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The TFIIH subunit p89 (XPB) localizes to the centrosome during mitosis

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Abstract. *Background*: The general transcription factor II H (TFIIH), comprised of a core complex and an associated CAK-complex, functions in transcription, DNA repair and cell cycle control. Mutations of the two largest subunits, p89 (XPB) and p80 (XPD), cause the hereditary cancer-prone syndrome xeroderma pigmentosum.

Methods: The TFIIH subunit p89 was monitored during interphase and cell division by immunofluorescence staining, GFP-fusion constructs including deletions, live cell imaging and immuno-precipitations.

Results: Here we demonstrate that during cell division, from prophase until telophase, the TFIIH core subunit p89, but not other subunits of TFIIH, associates with the centrosomes and the adjacent parts of the mitotic spindle. With overall constant levels throughout mitosis, p89 re-localizes to the newly formed nuclei by the end of mitosis. Furthermore, p89 interacts with the centrosomal protein γ-tubulin. Truncations of p89 result in an abnormal subcellular distribution during interphase and abolished centrosomal association during mitosis.

Conclusions: Our observations suggest a so far unappreciated role for p89 in cell cycle regulation, and may be the structural basis for a long known, but hitherto unexplained interaction between p89 and tubulin.

Keywords: TFIIH, mitosis, centrosome

1. Introduction

The general transcription factor II H (TFIIH) is a multi-subunit, multifunctional protein complex comprising a core and a three-subunit CAK complex (cdk7/cyclin H/MAT1). TFIIH has several distinct functions: transcription of RNA polymerase I and II, DNA repair (nucleotide excision repair (NER)), and with its three-subunit CAK complex also cell cycle control [7]. The core was long regarded as essential only for transcription and DNA repair. The two largest subunits of TFIIH, p89 (XPB) and p80 (XPD), have helicase activity, and they are encoded by genes comprising complementation groups of the xeroderma pigmentosum (XP) syndromes. Features of XP include skin sensitivity towards UV irradiation, dry skin and a dramatically higher incidence of skin cancer in sun exposed areas [20].

TFIIH is found in the nucleus and, albeit at lower levels, also in nucleoli [9,11]. TFIIH acts as a mediator of transcriptional repression during mitosis [8], providing a link between mitosis and transcription. No particular subcellular pattern has so far been reported for TFIIH during mitosis.

The centrosome contributes to efficient chromosomal segregation during cell division. It contains ring-like γ-tubulin complexes, which organize microtubules that make up the mitotic spindle. Impaired centrosome function or number may contribute to genetic instability and carcinogenesis [6]. Based on genetics analysis, a role of the gene product of the *Drosophila* p89 (XPB) homologue *haywire* [16] in general microtubule function has been postulated [17,18]. However, the exact nature of this interaction has not yet been elucidated. Here we monitored p89 throughout cell division and discovered an unexpected association of p89 with the centrosome and adjacent part of the mitotis spindle

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during mitosis, suggesting that with this p89 functions in the passage through the cell cycle.

2. Materials and methods

2.1. Cell culture, cell cycle manipulation and transfection

HeLa, COS and 293 cells were cultured in DMEM with 10% serum (Gibco-BRL). For GFP analysis, cells were transfected using FuGene 6 (Roche) with 1 μg plasmid/35 mm dish.

2.2. GFP fusion proteins and deletion constructs

A pSG5M/XPB plasmid (kindly provided by K. Kremer) was used as the source for human wild-type p89 cDNA (including 3′-UTR). XPB (p89) wild-type cDNA was recovered by restriction enzyme cutting and cloned into pEGFP-C1. Frame and orientation was checked by plasmid sequencing. Based upon the full-length XPB clone in pEGFP-C1 (pEGFP/XPB), several deletional subclones were established by restriction enzyme digestion as follows. Δ1-206 (clone A30): blunted lower fragment of SacI cut in blunted AccI/PstI (pEGFP/XPB) (Vector Fragment); contains XPB with first 206 codons missing. Δ1-298 (clone D1): AccIII cut pEGFP/XPB, vector fragment blunted and religated; contains XPB with first 298 codons missing. DEXHc (clone C27): AccI/PstI cut (pEGFP/XPB), smallest fragment (0.6 kb) contains DEXHc domain and was blunted and religated into blunted AccI/PstI pEGFP/XPB (Vector Fragment). Δ495-782 (clone F1): PstI cut (pEGFP/XPB), vector fragment religated; contains the first 494 codons of XPB including DEXHc, but without HELICc domain. Δ207-782 (clone E4): SacI cut of XPB-FL-EGFP and religation of upper fragment; contains the first 206 codons of XPB. A pET11d/p62 (GTF2H1) plasmid was used as source for human wild-type p62 cDNA. The full-length GTF2H1 (p62) coding region (from the initiating methionine to the stop codon) was PCR-amplified using Pfu DNA polymerase (Stratagene) and cloned into pEGFP-C1 using KpnI and XbaI sites. The cdk7 and cyclin H cDNAs were PCR-amplified using the corresponding I.M.A.G.E. clones (Invitrogen) as template, and cloned into pEGFP-C1 between HindIII and KpnI sites. All the clones were verified by plasmid sequencing and protein expression analysis.

2.3. Immunofluorescence microscopy

Adherent cells were grown overnight on coverslips. Cells were washed twice in 1 × PBS (5 min). For most staining procedures, cells were fixed for 5 min in 3% paraformaldeyde at room temperature. Prior to incubation with the primary antibodies, coverslips were incubated for with SDS (0.1%, 5 min) to unmask epitopes followed by blocking with 5% bovine serum albumin for 12 min. Primary antibodies were as follows: α-p89 (rabbit polyclonal, Santa Cruz, CA, USA), 1:100 dilution; α-γ-tubulin (rabbit polyclonal, Sigma), 1:200 dilution. Primary antibody incubation was performed 2 h. After two times washing in PBS for 5 min, cover slips were incubated for 1 h with the secondary antibodies (1:400 Alexa Fluor® 488 goat anti-rabbit and AlexaFluor® 647 goat anti-mouse, HOE33342 for nuclear staining; all from Invitrogen, Germany). Then, the slides were washed in PBS two times for 5 min and then mounted with fluorescent-free glycergel mounting medium (Dako-Cytomation, Hamburg, Germany). For nuclear staining on living cells, HOE33342 (1 μM) was added to the culture medium for 15 min prior to the measurements. Stained cells were analyzed with a filter wheel-based imaging system (Universal Imaging Corporation, Dowingtown, PA, USA) mounted on an inverted microscope (Axiovert 200, 40× objective, Zeiss, Jena, Germany) or with a confocal microscope (LSM 510; Zeiss, Jena, Germany) using sequential scanning (Plan Apochromat 63×/1.4 oil objective, excitation at 488 and 633 nm, emission at 505–530 and >650 nm, respectively). For nuclear staining, HOE33343 epi-fluorescence signal was superimposed.

2.4. Live cell imaging

Images were collected under thermostat conditions (37°C) using a filter wheel-based imaging system (Universal Imaging Corporation, Dowingtown, PA, USA) mounted on an inverted microscope (Axiovert 200, Zeiss, Jena, Germany). GFP-specific time series were analyzed using the Metafluor software (Universal Imaging, West Chester, PA, USA).

2.5. Immunoprecipitation

Cells extracts were prepared as described (Liu, 2001 #31). Binding reactions were rotated for 4 h at 4°C

in buffer BC-100 (20 mM HEPES, pH 7.9, 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 20 mM imidazole, 0.5 mM PMSF, 0.5 mM DTT) in the presence of 25 µg protein extract and either 250 µg mouse IgG (Santa Cruz, CA, USA), 250 µg monoclonal p89 antibody (AUSTRAL Biologicals) or 250 µg monoclonal γ-tubulin (GTU-88, Sigma). Protein A beads (Roche) were added, and incubation continued overnight. The protein A beads were washed four times in 200 mM salt buffer (20 mM HEPES, pH 7.9; 200 mM NaCl, 0.05% Triton X-100). Recovered proteins were subjected to electrophoresis and immunoblot analysis. Detection was performed with the following primary antibodies: SC-289 (Santa Cruz, CA, USA) for p89 (1:400 dilution), GTU-88 (Sigma) for γ-tubulin (1:4000 dilution).

3. Results

3.1. *p89 co-localizes with the centrosome during mitosis*

To test if TFIIH, like most sequence-specific transcription factors [15] is released from mitotic chromosomes, we monitored p89 throughout cell division in COS cells. Immunofluorescence (IF) staining of p89 revealed the expected nuclear localization in interphase (Fig. 1A), similar to the pattern described by others [11,21]. Like most other transcription factors, p89 dissociated from the mitotic chromatin. However, instead of a random distribution throughout the dividing cell, p89 decorated the mitotic spindle and the centrosomes (Fig. 1B–D). The same staining pattern was also

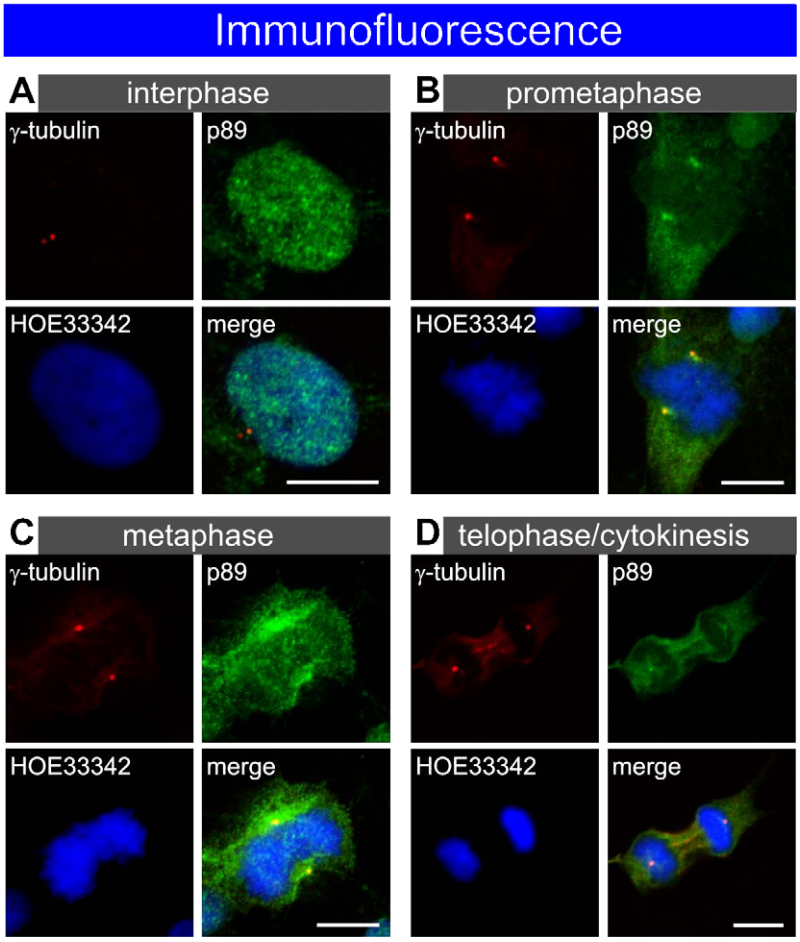

Fig. 1. The TFIIH subunit p89 localizes to the centrosome during mitosis. (A) Immunofluorescence staining in interphase cells shows p89 in a finely stippled pattern in the nucleus. No signal is detectable at the interphase centrosome. Starting from prometaphase (B), in metaphase (C) and until telophase/cytokinesis (D), a strong signal is detected at the centrosome and adjacent mitotic spindle. Scale bars = 10 µm. Red – γ-tubulin, green – p89; blue – HOE33342.

observed in other cell types (HeLa and 293 cells; not shown).

To authenticate the mitotic trafficking to the centrosome, p89 was transiently expressed as a GFP-p89 fusion protein in COS cells. GFP-p89 revealed the interphase pattern seen for endogenous p89 (Fig. 2A). In prophase, it was still detectable at the chromosomes, but in addition, started to decorate the centrosomes (Fig. 2B). In metaphase, the condensed chromatin was mostly devoid of fluorescence, but intensity at the centrosome and adjacent part of the mitotic spindle was very high (Fig. 2C–E). In telophase, GFP-p89 still localized to the centrosome, but also became detectable at the de-condensing chromosomes (Fig. 2F). Thus, the subcellular localization patterns of p89 observed by IF and GFP-labeling were mostly similar. Small differences in the subcellular localization patterns, e.g. in late phases of cell division, were most likely due to different expression levels of endogenous and transgenic p89.

3.2. *p89 interacts with γ-tubulin*

What triggers TFIIH to the centrosome? A screen in *Drosophila* designed to discover "genes involved in microtubule function" yielded *haywire,* the *Drosophila* p89 homologue, in addition to the easily anticipated tubulin genes [16–18]. Therefore, we tested for an association between p89 and centrosomal components in HeLa cells. A p89 antibody precipitated not only p89, but also γ-tubulin. Reciprocally, the γ-tubulin antibody co-precipitated p89, indicating that there may be a di-

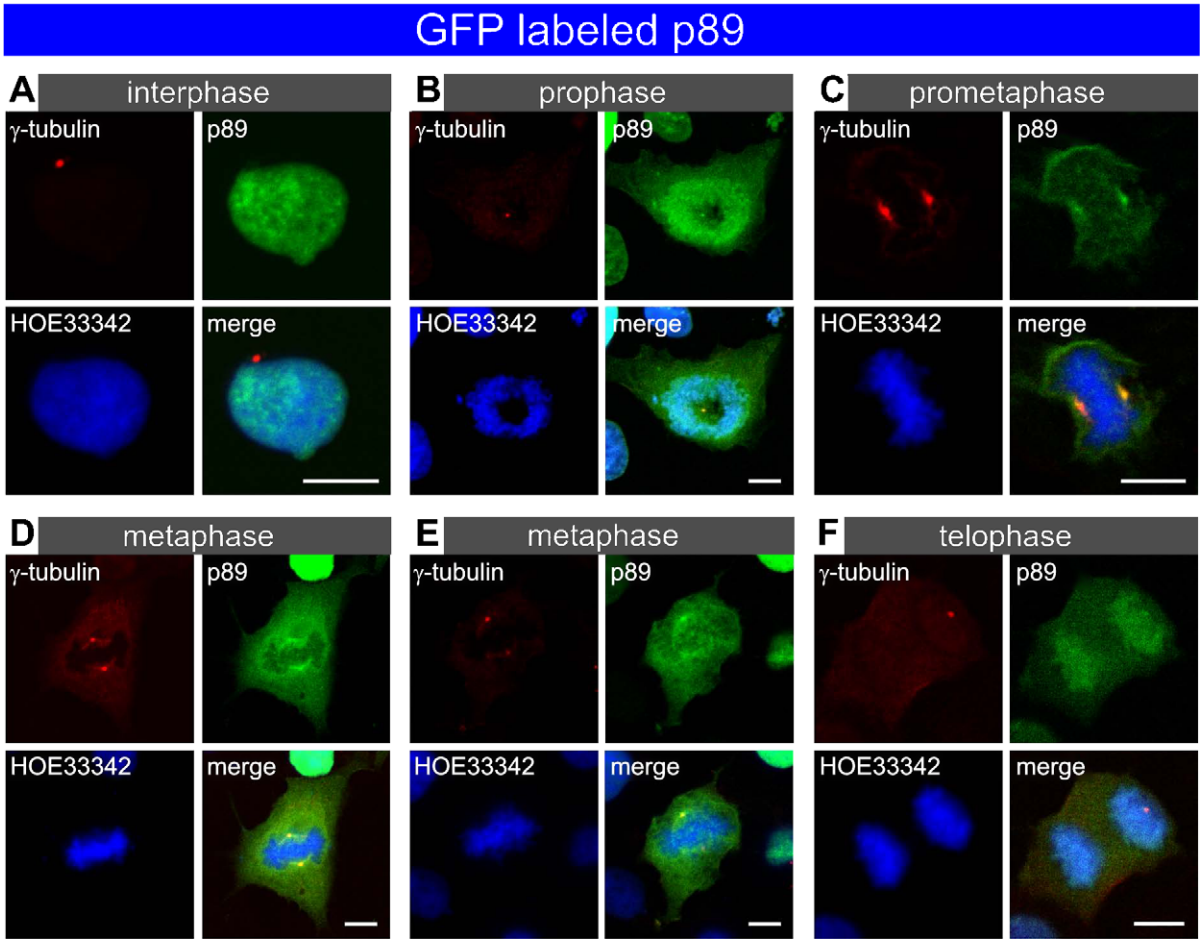

Fig. 2. GFP-p89 also localizes to the centrosome during mitosis. (A–F) GFP-p89 shows the same distribution during the cell cycle as endogenous p89. In interphase (A) GFP-p89 nearly exclusively localizes in the nucleus with no signal at the interphase centrosome. Starting from prophase (B), signals can be detected at the centrosomes. Starting from prometaphase throughout metaphase, staining of centrosomes is intense, whereas no chromosomal localization is found (C–E). In late telophase (F), intensity at the centrosomes decreases, and chromosomal association increases again. Scale bars = 10 μm. Red – γ-tubulin, green – GFP-p89; blue – HOE33342.

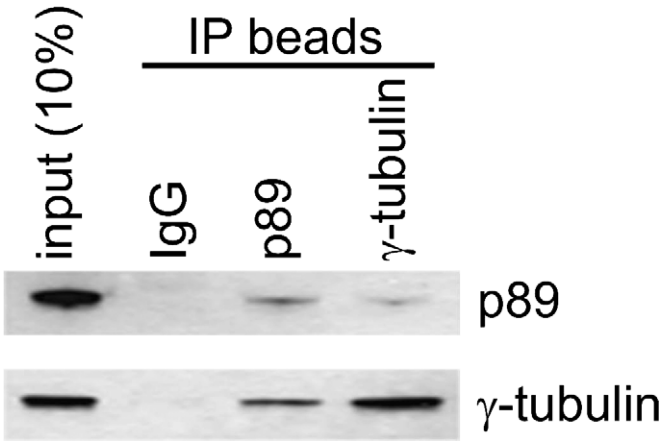

Fig. 3. p89 interacts with γ-tubulin. Immunoprecipitation with antibodies against p89 and γ-tubulin reveals a reciprocal co-immunoprecipitaiton, indicating an interaction between p89 and γ-tubulin. Mouse IgG: control antibody.

rect (or indirect) physical interaction between p89 and centrosomal components (Fig. 3).

3.3. Spatial re-distribution of p89 throughout cell division

We next sought to monitor GFP-p89 in living cells, in order to circumvent potential artifacts inherent to fixation and permeabilization. Live imaging of GFP-p89 transfected COS cells showed a nearly exclusively nuclear distribution of the fusion protein in interphase cells (Fig. 4A, panels 1, 11 and 12). Once cells entered mitosis, GFP-p89 was dispersed throughout the cell. Nearly no signal was detectable in projection to the metaphase plate (Fig. 4A, panels 4–7), indicating GFP-p89 displacement from mitotic chromosomes. During metaphase, highest fluorescence intensities were again at the centrosomes and spindle (Fig. 4A, panels 3 and 4). During telophase, there was an abrupt shift back to the newly forming nuclei (Fig. 4A, panels 7 vs. 8). By the end of cytokinesis, GFP-p89 was completely back in the newly formed nuclei (Fig. 4A, panels 9–12).

Proteolysis of regulatory proteins controls progression through mitosis. For cyclin B1, a mitosis-specific association with the centrosomal region has been described, and shown to correlate with its degradation [5]. To test if the p89 amount changes, fluorescence quantity of GFP-p89 was determined throughout cell division. Although there was a dramatic dispersion of fluorescence upon entry into mitosis (Fig. 4B, time points 1–3), the overall quantity remained nearly constant (Fig. 4B, bottom panel). Thus, the overall cellular level of p89 did not change significantly during mitosis.

3.4. Truncations of p89 affect its subcellular distribution

Several truncations of GFP-p89 were generated in order to determine which parts of p89 are relevant for its subcellular localization in COS cells. Truncations included either a part of the N-terminus (Δ1-206, Δ1-298), or both the N- and C-terminus, leaving only the DNA binding domain (DEXDc), or the C-terminus with loss of HELICc only (Δ495-782), or HELICc and DEXDc (Δ207-782) (Fig. 5A). These truncations revealed a differential subcellular localization during interphase and in mitosis (Fig. 5B and Table 1).

The nearly exclusive nuclear localization of p89 during interphase was altered in Δ1-206, resulting in additional cytoplasmic localization. The interphase distribution was only weakly affected in Δ495-782 and Δ207-782. Deleting large parts of the N- and C-terminus at the same time resulted in a toxic accumulation within the nucleus.

Truncating p89 also affected its distribution in mitosis. Deleting the N-terminus significantly reduced mitotic association with the centrosome (Δ1-298 more than Δ1-206). C-terminal deletion was less critical with Δ495-782 revealing a similar pattern as GFP-p89. Deleting both, large parts of the N- and C-terminus gave a signal similar to the GFP-mock control, abolishing any p89-typical localization.

3.5. Other TFIIH subunits do not show such an association with the centrosome

In order to investigate if the mitosis-specific association of p89 with the centrosome is common among

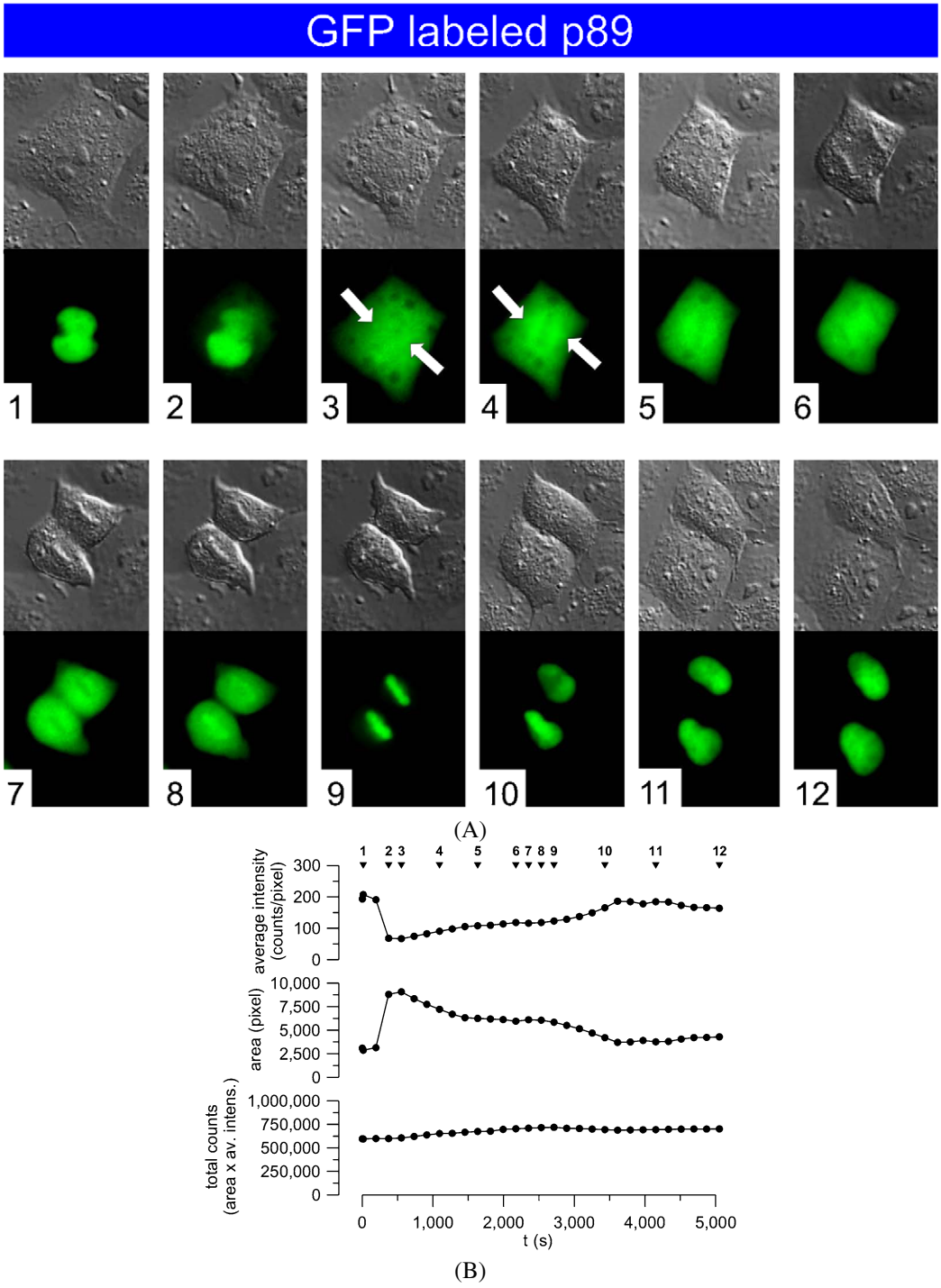

Fig. 4. Kinetics of p89 during the cell cycle. (A) Live imaging of GFP-p89 with time lapse series. During interphase, p89 is nearly exclusively in the nucleus. Upon prometaphase (1–2), at the same time the nuclear membrane breaks off, p89 detaches from the chromosomes to localize to the centrosomes (3). In late telophase, there is a sudden re-association of p89 with the chromatin (compare panels 7 and 8), and GFP-p89 localization is again confined to the newly forming nuclei (9–12). Panels: Differential interference contrast (DIC) microscopy (top) and corresponding GFP-p89 (bottom). Time points correspond to the arrowheads depicted in (B). (B) Quantification of fusion protein reveals spatial changes in intensity upon entry into mitosis, reflecting the re-distribution parallel to fall-off from the chromatin. However, overall intensity is nearly constant (lower panel).

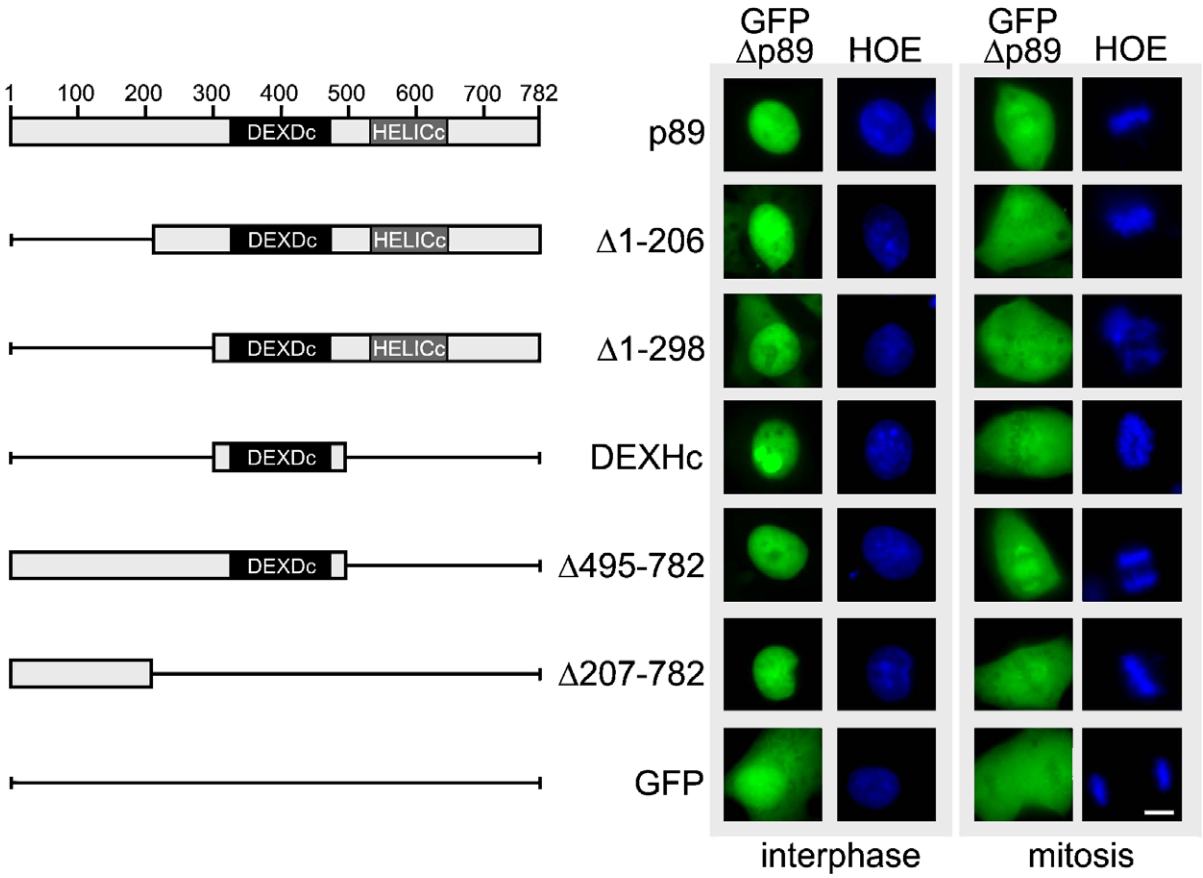

Fig. 5. Truncations of p89 affect its subcellular localization. (A) Deletions were generated by truncating part of the N-terminus (Δ1-206, Δ1-298), the N- and C-terminus, and the C-terminus with loss of HELICc only (Δ495-782), or HELICc and DEXDc (Δ207-782). (B) Deletions of N-terminal parts of p89 interfere with the nearly exclusive nuclear localization during interphase. Deletions of C-terminal parts of p89 hardly interfere with the nearly exclusive nuclear localization. Truncation of the N-terminus significantly reduces mitotic association with the centrosome, whereas C-terminal deletion is interfering less with centrosomal localization (Δ495-782). Deleting both, large parts of the N- and C-terminus of p89, not only reduces proper distribution, but also leads to toxic accumulation within the nucleus (DEXDc). GFP-p89 (top row) and GFP-mock (bottom row) are shown for comparison. Scale bar = 10 μm.

TFIIH subunits, we sought to monitor further subunits. Under standard conditions, IF staining did not show such a clear association of p62 with the mitotic spindle or centrosome. Transient expression of GFP-p62 revealed an interphase pattern similar as seen for p89 (Fig. 6A). Monitoring GFP-p62 during mitosis revealed different patterns depending on the treatment of cells. After fixation and permeabilization, the fusion protein revealed a signal at the centrosome (Fig. 6B). In contrast, when monitoring living cells, only briefly treated with HOE33342, no signal was detected at the centrosomes or the mitotic spindle (Fig. 6C). GFP-p89 monitored in parallel, still revealed a strong association (Fig. 6D). Thus, in contrast to p89, no unequivocal centrosome or spindle association was demonstrated for p62.

To monitor TFIIH subunits other than core components, GFP-fusion proteins for cyclin H and cdk7 were studied. The interphase patterns of these suggested integration into the TFIIH complex (Fig. 6E and G). During mitosis, neither for cyclin H nor for cdk7, a signal enhancement at the centrosome or adjacent spindle was observable (Fig. 6F and H). Thus, there was no evidence for an association of these CAK complex proteins with the spindle or centrosomes during mitosis. Therefore, the mitosis-specific association with the centrosome and spindle seems to be restricted to the p89 subunit.

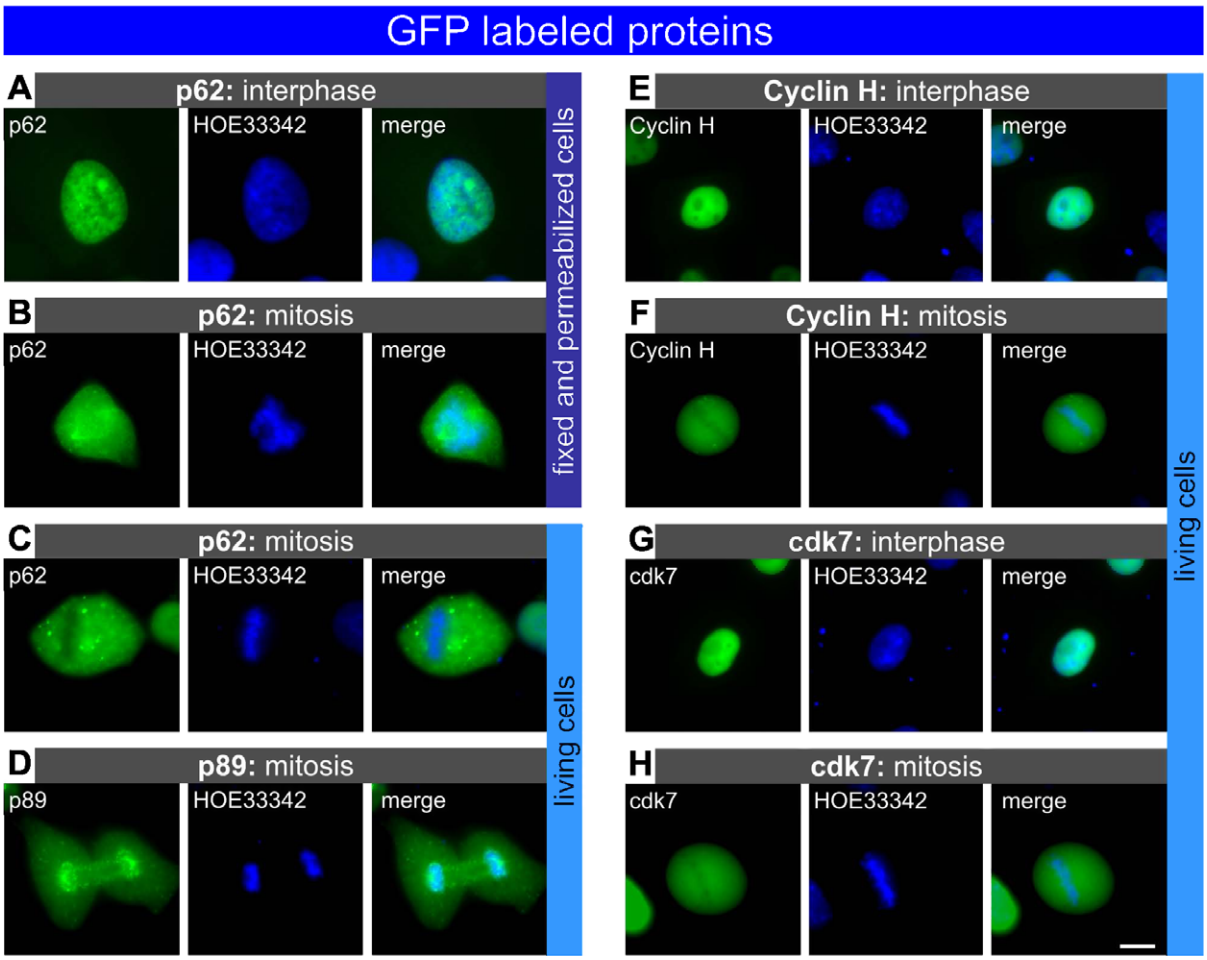

Fig. 6. Localization of other TFIIH subunits during mitosis. (A) Nuclear localization of GFP-p62 in interphase cells. During mitosis, GFP-p62 can be visualized at the centrosome only following (B) cell permeabilization, (C) but not in un-permeabilized living cells. (D) In contrast, GFP-p89 revealed the known centrosomal association also in living cells. GFP-labelled (E) cyclin H and (G) cdk7 showed a similar nuclear distribution in interphase. During mitosis, no significant spindle or centrosomal localization was detectable in (F) cyclin H- or (H) cdk7-transfected cells, respectively. Scale bar = 10 μm.

Table 1

Subcellular localization of the GFP-Δp89 constructs. Summary of a semi-quantitative analysis of the subcellular localization of the GFP-Δp89 constructs in interphase and mitosis in living cells. Signal intensities: − = none, + = weak, ++ = strong, +++ = very strong

	Interphase		Mitosis
	Nucleus	Cytoplasm	Centrosome
p89	+++	−	+++
Δ1-206	++	+	+
Δ1-298	++	+	+
DEXDc	++	+	−
Δ495-782	+++	−	+++
Δ207-782	+++	−	++
GFP	++	++	−

4. Discussion

The major finding of our study is a cell cycle dependent re-distribution of the TFIIH subunit p89 (XPB) with a mitosis-restricted localization at the centrosome. This observation suggests that the TFIIH core plays a previously unappreciated role in the regulation of the cell cycle.

We provide several independent lines of evidence that p89 is at the centrosome during mitosis: (1) Immunofluorescence staining of endogenous p89 with different antibodies reveals localization at the centrosome. (2) Transiently expressed GFP-p89 shows the same distribution. (3) Colocalization to the centrosome during mitosis is modified by deleting parts of p89.

(4) Co-immunoprecipitation of p89 and the centrosomal protein γ-tubulin indicates a biochemical interaction between these proteins. Thus, our data show that at least a fraction of p89 interacts with the centrosome during mitosis. It is unlikely that this mitosis-specific localization of p89 is a random event.

The association of p89 with the centrosome and adjacent part of the mitotic spindle is reminiscent to the localization of cyclin B1 during mitosis. For cyclin B1, this association has functionally been linked to proteosomal degradation of this protein [5]. Therefore, one might assume a similar role of the assembly of p89 with the centrosome. However, our quantitative analysis revealed that overall cellular p89 levels do not change significantly (Fig. 4). Biochemical analysis of p89 during mitosis also did not show any difference in p89 levels [1]. Therefore, centrosomal migration of p89 does obviously not lead to degradation of p89/TFIIH. If it is not turn-over bringing p89 to the centrosome, what else could it be?

Remarkably, other TFIIH subunits did not show the same affinity to the centrosome or mitotic spindle, which at first glance is surprising since it implies that the TFIIH complex is at least partially dissociated and free p89 exists. However, TFIIH is not inert during cell division, and several subunits are subject to modification. The CAK complex includes cdk7, which helps to trigger cell division, and so could modify cell cycle kinetics directly [22]. The cdk7 subunit is also hyperphosphorylated during mitosis, which goes along with an inhibition of transcriptional activity [1,14]. Evidence from *Drosophila* indicates that selective degradation of XPD/p80 permits entry into M-phase [4]. It is conceivable that p89 is also modified during mitosis and freed from the complex.

The functional significance of a transcription factor localizing at the centrosome is not self-evident. However, TFIIH serves multiple duties beyond transcription, in particular in cell cycle regulation and DNA repair. A number of other proteins involved in DNA repair and the control of genome integrity, such as Rb [19], Brca1 [10], XPC [2] and p53 [3], have been reported to localize to the centrosome, frequently in a mitosis-specific manner. Taking into account that TFIIH is known to interact with several of these, detecting p89 also at the centrosome is not that surprising. Our data might be relevant for understanding a genetic interaction described in *Drosophila*, the basis of which so far has not been solved. A genetic screen designed to recover proteins interacting with tubulin yielded *haywire*, the *Drosophila* homologue of p89. These results were attributed to an indirect transcriptional effect, albeit the possibility of a physical interaction between tubulin and p89 [17,18]. The co-localization and interaction of p89 with components of the centrosome and mitotic spindle shown here provide evidence for a direct interaction between p89 and tubulins. Our observations potentially have implications for understanding TFIIH-related tumorigenesis. The significance of impaired nucleotide excision repair (NER) for carcinogenesis in xeroderma pigmentosum (XP) is undisputed. However, defects in NER alone seem insufficient to explain the full cancer diathesis of XPB diseases, because not all DNA repair defects similarly predispose to carcinogenesis. XPB (and XPD) mutations that disturb NER, also have the potential to compound the pathology via transcriptional deregulation of critical target genes [12,13,24]. From this perspective, a single XP mutation may deliver multiple carcinogenic effects beyond defective NER: firstly, an apoptotic defect that impairs destruction of the genetically injured cell [23], secondly, a faulty regulation of critical genes. And finally, an XPB mutation may impede normal progression through G2/M. It therefore seems likely that deregulation of all these p89-dependent functions contributes to XPB-related carcinogenesis. Our data provide an attractive explanation for the genetic interaction between p89 and tubulin described in *Drosophila* [17,18]. Although we have not yet definitely proven that the physical interaction between p89 and the centrosome shown here indeed provides the basis for this genetic interaction, we nevertheless provide a molecular basis for further investigation. The fact that p89, a factor crucial for transcription, DNA repair and cell cycle control, interacts with the centrosome and spindle during mitosis, makes is possible that diverse cellular processes are temporally and spatially coupled by utilizing the dynamic re-distribution of p89. With this, p89 at the centrosome would provide a molecular platform to efficiently coordinate transcription, DNA repair and cell division.

5. Acknowledgements

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References

[1] S. Akoulitchev and D. Reinberg, The molecular mechanism of mitotic inhibition of TFIIH is mediated by phosphorylation of CDK7, *Genes Dev.* **12** (1998), 3541–3550.

[2] M. Araki, C. Masutani, M. Takemura et al., Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair, *J. Biol. Chem.* **276** (2001), 18665–18672.

[3] C.R. Brown, S.J. Doxsey, E. White and W.J. Welch, Both viral (adenovirus E1B) and cellular (hsp 70, p53) components interact with centrosomes, *J. Cell. Physiol.* **160** (1994), 47–60.

[4] J. Chen, S. Larochelle, X. Li and B. Suter, Xpd/Ercc2 regulates CAK activity and mitotic progression, *Nature* **424** (2003), 228–232.

[5] P. Clute and J. Pines, Temporal and spatial control of cyclin B1 destruction in metaphase, *Nat. Cell Biol.* **1** (1999), 82–87.

[6] S. Doxsey, D. McCollum and W. Theurkauf, Centrosomes in cellular regulation, *Annu. Rev. Cell Dev. Biol.* **21** (2005), 411–434.

[7] J.M. Egly, The 14th Datta lecture. TFIIH: from transcription to clinic, *FEBS Lett.* **498** (2001), 124–128.

[8] J.M. Gottesfeld and D.J. Forbes, Mitotic repression of the transcriptional machinery, *Trends Biochem. Sci.* **22** (1997), 197–202.

[9] D. Hoogstraten, A.L. Nigg, H. Heath et al., Rapid switching of TFIIH between RNA polymerase I and II transcription and DNA repair *in vivo*, *Mol. Cell* **10** (2002), 1163–1174.

[10] L.C. Hsu and R.L. White, BRCA1 is associated with the centrosome during mitosis, *Proc. Natl. Acad. Sci. USA* **95** (1998), 12983–12988.

[11] S. Iben, H. Tschochner, M. Bier et al., TFIIH plays an essential role in RNA polymerase I transcription, *Cell* **109** (2002), 297–306.

[12] A. Keriel, A. Stary, A. Sarasin, C. Rochette-Egly and J.M. Egly, XPD mutations prevent TFIIH-dependent transactivation by nuclear receptors and phosphorylation of RARalpha, *Cell* **109** (2002), 125–135.

[13] J. Liu, S. Akoulitchev, A. Weber et al., Defective interplay of activators and repressors with TFIH in xeroderma pigmentosum, *Cell* **104** (2001), 353–363.

[14] J.J. Long, A. Leresche, R.W. Kriwacki and J.M. Gottesfeld, Repression of TFIIH transcriptional activity and TFIIH-associated cdk7 kinase activity at mitosis, *Mol. Cell. Biol.* **18** (1998), 1467–1476.

[15] M.A. Martinez-Balbas, A. Dey, S.K. Rabindran, K. Ozato and C. Wu, Displacement of sequence-specific transcription factors from mitotic chromatin, *Cell* **83** (1995), 29–38.

[16] L.C. Mounkes, R.S. Jones, B.C. Liang, W. Gelbart and M.T. Fuller, A *Drosophila* model for xeroderma pigmentosum and Cockayne's syndrome: haywire encodes the fly homolog of ERCC3, a human excision repair gene, *Cell* **71** (1992), 925–937.

[17] C.L. Regan and M.T. Fuller, Interacting genes that affect microtubule function: the nc2 allele of the haywire locus fails to complement mutations in the testis-specific beta-tubulin gene of *Drosophila*, *Genes Dev.* **2** (1988), 82–92.

[18] C.L. Regan and M.T. Fuller, Interacting genes that affect microtubule function in *Drosophila melanogaster*: two classes of mutation revert the failure to complement between haync2 and mutations in tubulin genes, *Genetics* **125** (1990), 77–90.

[19] R.C. Thomas, M.J. Edwards and R. Marks, Translocation of the retinoblastoma gene product during mitosis, *Exp. Cell Res.* **223** (1996), 227–232.

[20] H. van Steeg and K.H. Kraemer, Xeroderma pigmentosum and the role of UV-induced DNA damage in skin cancer, *Mol. Med. Today* **5** (1999), 86–94.

[21] W. Vermeulen, E. Bergmann, J. Auriol et al., Sublimiting concentration of TFIIH transcription/DNA repair factor causes TTD-A trichothiodystrophy disorder, *Nat. Genet.* **26** (2000), 307–313.

[22] M.R. Wallenfang and G. Seydoux, cdk-7 Is required for mRNA transcription and cell cycle progression in *Caenorhabditis elegans* embryos, *Proc. Natl. Acad. Sci. USA* **99** (2002), 5527–5532.

[23] X.W. Wang, W. Vermeulen, J.D. Coursen et al., The XPB and XPD DNA helicases are components of the p53-mediated apoptosis pathway, *Genes Dev.* **10** (1996), 1219–1232.

[24] A. Weber, J. Liu, I. Collins and D. Levens, TFIIH operates through an expanded proximal promoter to fine-tune c-myc expression, *Mol. Cell. Biol.* **25** (2005), 147–161.