TFIIF, a basal eukaryotic transcription factor, is a substrate for poly(ADP-ribosyl)ation

Jean M. RAWLING and Rafael ALVAREZ-GONZALEZ*

Department of Microbiology and Immunology, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107-2699, U.S.A.

We have examined the susceptibility of some of the basal eukaryotic transcription factors as covalent targets for poly(ADP-ribosyl)ation. Human recombinant TATA-binding protein, transcription factor (TF)IIB and TFIIF (made up of the 30 and 74 kDa RNA polymerase II-associated proteins RAP30 and RAP74) were incubated with calf thymus poly(ADP-ribose) polymerase and [^{32}P]NAD$^+$ at 37 °C. On lithium dodecyl sulphate/PAGE and autoradiography, two bands of radioactivity, coincident with RAP30 and RAP74, were observed. No radioactivity co-migrated with TATA-binding protein or TFIIB. The phenomenon was dependent on the presence of nicked DNA, which is essential for poly(ADP-ribose) polymerase activity. Covalent modification of TFIIF increased with time of incubation, with increasing TFIIF concentration and with increasing NAD$^+$ concentration. High-resolution PAGE confirmed that the radioactive species associated with RAP30 and RAP74 were ADP-ribose polymers. From these observations, we conclude that both TFIIF subunits are highly specific substrates for covalent poly(ADP-ribosyl)ation.

INTRODUCTION

Poly(ADP-ribosyl)ation of proteins is a post-translational reaction that involves the sequential addition of NAD$^+$-derived ADP-ribose units to nuclear acceptor proteins in response to DNA strand breaks (for reviews, see references [1,2]). Poly(ADP-ribose) polymerase (PARP; EC 2.4.2.30) is the chromatin-bound DNA-dependent enzyme that catalyses this reaction [1–3]. Competitive inhibition of PARP [4,5], niacin deprivation [4,6,7], mutation leading to reduced PARP activity [8,9], expression of PARP antisense mRNA [10,11] and overexpression of the enzyme's DNA-binding domain [12,13] all suppress poly(ADP-ribose) synthesis. Under such circumstances, DNA base excision repair is compromised [6,9,10,12,13]. Moreover, cellular differentiation [14–17] is also impaired in the absence of poly(ADP-ribosyl)ation. However, the molecular function that the poly(ADP-ribosyl)ation pathway fulfils in these processes has yet to be elucidated. Poly(ADP-ribosyl)ation of proteins has profound effects on chromosomal architecture because most of the molecular targets for poly(ADP-ribose) are DNA-binding proteins. Therefore it is likely that the polyanionic nature of poly(ADP-ribose) affects the interaction of these polypeptides with DNA. In support of this hypothesis is the finding that PARP is associated with transcriptionally active DNA [18]. This phenomenon suggests an involvement of the protein-poly(ADP-ribosyl)ation pathway in gene expression. Indeed, Matsui et al. [19] previously described the inhibition of nick-induced mRNA transcription at the elongation stage, without interruption of specific transcription initiation, by transcription factor IIC (TFIIC). Interestingly, TFIIC was later identified as PARP [20].

Basal eukaryotic transcription factors orient RNA polymerase II (Pol II) for accurate site-specific mRNA transcript initiation and elongation (for reviews, see references [21–24]). The TATA-binding protein (TBP) of transcription factor IID (TFIID) locates and binds to the TATA box in the promoter element of a class II gene [25,26]. Transcription factor IIB (TFIIB) associates with TBP and directs Pol II [27] and transcription factor IIF (TFIIF) [28] to the initiation complex. RAP30, the small subunit of TFIIF, interacts with TFIIB and Pol II [29,30] and is essential for transcript initiation. Transcription factors IIE and IIH assist Pol II in clearing the promoter site [31]. Finally, RAP74 binding to RAP30 and Pol II is required for elongation of the nascent transcript [32].

Owing to the myriad of intricate protein–protein interactions involved in transcription, regulation of this process may be achieved via post-translational modification of components within the transcription assembly. This has already been demonstrated for the phosphorylation of some specific transcriptional activators (for reviews, see references [33,34]). However, less is known about the control of the basal transcription complex. It has been established that phosphorylation of the Pol II C-terminal domain, which is catalysed by TFIIH after transcript initiation [35], inhibits interaction of Pol II with the promoter [36]. The phosphorylation of the large TFIIF subunit, RAP74, modulates the degree to which TFIIF participates in transcription complex-formation [37] as well. This modification appears to stimulate both basal transcript initiation and elongation *in vivo* [37]. In a similar manner, other post-translational modifications of the general transcription factors may contribute to the regulation of basal gene expression.

In this study, we have explored the potential for some of the general transcription factors to act as targets for poly(ADP-ribosyl)ation. Human recombinant (hr) TBP, TFIIB and TFIIF (RAP30/RAP74) were exposed to calf thymus PARP in the presence of ^{32}P-labelled NAD$^+$ and DNA. These experiments demonstrate that both TFIIF subunits are highly specific substrates for poly(ADP-ribosyl)ation *in vitro*.

MATERIALS AND METHODS

Materials

hrTBP (~ 20 μg/ml), hrTFIIB (20 μg/ml) and hrTFIIF (hrRAP30 ~ 250 μg/ml; hrRAP74 ~ 500 μg/ml) were supplied

Abbreviations used: PARP, poly(ADP-ribose) polymerase; TFIIF, transcription factor IIF; hrRAP30/74, human recombinant 30 kDa and 74 kDa RNA polymerase II-associated proteins; pol II, RNA polymerase II; LDS, lithium dodecyl sulphate; TBP, TATA-binding protein.

* To whom correspondence should be addressed.

by Upstate Biotechnology (Lake Placid, NY, U.S.A.). Sephadex G-25 was obtained from Pharmacia (Piscataway, NJ, U.S.A.). Cellulose was bought from Whatman (Hillsboro, OR, U.S.A.). Lithium dodecyl sulphate (LDS) and activated calf thymus DNA (type XV) were provided by Sigma (St. Louis, MO, U.S.A.). [*adenylate*-^{32}P]NAD$^+$ (specific radioactivity 500 Ci/mmol) was procured from ICN (Costa Mesa, CA, U.S.A.). Dithiothreitol, electrophoresis reagents and molecular-mass markers were purchased from Bio-Rad (Hercules, CA, U.S.A.). All other chemicals used were of the highest purity commercially available.

Enzyme purification

PARP was partially purified to the DNA–cellulose step by the method of Zahradka and Ebisuzaki [38]. A crude homogenate of calf thymus was fractionated by two sequential $(NH_4)_2SO_4$ precipitations of 30 and 70% respectively. Insoluble material was desalted on Sephadex G-25, and the enzyme was subjected to DNA–cellulose chromatography. The final preparation contained 40 μg of protein/ml. The enzyme was shown to be approx. 90% pure as revealed by SDS/PAGE.

Determination of transcription factor modification by calf thymus PARP

The potential for poly(ADP-ribosyl)ation of hrTBP, hrTFIIB and hrTFIIF was assessed using a previously described method [39]. Transcription factors were incubated in the presence of calf thymus PARP, nicked calf thymus DNA and [^{32}P]NAD$^+$ at 37 °C. The reaction was stopped by the addition of trichloroacetic acid (20%, w/v, final concentration). Incorporation of ^{32}P into the trichloroacetate-precipitable material was measured by Čerenkov counting. Proteins were separated by LDS/PAGE on a 10% acrylamide gel, as detailed [39]. Coomassie Blue-stained gels were autoradiographed using Kodak XAR-5 X-ray film. Band intensities on the films and gels were determined using the Intelligent Quantifier software from Bio Image (East Lansing, MI, U.S.A.).

Identification of ADP-ribose polymers bound to TFIIF

The method of Alvarez-Gonzalez and Jacobson [40] was used to determine the size distribution of ADP-ribose polymers attached to TFIIF. After LDS/PAGE of modified hrRAP30 and hrRAP74 as described above, Coomassie Blue-stained bands were cut from the gel. The bands were homogenized in 100 mM ammonium acetate, pH 4.5, and incubated at 37 °C for 60 min to elute the proteins. Ammonium formate was eliminated from the samples via lyophilization. To chemically detach the ADP-ribose polymers from their acceptor proteins, samples were incubated in 0.1 M NaOH/20 mM EDTA for 2 h at 60 °C. To resolve the different ADP-ribose polymer species, samples were neutralized with 0.1 M HCl and electrophoresed on TBE (100 mM Tris/borate, 5 mM EDTA, pH 8.3)/20% acrylamide gels. Gels were exposed to X-ray film at −80 °C.

RESULTS AND DISCUSSION

In this study we explored the potential for basal eukaryotic transcription factors to be poly(ADP-ribosyl)ated in a pure enzyme system. Purified hrTBP, hrTFIIB and hrTFIIF were incubated with calf thymus PARP, nicked calf thymus DNA and [^{32}P]NAD$^+$ (1 μM) at 37 °C for 5 min. A relatively low NAD$^+$ concentration was used, because at this level PARP synthesizes predominantly ADP-ribose oligomers rather than long highly branched molecules [39] which can inactivate the enzyme on automodification. The proteins were electrophoresed under acidic conditions to prevent the degradation of potentially alkali-labile protein–ADP-ribose conjugates. Gels were exposed to X-ray film. As shown in Figure 1, most of the radioactivity was present at the origin of the gel in all lanes. This represents the auto-

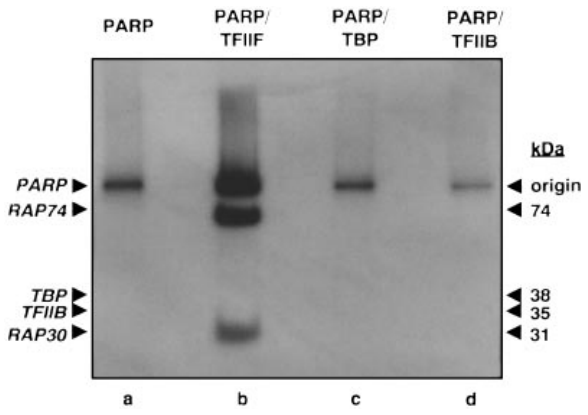

Figure 1 Poly(ADP-ribosyl)ation of basal transcription factors

hrTBP, hrTFIIB and hrTFIIF were incubated with calf thymus PARP (14 nM), nicked calf thymus DNA (20 μg/ml) and [^{32}P]NAD$^+$ (1 μM) at 37 °C for 5 min. *Lane a*, PARP control; *lane b*, PARP/TFIIF; *lane c*, PARP/TBP; *lane d*, PARP/TFIIB. The results shown are representative of three separate experiments with very similar results.

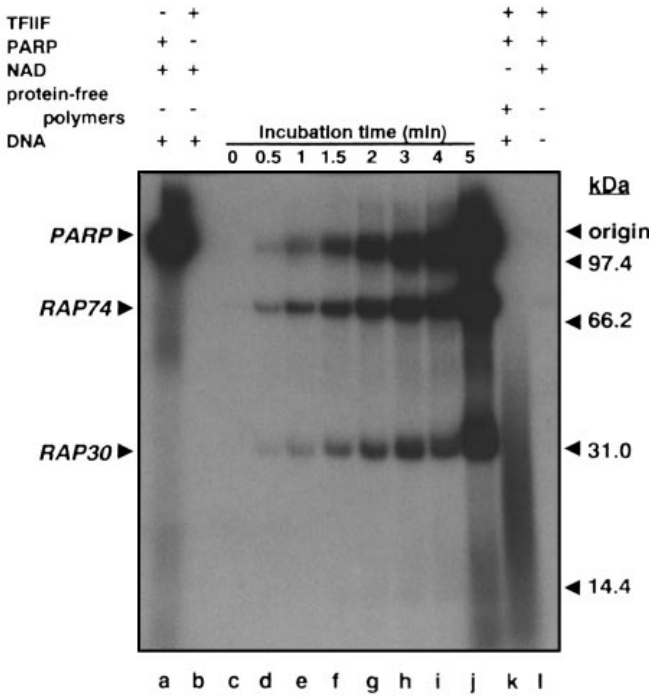

Figure 2 Kinetics of the poly(ADP-ribosyl)ation of TFIIF

hrRAP30 (38 nM) and hrRAP74 (41 nM) were incubated with calf thymus PARP (14 nM), nicked calf thymus DNA (20 μg/ml) and [^{32}P]NAD$^+$ (1 μM) at 37 °C. Proteins were separated by LDS/PAGE and autoradiographed. *Lane a*, PARP control, no TFIIF; *lane b*, TFIIF control, no PARP; *lanes c–j*, 0, 30 s, 60 s, 90 s, 2 min, 3 min, 4 min and 5 min of incubation respectively; *lane k*, 5 min incubation of TFIIF plus free radiolabelled ADP-ribose polymers; *lane l*, TFIIF/PARP control, no DNA.

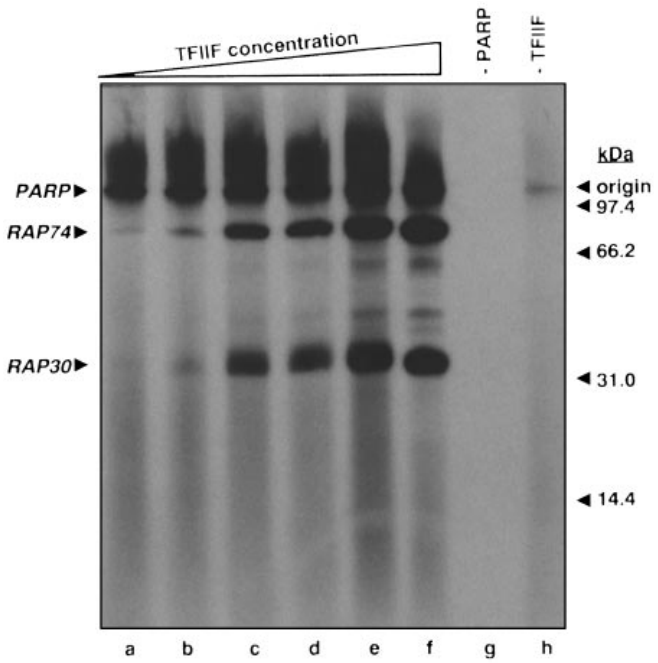

Figure 3 Poly(ADP-ribosyl)ation of TFIIF is TFIIF-concentration-dependent

Increasing concentrations of hrRAP30 and hrRAP74 were incubated in the presence of PARP (14 nM), nicked calf thymus DNA (20 μg/ml) and [^{32}P]NAD$^+$ (1 μM) at 37 °C for 5 min. An autoradiograph of proteins separated by LDS/PAGE is shown. *Lanes a–f*, 0.8/0.8, 1.5/1.6, 7.7/8.1, 19.2/20.3, 38.3/40.5 and 76.6/81.0 nM RAP30/RAP74 concentrations respectively per lane; *lane g*, TFIIF control, no PARP; *lane h*, PARP control, no TFIIF.

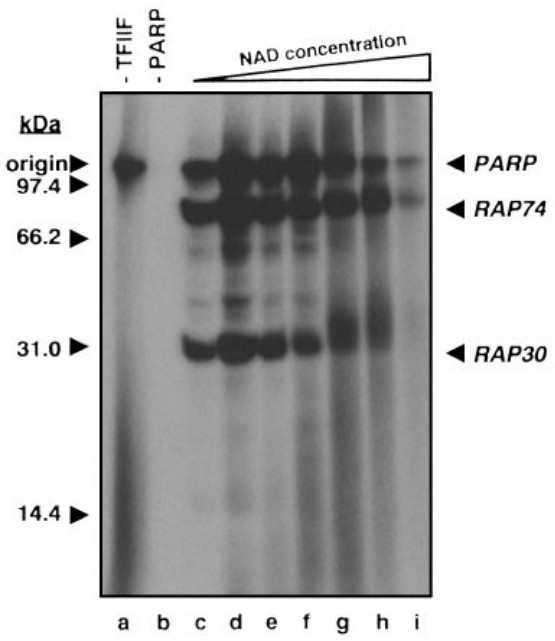

Figure 4 Poly(ADP-ribosyl)ation of TFIIF is NAD$^+$-concentration-dependent

Increasing concentrations of [^{32}P]NAD$^+$ were added to a mixture of hrRAP30 (38 nM) and hrRAP74 (41 nM), PARP (14 nM) and nicked DNA (20 μg/ml). Samples were incubated for 5 min at 37 °C. An autoradiograph of proteins separated by LDS/PAGE is shown. *Lane a*, PARP control, no TFIIF; *lane b*, TFIIF control, no PARP; *lanes c–i*, NAD$^+$ concentrations of 87 nM, 200 nM, 500 nM, 1 μM, 10 μM, 50 μM and 100 μM respectively.

poly(ADP-ribosyl)ation activity of PARP which, at 116 kDa, did not migrate into the 10% gel [38]. Two other bands were evident in the sample containing TFIIF (Figure 1, lane b). These migrated at 30 and 74 kDa, coincident with the Coomassie-stained bands which represented RAP30 and RAP74 respectively (not shown). No radioactivity was present in association with either TBP (Figure 1, lane c) or TFIIB (Figure 1, lane d). Both TFIIF subunits are therefore specific substrates for poly(ADP-ribosyl)ation, since neither of the other transcription factors was modified. As indicated above, the majority of poly(ADP-ribose) acceptors are DNA-binding proteins. However, TBP, the central DNA-binding participant in the preinitiation complex, was not modified by PARP. In contrast, RAP30, which contains a DNA-binding domain [41], was efficiently poly(ADP-ribosyl)ated. Our observation that RAP74 is also radiolabelled supports the suggestion that this polypeptide is able to associate with DNA [32].

We next proceeded to characterize the radioactive labelling of the TFIIF subunits. As shown in Figure 2, there was no modification of hrRAP30 or hrRAP74 in the absence of PARP (lane b) or nicked calf thymus DNA (lane l); PARP requires nicked DNA for activation [42]. Furthermore the association of radioactivity with the TFIIF subunits was not merely a result of non-covalent physical association between the proteins and poly(ADP-ribose), since incubation of TFIIF with protein-free ^{32}P-labelled ADP-ribose polymers did not result in the localization of a radiolabelled band at these two sites (Figure 2, lane k). These controls confirm that hrRAP30 and hrRAP74 were covalently modified by PARP.

Figure 2 also shows that poly(ADP-ribose) incorporation increased with the time of incubation up to 5 min (lanes c–j). Relative rates of protein modification were quantified by densitometric scanning from 0 to 5 min. In keeping with previous results [43,44], PARP was the preferred acceptor substrate as indicated by the delay in modification of the TFIIF subunits (results not shown). This delay may have been necessary for the heterodimerization of PARP with either hrRAP30 or hrRAP74 to occur. We also observed that hrRAP74 was modified in preference to hrRAP30. Interestingly, hrRAP74 contains a central peptide domain, 50% of which is composed of glutamate and aspartate residues [45]. These amino acids are the main residues modified on poly(ADP-ribose) acceptor proteins characterized to date [46–49], so the acidic regions in hrRAP74 may contribute to its suitability as a PARP substrate. Preliminary results indicate that the ADP-ribose polymers bound to both TFIIF subunits are labile during mild alkali treatment or prolonged exposure to neutral hydroxylamine (results not shown), further suggesting that carboxylate groups are the acceptors for the polymers [50,51].

The poly(ADP-ribosyl)ation of TFIIF also increased with increasing concentrations of hrRAP30/hrRAP74 (Figure 3, lanes a–f). It should be noted that even at the lowest TFIIF concentration used in this experiment (0.8 nM RAP30; 0.8 nM RAP74), a situation in which there was a 17:1 ratio between PARP and each TFIIF subunit, modification of both hrRAP30 and hrRAP74 was possible (Figure 3, lane a). A progressive accumulation of ADP-ribose polymers was evident as the TFIIF concentration increased (Figure 3, lanes a–f).

We next examined the effect of increasing NAD$^+$ concentrations on the poly(ADP-ribosyl)ation of TFIIF (Figure 4, lanes c–i). In this experiment, equal amounts of ^{32}P-labelled NAD$^+$ were diluted with progressively greater quantities of unlabelled NAD$^+$. Thus, at higher NAD$^+$ concentrations, the specific

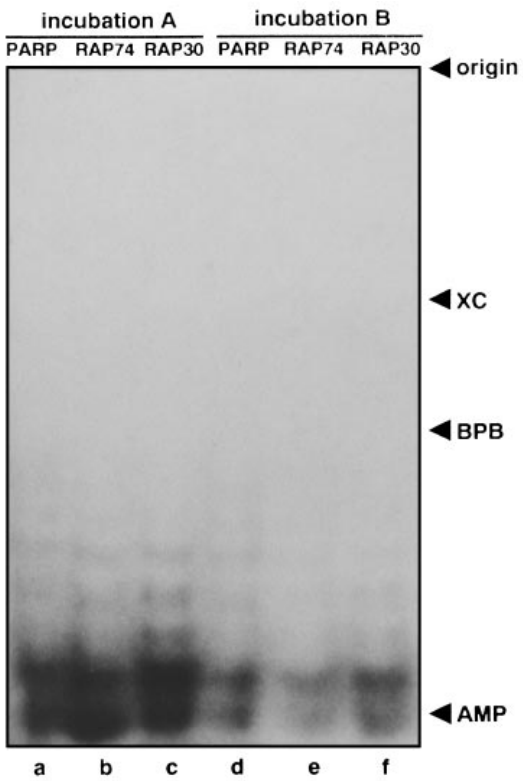

Figure 5 Profile of ADP-ribose polymers covalently attached to TFIIF

hrRAP30 and hrRAP74 were incubated with PARP, nicked DNA and 1 μM NAD^+ over 5 min at 37 °C. Proteins were separated by LDS/PAGE and then eluted from gel slices. Poly(ADP-ribose) was chemically detached from the acceptor proteins and separated by TBE high-resolution gel electrophoresis. Poly(ADP-ribose) synthesized in two separate incubations (*lanes a–c; lanes d–f*) is shown. *Lanes a, d,* ADP-ribose polymers attached to PARP; *lanes b, e,* ADP-ribose polymers attached to RAP74; *lanes c, f,* ADP-ribose polymers attached to RAP30. Electrophoretic migration of xylene cyanol (XC) [$(ADP\text{-}ribose)_{20}$], Bromophenol Blue (BPB) [$(ADP\text{-}ribose)_8$] and AMP is indicated.

radioactivity of the NAD^+ was lower. The band intensities of modified TFIIF subunits and PARP were therefore products of (a) the change in specific radioactivity and (b) the quantity of ADP-ribose polymers incorporated into each protein [52]. Although the intensities of the protein bands at elevated NAD^+ concentrations decreased (Figure 4, lane g, 10 μM NAD^+; lane h, 50 μM NAD^+; lane i, 100 μM NAD^+), there was undoubtedly more poly(ADP-ribose) incorporation at these NAD^+ levels since the RAP30 and RAP74 bands exhibited decreased and more diffuse electrophoretic mobilities. In the presence of greater NAD^+ concentrations, PARP synthesizes longer branched ADP-ribose molecules [39], which would have caused the shift in gel mobility.

The DNA-dependence, time course and dose–responses all support the conclusion that PARP was synthesizing covalently bound ADP-ribose polymers on to TFIIF. In order to confirm this hypothesis, we examined the radioactive species attached to TFIIF. hrRAP30 and hrRAP74, modified by PARP in the presence of 1 μM NAD^+, were electrophoresed under acidic conditions. The protein bands were excised from the gel and the radioactivity was detached from the proteins with alkali. Using a TBE/20 % acrylamide gel, we separated the radioactive species. The results are shown in Figure 5. ADP-ribose monomers, which are converted into AMP under the alkaline conditions of release [39], are present at the bottom of the autoradiograph. A ladder of sequentially larger ADP-ribose polymers rises above AMP. No long ADP-ribose polymers were produced under these conditions because of the low NAD^+ concentration used [39]. The ADP-ribose polymers associated with PARP, up to four residues in length (Figure 5, lanes a and d), were of identical size with those attached to RAP74 and RAP30 (four residues long; Figure 5, lanes b, c, e and f).

We have demonstrated here, for the first time, the susceptibility of a basal transcription factor to poly(ADP-ribosyl)ation. Furthermore, the modification of the factor, TFIIF, is highly specific. The majority of poly(ADP-ribose) acceptors are DNA-binding proteins, yet TBP was not poly(ADP-ribosyl)ated. TFIIB, another general transcription factor, was also ineffective as a PARP substrate. TFIIF is involved in the initiation and elongation of RNA transcripts. RAP30 interacts with Pol II [30]. It also contains a DNA-binding domain, which may be important for either preventing Pol II association with non-specific DNA sequences or stabilizing the transcription preinitiation complex [53]. RAP74 appears to be necessary for promoter clearance and transcript elongation [32,54]. Its central acidic domain is postulated to regulate N-terminal interactions with RAP30 and C-terminal associations with Pol II [32]. The post-translational addition of a large polyanion such as poly(ADP-ribose) to RAP30 or RAP74 would undoubtedly affect the protein–protein interactions in which TFIIF participates. It has previously been shown that PARP can prevent random transcription at DNA strand breaks, without interfering with site-specific mRNA transcript initiation [19,20]. Poly(ADP-ribosyl)ation of RAP30 and RAP74 may be an important component of this phenomenon, and may provide a link between DNA transcription and repair.

Finally, it is important to note that Wang et al. [55] recently reported that mice homozygous for the deletion of PARP were healthy, normal and fertile, possibly suggesting that protein-poly(ADP-ribosyl)ation has little, if any, role in basal transcriptional and replicative processes. However, one should keep in mind that the protein-poly(ADP-ribosyl)ation pathway is substantially enhanced when DNA-strand breaks are introduced as a result of DNA damage during DNA excision repair. Therefore the covalent poly(ADP-ribosyl)ation of TFIIF may be physiologically relevant during DNA excision repair after DNA damage. The utilization of cell lines derived from these knock-out mice [56] may prove valuable in the functional characterization of the poly(ADP-ribosyl)ation of TFIIF.

This work was supported by grants from the Texas Advanced Research program (no. 009768-14) and the National Institutes of Health (no. GM45451).

REFERENCES

1 Althaus, F. R. and Richter, C. (1987) Mol. Biol. Biochem. Biophys. **37**, 1–125
2 Lautier, D., Lagueux, J., Thibodeau, J., Menard, L. and Poirier, G. G. (1993) Mol. Cell. Biochem. **122**, 171–193
3 De Murcia, G., Huletsky, A. and Poirier, G. G. (1987) Biochem. Cell Biol. **66**, 626–635
4 Durkacz, B., Omidiji, O., Gray, D. A. and Shall, S. (1980) Nature (London) **283**, 593–596
5 Banasik, M., Komura, H., Shimoyama, M. and Ueda, K. (1992) J. Biol. Chem. **267**, 1569–1575
6 Jacobson, E. L., Nunbhakdi-Craig, V., Smith, D. G., Chen, H. Y., Wasson, B. L. and Jacobson, M. K. (1992) in ADP-Ribosylation Reactions (Poirier, G. G. and Moreau, P., eds.), pp. 153–162, Springer-Verlag, New York
7 Rawling, J. M., Jackson, T. M., Driscoll, E. R. and Kirkland, J. B. (1994) J. Nutr. **124**, 1597–1603
8 Chatterjee, S., Hirschler, N. V., Petzold, S. J., Berger, S. J. and Berger, N. A. (1989) Exp. Cell Res. **184**, 1–15
9 Chatterjee, S. and Berger, N. A. (1994) Mol. Cell. Biochem. **138**, 61–69

10 Ding, R., Pommier, Y., Kang, V. H. and Smulson, M. E. (1992) J. Biol. Chem. **267**, 12804–12812
11 Smulson, M. E., Kang, V. H., Ntambi, J. M., Rosenthal, D. S., Ding, R. and Simbulan, C. M. G. (1995) J. Biol. Chem. **270**, 119–127
12 Kupper, J. H., Muller, M., Jacobson, M., Tatsumi-Miyajima, J., Coyle, D. L., Jacobson, E. L. and Burkle, A. (1995) Mol. Cell. Biol. **15**, 3154–3163
13 Schreiber, V., Hunting, D., Trucco, C., Gowans, B., Grunwald, D., de Murcia, G. and Menissier-de Murcia, J. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 4753–4757
14 Farzaneh, F., Zalin, R., Brill, D. and Shall, S. (1982) Nature (London) **300**, 362–366
15 Althaus, F. R., Lawrence, S. D., He, Y. Z., Sattler, G. L., Tsukada, Y. and Pitot, H. C. (1982) Nature (London) **300**, 366–368
16 Johnstone, A. P. and Williams, G. T. (1982) Nature (London) **300**, 368–370
17 Jost, J. P. and Jost, Y. C. (1984) J. Biol. Chem. **269**, 10040–10043
18 Hough, C. J. and Smulson, M. E. (1984) Biochemistry **23**, 5016–5023
19 Matsui, T., Segall, J., Weil, P. A. and Roeder, R. G. (1980) J. Biol. Chem. **255**, 11992–11996
20 Slattery, E., Dignam, J. D., Matsui, T. and Roeder, R. (1983) J. Biol. Chem. **258**, 5955–5959
21 Conaway, R. C. and Conaway, J. C. (1993) Annu. Rev. Biochem. **62**, 161–190
22 Buratowski, S. (1994) Cell **77**, 1–3
23 Aso, T., Conaway, J. W. and Conaway, R. C. (1995) FASEB J. **9**, 1419–1428
24 Struhl, K. (1996) Cell **84**, 179–182
25 Kim, J. L., Nikolov, D. B. and Burley, S. K. (1993) Nature (London) **365**, 520–527
26 Kim, Y., Geiger, J. H., Hahn, S. and Sigler, P. B. (1993) Nature (London) **365**, 512–520
27 Buratowski, S., Hahn, S., Guarente, L. and Sharp, P. A. (1989) Cell **56**, 549–561
28 Ha, I., Roberts, S., Maldonado, E., Sun, X., Kim, L. U., Green, M. and Reinberg, D. (1993) Genes Dev. **7**, 1021–1032
29 Flores, O., Lu, H., Killeen, M., Greenblatt, J., Burton, Z. F. and Reinberg, D. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 9999–10003
30 Garrett, K. P., Serizawa, H., Hanley, J. P., Bradsher, J. N., Tsuboi, A., Arai, N., Yokota, T., Arai, K.-I., Conaway, R. C. and Conaway, J. W. (1992) J. Biol. Chem. **267**, 23942–23949
31 Goodrich, J. A. and Tjian, R. (1994) Cell **77**, 145–156
32 Wang, B. Q. and Burton, Z. F. (1995) J. Biol. Chem. **270**, 27035–27044
33 Hunter, T. and Karin, M. (1992) Cell **70**, 375–387
34 Pennypacker, K. R. (1995) Pharmacology **51**, 1–12
35 Lu, H., Zawel, L., Fisher, L., Egly, J. M. and Reinberg, D. (1992) Nature (London) **358**, 641–645
36 Chesnut, J. D., Stephens, J. H. and Dahmus, M. E. (1992) J. Biol. Chem. **267**, 10500–10506
37 Kitajima, S., Chibazakura, T., Yonaha, M. and Yasukochi, Y. (1994) J. Biol. Chem. **269**, 29970–29977
38 Zahradka, P. and Ebisuzaki, K. (1984) Eur. J. Biochem. **142**, 503–509
39 Mendoza-Alvarez, H. and Alvarez-Gonzalez, R. (1993) J. Biol. Chem. **268**, 22575–22580
40 Alvarez-Gonzalez, R. and Jacobson, M. K. (1987) Biochemistry **26**, 3218–3224
41 Tan, S., Aso, T., Conaway, R. C. and Conaway, J. W. (1994) J. Biol. Chem. **269**, 25684–25691
42 Benjamin, R. C. and Gill, D. M. (1980) J. Biol. Chem. **255**, 10502–10508
43 Jump, D. B., Butt, T. R. and Smulson, M. (1979) Biochemistry **18**, 983–990
44 Ogata, N., Ueda, K., Kawaichi, M. and Hayaishi, O. (1981) J. Biol. Chem. **256**, 4135–4137
45 Aso, T., Vasavada, H. A., Kawaguchi, T., Germino, F. J., Ganguly, S., Kitajima, S., Weissman, S. M. and Yasukochik, Y. (1992) Nature (London) **355**, 461–464
46 Ogata, N., Ueda, K. and Hayaishi, O. (1980) J. Biol. Chem. **255**, 7610–7615
47 Ogata, N., Ueda, K., Kagamiyama, H. and Hayaishi, O. (1980) J. Biol. Chem. **255**, 7616–7620
48 Burzio, L. O., Riquelme, P. T. and Koide, S. S. (1979) J. Biol. Chem. **254**, 3029–3037
49 Suzuki, H., Quesada, P., Farina, B. and Leone, E. (1986) J. Biol. Chem. **261**, 6048–6055
50 Nishishuka, Y., Ueda, K., Yoshihara, K., Yamamura, H., Takeda, M. and Hayaishi, O. (1969) Cold Spring Harbor Symp. Quant. Biol. **34**, 781–786
51 Bredehorst, R., Wielckens, K., Gartemann, A., Lengyel, H., Klapproth, K. and Hilz, H. (1978) Eur. J. Biochem. **92**, 129–135
52 Alvarez-Gonzalez, R. (1994) Mol. Cell. Biochem. **138**, 213–219
53 Tan, S., Conaway, R. C. and Conaway, J. W. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 6042–6046
54 Chang, C. H., Kostrub, C. F. and Burton, Z. (1993) J. Biol. Chem. **268**, 20482–20489
55 Wang, Z.-Q., Auer, B., Stingl, L., Berghammer, H., Haidacher, D., Schweiger, M. and Wagner, E. (1995) Genes Dev. **9**, 509–520
56 Heller, B., Wang, Z.-Q., Wagner, E. F., Radons, J., Buerkle, A., Fehsel, K., Burkart, V. and Kolb, H. (1995) J. Biol. Chem. **270**, 11176–11180

Received 6 September 1996/20 December 1996, accepted 14 January 1997