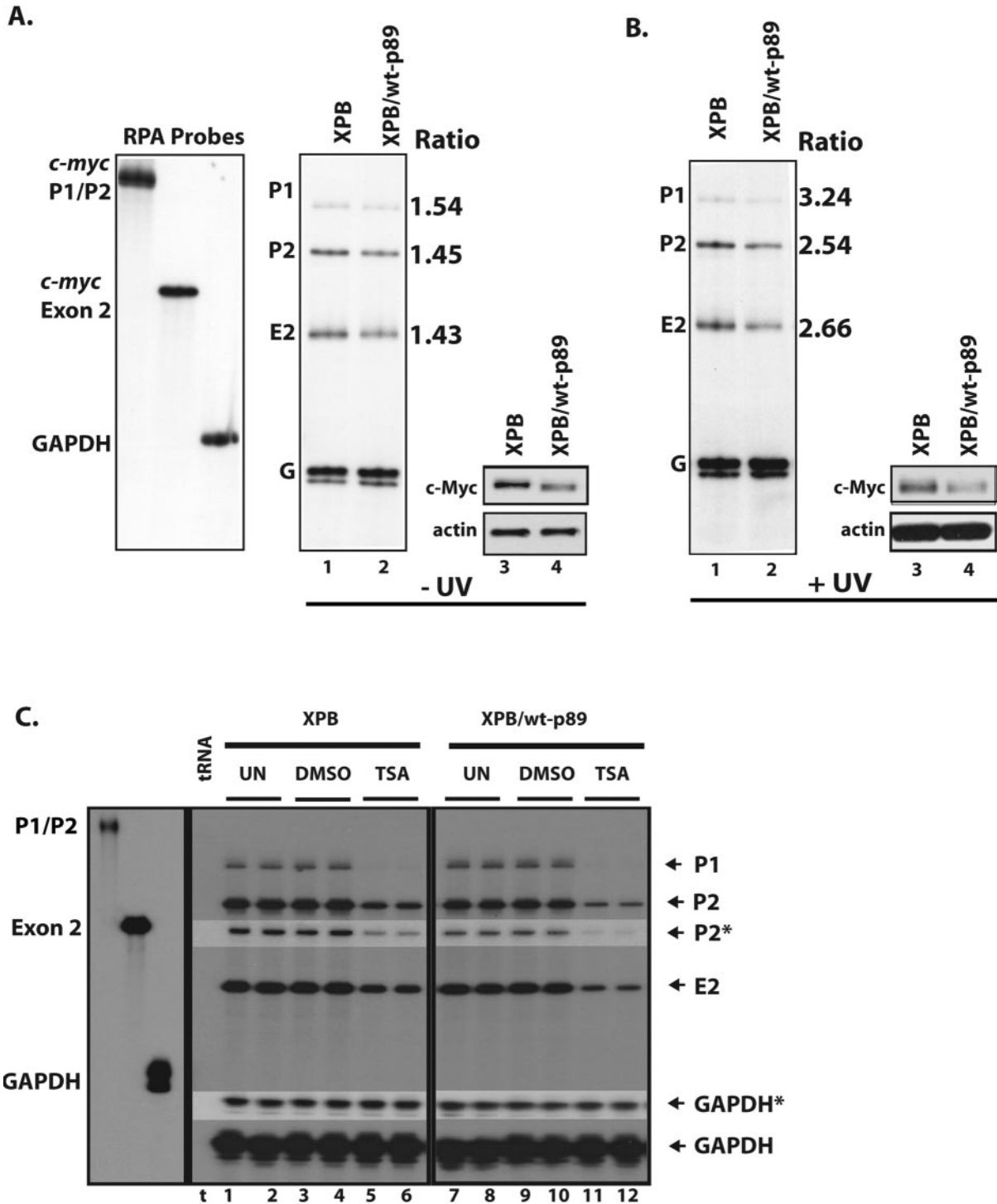


FIG. 4. Steady-state endogenous *c-myc* levels are disturbed in XPB cells. (A) (Left) *c-myc* mRNA is expressed at higher steady-state levels in XPB than in XPB/wt-p89 cells (lanes 1 and 2). RNase protection with *c-myc* P1-P2 and exon 2 riboprobes is shown. Quantification of *c-myc* mRNA (normalized to *gapdh* mRNA) reveals 1.4- to 1.6-fold higher levels of *c-myc* mRNA in XPB than in XPB/wt-p89 cells. (Right) Comparison of *c-Myc* protein levels in XPB versus XPB/wt-p89 cells. Concordant with RNA levels, XPB cells have more *c-Myc* than cells expressing wild-type p89 (lanes 3 and 4). (B) (Left) Analysis of *c-myc* expression in response to UV irradiation by RNase protection 3 h after UV exposure (8 J/m<sup>2</sup>). Quantification of *c-myc* mRNA (normalized to *gapdh* mRNA) demonstrates 2.5- to 3.2-fold-higher levels of *c-myc* mRNA in XPB than XPB/wt-p89 cells. (Right) *c-Myc* protein levels parallel mRNA levels (lanes 3 and 4). (C) Analysis of the response of *c-myc* mRNA to TSA treatment. Cells were treated with TSA (500 ng/ml) or dimethyl sulfoxide (DMSO) (as the vehicle) for 4 h. The RPA reveals that *c-myc* mRNA is down-regulated more efficiently in XPB/wt-p89 than in XPB cells (lanes 5 and 6 versus 11 and 12). Undigested probes are shown in the left panel. Bands marked with an asterisk were from a shorter exposure to verify intensity.

UV irradiation, or does the XPB mutation generally impair *c-myc* repression due to a transcription defect at the promoter? Histone deacetylase inhibitors such as trichostatin A (TSA) and butyrate reduce *c-myc* promoter activity and induce differentiation in susceptible cells (2, 70). Indeed, after 4 h of TSA treatment, *c-myc* was down-regulated less effectively in XPB cells than in XPB/wt-p89; XPB/wt-p89 cells as well as BJAB cells (data not shown) exhibited the expected decline in *c-myc* RNA (Fig. 4C, lanes 5 and 6 versus lanes 11 and 12) (6). Similar results were seen upon histone deacetylase inhibition with butyrate (data not shown). Thus, under all conditions examined, the uncorrected cells expressed more c-Myc than the corrected cells. Though modest, the differences in *c-myc* expression due to the p89 mutation in XPB might be significant, since even subtle changes in c-Myc levels have been associated with alteration of cell size, proliferation, apoptosis, and organismal phenotypes. Although a defective basal transcription factor might be expected to impair transcription, failure to receive repressor input or to holdback at the *c-myc* promoter might yield a net increase in expression. The changes in *c-myc* expression seen in XPB cells might be due to defective TFIID operating directly at the *c-myc* promoter or could be an indirect effect of the TFIID mutation. If direct, the increased levels of *c-myc* expression could be due to sporadic hyperactivity from just a few *c-myc* promoters, while the remaining *c-myc* genes maintain normal promoter structure and function; in this case, the structure, function, and composition of complexes at the *c-myc* promoter would be similar between these cells. Alternatively, TFIID may act uniformly at each *c-myc* promoter to hold expression to close tolerances; in this case, the mutant TFIID should cause conformational, compositional, and functional differences when the *c-myc* promoters in XPB and XPB/wt-p89 cells are compared. Therefore, the *c-myc* promoters of XPB and XPB/wt-p89 cells were examined.

**TFIID mutations impose major conformational, compositional, and functional changes at the *c-myc* promoter.** Either by helping to set the trajectory of promoter DNA within transcription complexes or via its intrinsic helicase activity, TFIID is equipped to manipulate DNA structure at transcription start sites. To assess the *c-myc* P2 promoter configuration in vivo, footprinting was performed using potassium permanganate as a DNA conformation-sensitive chemical probe; nucleotide +58 (using nucleotide +53 as a reference) was dramatically hyperreactive in cells with wild-type p89 but was much less reactive in XPB cells with mutant p89 (Fig. 5A). The start site region, in contrast, was somewhat less reactive in the corrected cells than in the mutant cells; thus, the overall reactivity was more closely localized to the initiation site in the absence of fully functional TFIID. To be detectable, these changes must occur at a significant portion of the total population of *c-myc* promoters.

The structural perturbation at +58 seen in p89 wild-type but not mutant cells suggested that some component opening the promoter was defective or missing from this site. ChIP revealed RNA polymerase bound to the *c-myc* promoters of both cell lines (Fig. 5B, lane 4). Following heat shock treatment as a control (see below), RNA polymerase was reduced at the *c-myc* promoter consistent with the expected down-modulation of most transcription units (Fig. 5B, lane 10). In contrast, promoter-engaged TFIID was efficiently recovered only from

the XPB/wt-p89 cells, and the *c-myc* promoters in the XPB cells harbored only very low levels of TFIID with antibodies against p89 and cyclin H (Fig. 5B, lane 3, and Fig. 5C). Apparently, mutant TFIID is inefficiently recruited, prematurely discharged, or ineffectively retained at the *c-myc* promoter; nevertheless, these TFIID-underloaded promoters support the same or higher levels of c-Myc.

To find out if the mutant TFIID provoked changes in the activity, composition, or DNA conformation at promoters besides *c-myc*, the *hsp70* promoter was also examined by RPA, in vivo footprinting with potassium permanganate, and ChIP. Starting from similar low basal levels, a pulse of heat shock effectively induced *hsp70* expression in both XPB and XPB/wt-p89 cells; levels subsequently declined in both cell lines, although subtle details of the temporal profile may distinguish the two (*hsp70* mRNA may linger in the mutant cells), as assayed by RPA (data not shown). Belying the overall similarity of the heat shock response of *hsp70* in XPB (or XPB<sub>o</sub>) and XPB/wt-p89 cells, ChIP revealed the same striking difference found with the *c-myc* promoter; whether basal or heat shocked, the *hsp70* promoter from the XPB mutant was dramatically deficient in TFIID (Fig. 5B, lanes 3 and 9). In contrast, the *hsp70* promoter from both the mutant and wild-type cells was effectively charged with RNA polymerase in both cell lines independent of heat shock, albeit with minor differences (Fig. 5B, lanes 4 and 10). Though substantially devoid of TFIID, the XPB *hsp70* promoter showed no unequivocal structural or functional changes attributable to the lack of TFIID when probed with potassium permanganate in vivo. Apparently, the p89 mutation results in either less-efficient loading or more-efficiently discharge of TFIID from the *hsp70* promoter without dramatically perturbing transcription, even during heat shock.

**The zone of transcription holdback is diminished in XPB.** If FBP, FIR, or other transacting factors use TFIID to influence transcription between initiation and promoter escape at the *c-myc* promoter, then the distribution of engaged RNA polymerases might distinguish XPB and XPB/wt-p89 cells. To monitor the distribution of active transcription complexes at the *c-myc* promoter, nascent RNAs were labeled in nuclear run-on assays and hybridized to a panel of immobilized promoter segments (6). This method maps the zones of holdback or release of paused RNA polymerases, although the kinetics of transcription, the length of the labeled products, the duration of labeling reactions, and the size of the immobilized DNA sequences limit resolution.

In XPB cells and XPB/wt-p89 cells, as in most cell types, *c-myc* P1 promoter usage was very weak compared to P2. The run-on RNAs from the mutant and wild-type cells hybridized differentially to the sequential DNA segments downstream of P2 (Fig. 5D, lane 1 versus 2 and lane 3 versus 4). Prior to complementation, the maximum run-on hybridization signal mapped to the segment most proximal to P2 (nucleotides +1 to +50 (Fig. 5D, lanes 1 and 3). A dramatic fall-off of hybridization to the second segment (+51 to +100) indicated that without the TFIID promoter escape occurs close to the promoter. Upon restitution of TFIID, the zone of hybridization prior to the drop-off expanded to include the second segment oligonucleotides (Fig. 5D, lanes 2 and 4), suggesting repositioning of some paused transcription complexes to sites further downstream or an extended zone of holdback in the presence of functional



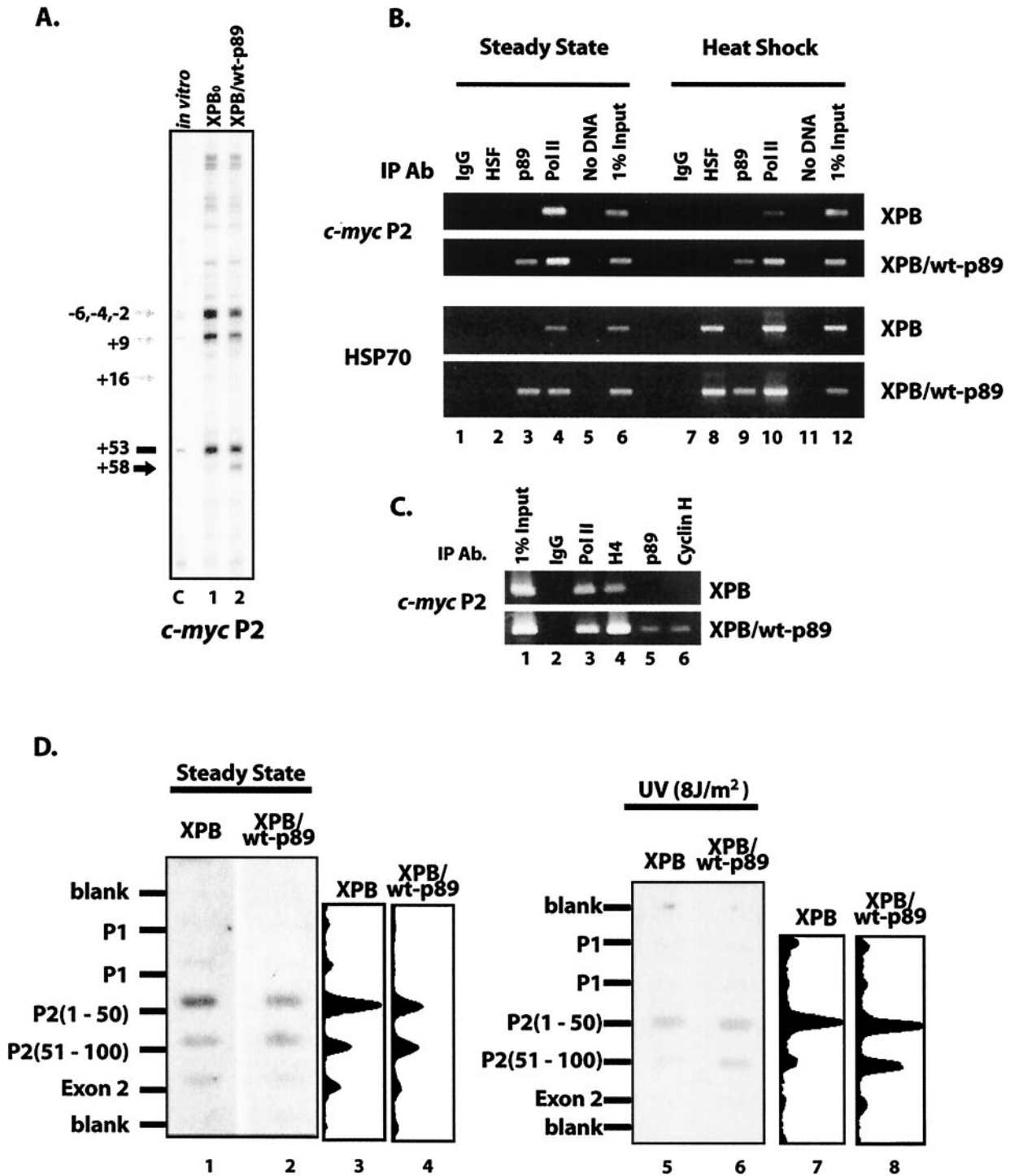


FIG. 5. Holdback of *c-myc* transcription occurs in a broader zone in XPB/wt-p89 than in XPB cells. (A) Conformation-sensitive in vivo footprinting of the *c-myc* P2 promoter with  $\text{KMnO}_4$  shows that the region around +58 is hyperreactive (black arrow) in cells with wild-type p89; modest start-site hyporeactivity (grey arrows) is seen in XPB/wt-p89 versus XPB cells, consistent with a downstream shift of transcription complexes in XPB/wt-p89 cells. Blank regions are hyporeactive, due to a paucity of thymidines. Lane C, genomic DNA treated with  $\text{KMnO}_4$  in vitro for 1 min at 25°C. (B) ChIP analysis reveals diminished TFIIH binding at the *c-myc* P2 promoter in XPB cells, unless complemented with wild-type p89 (top panels, lanes 3); the same occurs at the *hsp70* promoter (bottom panels, lanes 3). Binding of heat shock factor following heat shock is equivalent in both cells (lanes 8). Anti-p89 used in ChIP experiments recognizes both wild-type and truncated forms of p89. (C) ChIP analysis of the *c-myc* promoter in XPB and XPB/wt-p89 cells with  $\alpha$ -p89 and  $\alpha$ -CycH (Austral). (D) Nuclear run-on shows differential holdback at the major *c-myc* P2 promoter under steady-state conditions (left), as well as after UV irradiation (right). Hybridization with the second consecutive (downstream) P2 oligonucleotide is weaker for XPB cells, indicative of premature release of polymerase in cells with impaired TFIIH (lanes 1 and 3 versus 2 and 4). Differential release is exaggerated following UV irradiation (lanes 5 and 7 versus 6 and 8). Scans demonstrate relative intensities of the steady-state panel.

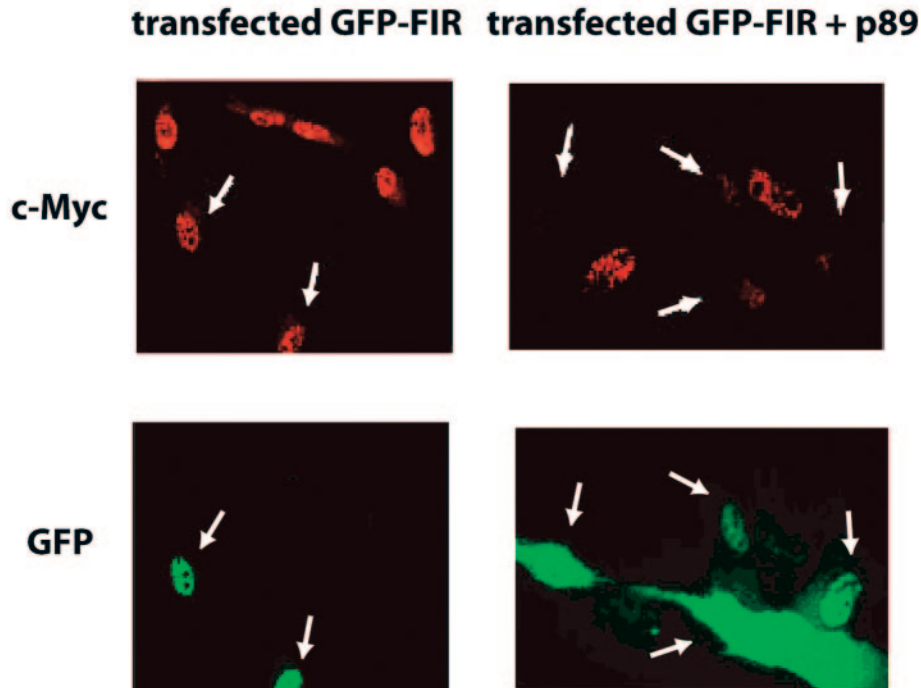


FIG. 6. FIR requires wild-type TFIH to repress endogenous *c-myc*. Transfected green fluorescent protein-tagged FIR was coexpressed in the presence or absence of wild-type p89 and immunostained for c-Myc. In XPB cells, FIR expression alone does not alter levels of c-Myc, but coexpression of wild-type p89 enables FIR repression of c-Myc via wild-type TFIH. Arrows indicate transfected cells.

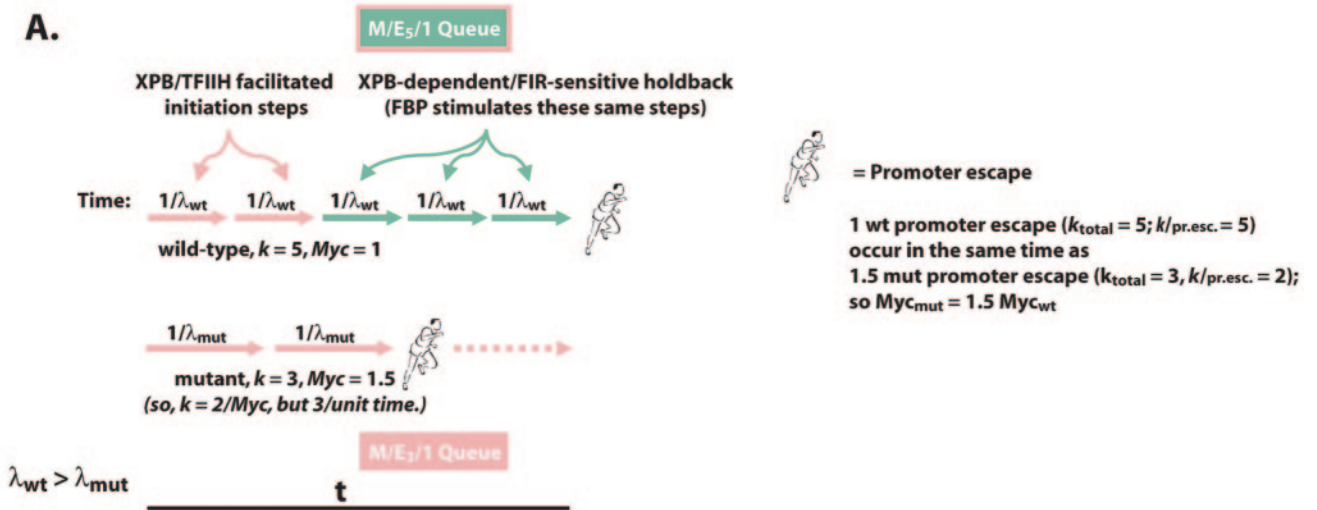
TFIIH. Following UV irradiation, there was a greater reduction in the signal at P2 nucleotides +51 to +100 than in the signal at P2 nucleotides +1 to +50, in the XPB cells than in the XPB/wt-p89 cells (Fig. 5D, lanes 5 and 7, versus lanes 6 and 8). These results are consistent with the transition to elongation occurring within a narrower zone, closer to the start site in the mutant cells.

Premature release of the paused polymerase may explain the delayed shutoff of *c-myc* mRNA seen in XPB but not in XPB/wt-p89 cells following UV irradiation.

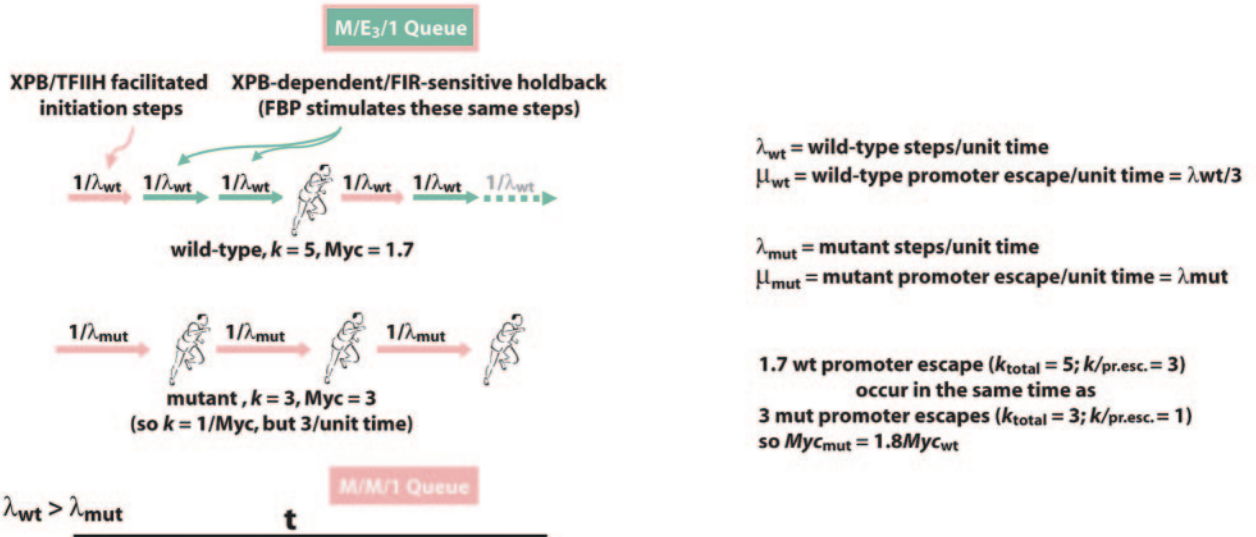
**FIR requires wild-type TFIH to repress endogenous *c-myc*.** Why are the constitutional and conformational differences between the *c-myc* promoters of XPB and XPB/wt-p89 cells as-

FIG. 7. Model describing the initiation to promoter escape transition as a series of steps, some accelerated and some slowed by properly functioning TFIH. Assume that each transcript begins with the temporally random arrival of a transcription complex (M, a Markovian process). Subsequent transcript synthesis is controlled by a series of  $k$  steps leading to promoter escape. The rate of promoter escape is  $\mu$ . Each individual step is a Markovian and Poisson process. Assume that the steps are kinetically equivalent (they may or may not be mechanistically equivalent). A series of  $k$  Poisson steps defines an Erlang  $k$  ( $E_k$ ) distribution. Moreover, if each step is a Poisson step, then the frequency of the duration of each step is a  $\gamma$ -distribution (and so each is exponentially distributed). For an  $E_k$  distribution with a mean interval until promoter escape of  $1/\mu$ , the variance on this interval is  $1/k\mu^2$  and the coefficient of variation is  $k^{-1/2}$ . Let transcripts be made on average at  $\mu$  transcripts per unit of time, and steps occur on an average of  $\lambda$  steps per unit of time; so  $k\mu = \lambda$ . Then the time for 1 step is  $1/\lambda$ ; the time for 1 transcript is  $1/\mu = K(1/\lambda) = k/\lambda$ . Because the steps occur through a Poisson process, the mean number of steps in a given time  $t = \lambda t = k\mu t$  with variance  $\lambda t = k\mu t$ , and with a coefficient of variation  $c = (\lambda t)^{-1/2}$ . The mean number of transcripts in this time is  $(\lambda/k)t = \mu t$  and  $c = (k\mu t)^{-1/2}$ . At  $t = 1/\mu$ ,  $c = k^{-1/2}$ . The fluctuation of the number of promoter escapes per cell decreases as the number of steps required for each promoter escape increases. From the  $c$  values for the wild-type and mutant cells (observed by flow cytometry) (Fig. 3), the respective  $k$  values are 5 and 3.5; but since there is 1.5-fold more c-Myc in the mutant cells, there are 1.5-fold more promoter escapes in the same interval, so  $k/c$ -Myc is 2.4 for the mutant cells. So the mutant cells have approximately two to three fewer p89/XPB-dependent steps than the wild-type cells. The model is consistent with an early role for XPB in initiation and as a regulator of several subsequent steps leading to promoter escape as described for panels A and B. (A) Predicted distribution of c-Myc based on five steps leading to promoter escape in the wild-type cells versus two steps in the mutant, but in two-thirds the time in the mutant, so that on average the mutant cells have 1.5-fold more c-Myc than the wild-type cells (hence the area under the wild-type curve in panel C is two-thirds of that under the mutant curve). In panel B, promoter escape with wild-type TFIH uses one initiation step and then two pauses, whereas as the pauses are lost in XPB, although the initiation step is slower (the TFIH mutation influences the response to both activators and repressors). Again, five steps occur in the wild type in the same time that three steps occur in XPB. This scheme supports 1.7-fold more c-Myc in XPB than in wild-type cells. In both panels A and B, salmon-colored steps are wild-type XPB-facilitated initiation steps (and so occur faster with wild-type TFIH), and green-colored steps represent TFIH-dependent pauses. (C) An Erlang process composed of multiple steps confers temporal regularity.  $E_1$  is a  $\gamma$ -distribution value (related to Poisson  $n = 1$ ), and events occur randomly. Promoter escape becomes more regular as  $k$  increases, even as the mean is held constant. Note that  $M/E_k/1$  indicates Markovian (random) entry (in this case, PIC formation) into a queue;  $E_k$  indicates a queue composed of  $k$  Markovian ( $\gamma$ -distribution) steps where the probability of a backward step is minimal, and 1 indicates that only one product (nascent transcript) is manufactured at a time before promoter preescape. Adapted from Ivo Adan and Jacques Resing, *Queueing theory* (<http://www.cs.duke.edu/~fishhai/misc/queue.pdf>).

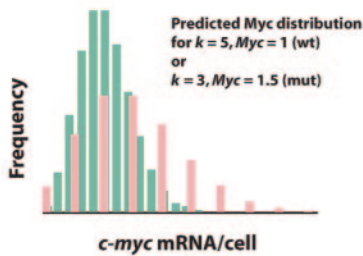
**A.**



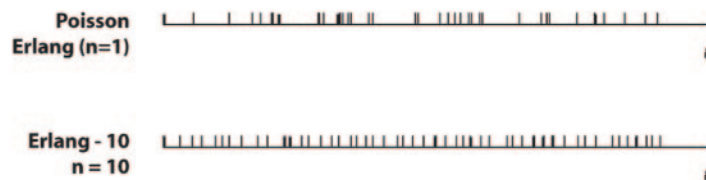
**B.**



**C.**



**D.**



sociated only with modest differences in *c-myc* mRNA levels? FBP and FIR operate through TFIID to activate and repress transcription (41). Moreover, FBP modulation of endogenous *c-myc* is impaired by mutation of p89 in XPB cells (41). If FIR repression of *c-myc* were equivalently compromised in XPB disease, then one mutation would disable offsetting activating and repressing systems. To assess the influence of XPB/wt-p89 and FIR on endogenous *c-myc* expression, green fluorescent protein-tagged FIR was cotransfected into XPB fibroblasts with and without wild-type p89 or the appropriate empty vector. Immunostaining experiments showed that in the background of mutated p89, FIR failed to repress c-Myc levels (Fig. 6), but FIR sharply depressed endogenous c-Myc levels when cotransfected with wild-type p89. Thus, for FIR to repress endogenous c-Myc, TFIID must be wild type. The simultaneous loss of FBP activation and FIR repression marginally shifts mean c-Myc levels within the total population but impairs fine tuning of c-Myc, leading to coarse regulation at the single-cell level. The operation of other activators and repressors operating through TFIID may be similarly impaired.

## DISCUSSION

The six-subunit core of TFIID serves double duty, participating in NER as well as transcription (5, 12, 64, 71). Though XP has been considered a disease of defective DNA repair, mutations in XPB and XPD have the potential to compound the pathology via transcriptional deregulation of critical target genes (9, 30, 72). Mutations in XPB and XPD that disturb NER helicase subunits differentially affect DNA repair, basal transcription, or the response of TFIID to activators and repressors (30, 71).

**What does TFIID do in transcription?** The role of TFIID in the early stages of the transcription cycle has been studied in detail with only a small number of promoters (often artificial), mainly during basal transcription. As the last basal factor recruited to the preinitiation complex, TFIID does not participate in promoter recognition. The most obvious contribution of TFIID to transcription initiation is opening the transcription bubble via helicase activity. Yet most RNA polymerases (prokaryotic and eukaryotic, other than RNA polymerase II) have the intrinsic ability to open the template and so initiate transcription without recruiting an active, extrinsic helicase. Alone, purified RNA polymerase II also transcribes duplex DNA perfectly well, albeit nonspecifically in vitro. Moreover, preinitiation complexes assembled on some supercoiled templates support transcription without TFIID. Possibly, TFIID and XPB helicase activity operates at several stages between initiation and promoter escape (11, 25, 26, 31, 48). Even in the simplest phage systems, this transition involves a complicated structural reorganization of the template-bound transcription machinery (65, 77). On some RNA polymerase II promoters in vitro, promoter escape, and not preinitiation complex formation, is the slow step in transcript synthesis (14, 33, 34). The data in this work indicate that in vivo, functional TFIID expands the zone at the endogenous *c-myc* promoter in which nascent transcript extension is regulated.

**TFIID oversees the transition from initiation to promoter escape.** These data and several previous key observations support the notion that TFIID helps to supervise postinitiation,

preescape *c-myc* transcription. (i) Most dramatically, by ChIP, TFIID is almost absent from the *c-myc* promoter in the mutant cells, indicating that it is either inefficiently recruited or rapidly discharged. Surprisingly, this near-absence does not diminish overall levels of c-Myc. Alternatively transcriptionally generated supercoils might render TFIID optional. (ii) By nuclear run-on, the paused polymerase at the *c-myc* promoter in XPB cells is released from holdback closer to the start site (within the first 50 nucleotides) than in normal cells that release downstream of +51. (iii) In cells with wild-type TFIID, permanganate footprinting highlights an altered DNA conformation in the region spanning +53 to +58, consistent with an expanded zone of holdback compared with the XPB cells. (iv) FBP and FIR require wild-type TFIID to modulate endogenous *c-myc* levels; these factors act at several postinitiation (but prepromoter) escape steps (41). (v) Mean c-Myc levels are modestly elevated but markedly variable in XPB mutant cells versus wild-type cells, suggesting an inability to finely adjust *c-myc* expression. (vi) Though footprinting and nuclear run-on studies reveal that in most situations the *c-myc* promoter is charged with polymerase (and so recruitment must not be rate limiting), no discrete pause site, pause sequence, or pause signals have yet been identified for the *c-myc* promoter (75). One way to account for the lack of a defined pause would be the asynchronous, slow progression of the *c-myc* transcription complex through several pauses prior to promoter escape.

**A model for *c-myc* transcription.** Together, the above facts support the following model. After initiation, the transcription apparatus, without proper TFIID, supports incremental transcript growth with pauses occurring at several, possibly variable, positions until promoter escape occurs early, well within the first 50 nucleotides. On some promoters in vitro, multiple sequential postinitiation, prepromoter escape transcription pauses have been observed; the exact number and sites of pausing are sequence dependent (14, 51, 52). If preinitiation complex formation is rapid on the *c-myc* promoter, as all evidence suggests, then promoter escape may be imagined as a queue of promoter-specific pauses. The duration of each individual pause and the interval from initiation until promoter escape are assumed to be Markovian, Poisson processes. Figure 7 shows two similar schemes compatible with the data. The total time of promoter escapes is then the sum of the intervals between the individual pauses, and the variance of this time is the summation of the variances at each pause. For simplicity, if each pause is imagined to be kinetically equivalent (although the general result is conceptually similar even relaxing this constraint), then the distribution of the time for promoter escape is described by an Erlang distribution, as follows.

$$f(t) = \frac{(\mu k)^k}{(k-1)!} t^{k-1} e^{-k\mu t} \quad (1)$$

The distribution is characterized by the number of pauses ( $k$ ) and the average rate of promoter escape ( $\mu$ ), which is the reciprocal of the mean interval between sequential escapes ( $1/\mu$ ). The variance ( $1/k\mu^2$ ) of the Erlang distribution decreases inversely with  $k$ ; thus, the output becomes more regular as the number of steps goes up. The variance of c-Myc in XPB cells is threefold higher than in XPB/wt-p89. A combination of

fewer and/or longer pauses could account for the increased variance.

In this scheme, wild-type TFIIH expands the zone of hold-back, increases the total number of pauses (by two or three) (Fig. 7B versus 7A), and steadies promoter output. Yet this same stability might oppose the changes in *c-myc* expression demanded in vital situations. Unlike a pathway regulated at a single rate-limiting step, accelerating or decelerating any single link in this chain of kinetically equivalent steps has limited influence on the net reaction rate. If each transfactor were dedicated to the control of only a single step, then whole sets would be needed to drive up expression. In this situation, the step-specific transfactors would exhibit strong synergy, and incremental regulation would be difficult to achieve. To account for *c-myc* induction, activators modulating TFIIH at several stages, such as FBP, could influence expression alone and would cooperate additively with mechanistically similar activators to advance the transcription complex incrementally toward promoter escape; repressors such as FIR would behave oppositely. TFIIH would then govern the transcription machinery as it ratchets its way along the template before the transition to elongation. A mutation that simultaneously disables activation by FBP and repression by FIR would then not shift mean *c-Myc* levels, but *k* would be reduced by 2, coarsening expression. Current structures of multisubunit RNA polymerases have not revealed a power stroke coupling nucleoside triphosphate hydrolysis with translocation (7, 36), and so nascent strand growth may be accomplished with a diffusion-driven sliding clamp, a Brownian ratchet (15). Activated XPB helicase may help to thread premelted template into the transcription apparatus and so facilitate forward diffusion, whereas repressors driving TFIIH to its ground state would impose additional impediments to translocation. Thus, TFIIH would serve as a signal integrator incrementally hastening or delaying progression to promoter escape. The involvement of TFIIH throughout all the stages of early transcript synthesis makes it an attractive target for regulation at multiple steps of the transcription cycle. Besides modulation of helicase activity, sequential action of TFIIH's Cdk7 to multiply phosphorylate the CTD could serve an entirely analogous role buffering against stochastic bursts of promoter firing; in fact, any pre-promoter escape step occurring on the same time scale would serve similarly. Recent evidence supports the notion that TFIIH may travel with the RNA polymerase through an expanded zone (60). The extent of TFIIH participation in the fine regulation of promoter escape may depend on the nature of the nearby DNA and chromatin-bound factors.

The XPB mutation removes the influence of at least some of the regulators operating through TFIIH, and so *c-myc* gene expression becomes more coarse. The influence of FIR in the presence of wild-type p89 exceeds that of FBP, and then the XPB mutation additionally nudges *c-myc* to higher levels, as observed. At other genes, the level of expression and the particular set of transfactors needed for proper regulation would determine the susceptibility to TFIIH mutations. Promoters regulated primarily at earlier stages of the transcription cycle (chromatin remodeling, preinitiation complex formation, or initiation) operating through transcription factors and coactivators targeting early steps in the transcription cycle such as occurs at the beta interferon gene would be less dependent on

fully functional TFIIH for proper regulation (42, 66). Another mechanism to escape the TFIIH dependence may be to bypass particular TFIIH functions. Recently, it has been shown that the yeast heat shock factor can mediate phosphorylation of the CTD of the largest subunit of RNA polymerase II, thus making transcription independent of proper TFIIH function (58). This observation is in line with the lack of effect of the XPB mutation on the *hsp70* promoter observed here. Thus, deficits in the general transcription factor TFIIH would be revealed as gene-specific defects in the expression of targets most dependent on TFIIH for proper regulation.

The XPB11BE mutation modestly increases mean *c-Myc* levels, but dramatically expands the cell-to-cell variation of this key regulator of proliferation, growth, differentiation, and apoptosis. Do fluctuating levels of *c-myc* matter? Although the answer to this question is not yet known, it is apparent that increased absolute levels of expression are insufficient to account for the pathology elicited by deregulated *c-myc* genes; average *c-Myc* levels in tumors such as Burkitt's lymphoma may be only modestly elevated or even normal, belying the critical role for *c-myc* deregulation in the pathogenesis of cancer (29, 57). Many mRNAs are present at very low copy numbers, so low that the notion of "the average cell" has been questioned, due to the almost endless combinations that may be generated by variable regulation and stochastic fluctuations (39). For many, if not most, genes the organism is robust enough to withstand the vagaries of hit-or-miss expression. Yet it would be surprising if mechanisms had not evolved to ensure uniform, low-level expression of some important genes.

#### ACKNOWLEDGMENTS

We thank Lance Liotta, Lyle Ungar, Louis Staudt, Deborah Boles, and Susan Mackem for helpful discussions and critical review of the manuscript. XP11BE lymphoblasts were from the Coriell Institute of Medical Research, Camden, N.J.

This study was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) to A.W. (AW2397/1-1 and AW2397/2-1).

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