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TFIIH: from transcription to clinic

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Abstract Once a large proportion of the genes responsible for genetic disorders are identified in the post-genome era, the fundamental challenge is to establish a genotype/phenotype relationship. Our aim is to explain how mutations in a given gene affect its enzymatic function and, in consequence, disturb the life of the cell. Genome integrity is continuously threatened by the occurrence of DNA damage arising from cellular exposure to irradiation and genotoxic chemicals. This mutagenic or potentially lethal DNA damage induces various cellular responses including cell cycle arrest, transcription alteration and processing by DNA repair mechanisms, such as the nucleotide excision repair (NER) pathway. Disruption of NER in response to genotoxic injuries results in autosomal recessive hereditary diseases such as Xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD). One of the most immediate consequences of the induction of strand-distorting lesions is the arrest of transcription in which TFIIH plays a role in addition to its role in DNA repair. The observations made by clinicians close to XP, TTD and CS patients, suggested that transcription defects responsible for brittle hair and nails for TTD, or developmental abnormalities for CS, resulted from TFIIH mutations. Here a story will be related which could be called 'a multi-faceted factor named TFIIH'. As biochemists, we have characterized each component of TFIIH, three of which are XPB and XPD helicases and cdk7, a cyclin-dependent kinase. With the help of structural biologists, we have characterized most of the specific three-dimensional structures of TFIIH subunits and obtained its electron microscopy image. Together these approaches help us to propose a number of structure–function relationships for TFIIH. Through transfection and microinjection assays, cell biology allows us to determine the role of TFIIH in transcription and NER. We are thus in a position to explain, at least in part, transcription initiation mechanisms and their coupling to DNA repair. We now know how the XPB helicase opens the promoter region for RNA synthesis and that one of the roles of XPD helicase is to anchor the cdk7 kinase to the core-TFIIH. In XP and CS associated patients, we have demonstrated that some XPD mutations prevent an optimal phosphorylation of nuclear receptors by cdk7 with, as a consequence, a drop in the expression of genes sensitive to hormone action. We have thus shown that hormonal responses operate through TFIIH. Careful analysis of each TFIIH subunit also shows how the p44 Ring finger participates in certain promoter escape reactions. We are also able to localize the action of TFIIH in the sequence of events that lead to the elimination of DNA lesions. Thanks to the combination of these different approaches we are obtaining a much clearer picture of the TFIIH complex and its integration into the life of the cell.

1. Introduction

The synthesis of messenger RNA in eukaryotes by RNA pol II is a key step in the gene expression pathway which conditions the 'normal' life of a cell and the development of an individual. Accurate synthesis will depend not only on the integrity of the gene to be transcribed but also on the numerous factors involved in the various processes that allow mRNA synthesis and, thereafter, the production of biologically active proteins. Dysregulation of this pathway results in different disorders in man. On the one hand, mutations that affect components of the transcription apparatus including the RNA polymerases, their associated factors and/or their target sites will lead to genetic disorders, some of which are already identified [1]. On the other hand, the inaccurate repair of damage encountered on the transcribed strand of a given gene, will either modify the rate of RNA synthesis and/or the nature (the quality) of the RNA. Such inaccurate DNA repair may be at the origin of mutations. Damage on the transcribed strand may also stop RNA pol II, although it is also possible that RNA pol II reading through the damaged DNA will lead to the production of modified proteins. Defects in the mechanism which couples transcription to repair gives rise in humans to phenotypes associated with the Cockayne syndrome (CS) genetic disorders.

In this review, I will focus on TFIIH, a factor involved in both transcription and DNA repair [2–4]. I will try to demonstrate how, having at hand several biochemical assays and observations of patients by clinicians, we were able not only to establish genotype/phenotype relationships, but also to better understand the mechanism of transcription initiation and, to a certain extent, DNA repair. Our work was facilitated by the discovery that mutations in two subunits of TFIIH were at the origin of Xeroderma pigmentosum (XP), previously defined as DNA repair disorders. XP results from mutations in the XPB and XPD helicases, the two largest subunits of

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TFIIH, and exhibits several clinical features such as UV-sensitivity, cutaneous hyperpigmentation and a skin cancer predisposition, neurodegeneration and progressive mental retardation that may also be attributed to transcriptional deficiencies. In some cases, XP presents clinical features characteristic of CS, caused by a dysfunction in the CSA and CSB proteins which, it has been suggested, are involved in the coupling between transcription and nucleotide excision repair (NER). Indeed, it was found a few years ago that in the case of genotoxic attack, actively transcribed genes are preferentially, and therefore more rapidly, repaired than the non-transcribed ones [5,6]. The most commonly described features of CS are a moderate UV-sensitivity without cancer proneness, growth retardation (especially cachectic dwarfism), sexual immaturity, neurodysmyelination and progressive mental degeneration. In other cases the XPB and XPD proteins are associated with trichothiodystrophy disorder (TTD). The most typical clinical features of these patients are brittle hair and nails. With the exception of photo-sensitivity, this large spectrum of symptoms cannot be explained by a DNA repair defect alone but might also reflect a transcriptional deficiency.

In fact the TFIIH story started in 1989 [7], when within the framework of our studies on the mechanisms that govern transcription initiation, we attempted to purify a factor, at first named BTF2, from a protein fraction which was shown to be indispensable to restore basal transcription activity in vitro. Starting from 500 l of HeLa cells, it took two and a half years at +4°C to obtain, after seven purification steps, a protein complex containing nine polypeptides ranging from 90 to 30 kDa. The following steps would have been simply to characterize each of the nine subunits (Fig. 1), if we had not identified ERCC3/XPB, an excision repair cross complementary protein [8], involved in NER and thus part of the DNA repair apparatus set in action upon genotoxic attack [9]. Was this a contamination, an artefact or a bona fide partner of the large TFIIH complex? An antibody directed against XPB, proved that this subunit was indeed part of TFIIH and that it was, according to in vivo microinjection experiments and in vitro reconstituted systems, implicated in both NER and transcription [10]. This finding helped to establish definitively the connection between transcription and repair which in fact had been suspected for several years. This also explained the rarity of XPB patients as most of the mutations in this gene would be incompatible with life. With the motto, ''one polypeptide per student and/or post-doc'', we have identified each of the nine subunits of TFIIH [11,12]; one was XPD, a helicase [13] in which a mutation gives rise to another form of XP (the XPD group). Again our work stimulated enthousiasm when we identified cdk7 (a cyclin-dependent kinase) as part of TFIIH and thus responsible for phosphorylation of the carboxy-terminal domain (CTD) of the largest subunit of RNA pol II [14–16]. Whether the fact that the TFIIH kinase is cyclin-dependent reflects a connection between TFIIH as a repair factor and being involved in cell cycle regulation, remains to be further documented.

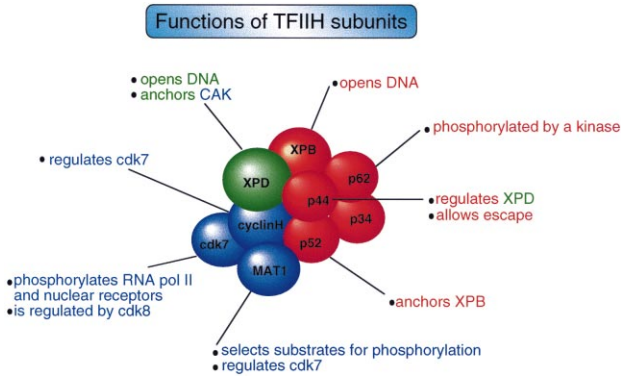

Fig. 1. Summary of the enzymatic functions of TFIIH subunits.

2. TFIID: a three-dimensional (3D) picture

Having the possibility to produce recombinant as well as endogeneous TFIIH, one of our objectives was to establish the structure–function relationships of TFIIH. This would also allow the design of drugs that can interfere with the enzymatic activities of TFIIH and could circumvent the transcription and/or NER defects observed in XP patients. Up to now we have failed to obtain a crystal structure of TFIIH. We have, however, characterized most of the specific 3D structures of the TFIIH subunits [17,18]. We also succeeded in preparing a highly purified and concentrated human TFIIH fraction to obtain electron microscopy pictures. The 3D model designed from electron microscopy analysis shows that TFIIH is organized into a ring-like structure, with a hole whose size is able to accommodate a double-stranded DNA molecule, and from which an almost spherical bulge of protein density protrudes out [19,20]. The human recombinant core-TFIIH subcomplex, which only contains five subunits, has a circular architecture that can be superimposed on the ring found in human TFIIH, suggesting that the cdk-activating kinase (CAK) constitutes the protruding domain. The quaternary organization of TFIIH subunits can be partially inferred from immunolabeling combined with co-immunoprecipitation experiments. The cdk7 kinase was shown to be located in the protruding domain, whereas p44 is located within the ring structure, at the basis of the protruding protein density, and is flanked on either side by the XPB and XPD helicases. It is interesting to note that the location of XPD, close to the CAK-containing bulge, is consistent with the proposal that it could bridge (or stabilize) the interaction between the CAK and the core-TFIIH. Such information will also explain some defects within XPD patients (see below). The discrepancies in the subunit composition and quaternary organization between the yeast and human TFIIH subcomplexes [21] probably reflect some subtle differences concerning the role of their various subunits in the DNA repair and transcription activities of TFIIH.

Once the TFIIH subunits had been identified, we investigated their role in both transcription and NER [22,23]. This work was made possible due to the availability of TFIIH immunopurified from the numerous cell lines derived from XPB and XPD patients. In addition we had set up in vitro assays to evaluate not only transcription and NER activity but also helicase activity [24]. Moreover, we are now able to overexpress recombinant TFIIH with mutations in any of the subunits, using the baculovirus/insect cell systems [25].

3. XPB, the helicase of transcription

Once the pre-initiation complex is formed (upon arrival of the last basal transcription factor), opening of the promoter around the start site occurs in an ATP-dependent manner to

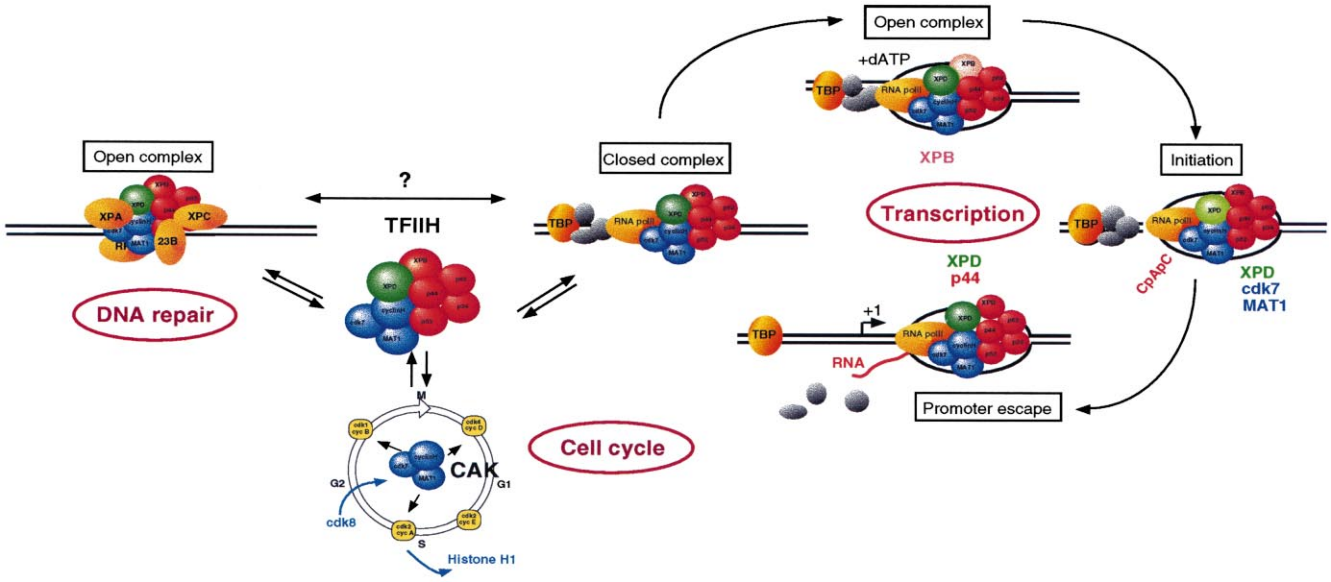

Fig. 2. TFIIH at the crossroad of transcription, DNA repair and cell cycle regulation.

allow further reading of the coding strand by RNA pol II [26]. This function is mainly due to the ATP-dependent XPB helicase (Fig. 2). Whether such an opening is regulated by proteins of either the TFIIH factor, the pre-initiation complex, the activation machinery or external stimuli is not yet clear. However, another subunit, p52 exhibits some connections with XPB since it allows its integration within TFIIH (our unpublished data). One may also wonder as to the consequence of the interaction with XPB and stimulation by TFIIE of the ATPase and kinase activities of TFIIH on the transcription reaction [27]. One should mention that XPG, an endonuclease involved in DNA repair, also targets XPB. This may explain some of the clinical features observed in XPG patients (having a mutation in the XPG) which have not yet been investigated. These observations, however, should not obscure the crucial role of XPB in the DNA repair reaction, in which it allows the opening around the DNA damage [28]. However, it is clear that abolishing the XPB helicase function, by mutating its ATP binding site, completely abolishes its function in both transcription and DNA repair [25].

4. A secondary role of XPD: opening and anchoring

The mammalian TFIIH revealed a highly conserved structure and function from yeast to man which can be resolved into several functional subcomplexes: the core-TFIIH, composed of five subunits (XPB, p62, p52, p44 and p34), and the CAK, composed of cdk7, cyclin H and MAT1. The remaining XPD helicase subunit can be found associated with either the core-TFIIH or the CAK and is believed to bridge CAK to the core-TFIIH. Beside its helicase activity, XPD allows anchoring of CAK to the core-TFIIH (Fig. 2) through an interaction with p44, another subunit of TFIIH [29]. Upon interaction with p44, XPD helicase activity increases. Contrary to what was observed with XPB, XPD is dispensable for the transcription reaction: TFIIH that carries a mutation in the XPD helicase ATP binding site or that lacks the XPD subunit, allows (although to a lower extent) RNA synthesis, when added to an in vitro transcription assay that contains all the basal transcription factors. It is clear, however, that when XPD is present, it allows CAK anchoring to TFIIH and optimal transcriptional activity. Mutations in XPD or in p44 which modify the XPD–p44 interaction strongly modulate the composition of TFIIH by affecting the overall architecture of TFIIH [30]. It is also interesting to note the additional role of p44 in promoter escape. Indeed, mutations that abolish the ring finger structure of p44 allow the first phosphodiester bond formation, when the mutated recombinant TFIIH is added to an in vitro transcription assay, but reduce RNA elongation.

5. Phosphorylation by CAK is essential

Most basal transcription factors are phosphorylated (at least in vitro). Cyclin H and cdk7, part of CAK, are substrates for other cyclin-dependent kinases [31], whereas they phosphorylate components of the transcription machinery. The CTD of the largest subunit of RNA pol II, which contains several repeats of a serine/threonine-rich heptapeptide, represents an ideal substrate for serine/threonine kinases. CTD phosphorylation/dephosphorylation follows certain steps of the transcription reaction. Indeed it has been demonstrated that RNA pol II engaged in the elongation step, is hyperphosphorylated, whereas initiation requires a hypophosphorylated RNA pol II [32]. Mutations in either cdk7 or MAT1 decrease both CTD phosphorylation and basal transcription (Fig. 2). How this phosphorylation process is connected with the other (helicase and ATPase) enzymatic functions of TFIIH and the transcription complex is not yet established. In fact, in vitro CTD phosphorylation requires neither promoter opening nor formation of the first phosphodiester bond: CAK stimulates significantly the synthesis of the first phosphodiester bond; this involves only a structural contribution of the complex and not its enzymatic activity [33].

Since transcription regulators interact directly, or via cofactors, with the basal transcription machinery, it was hypothesized that kinases such as cdk7, present within this machinery, would participate in the regulation of transcription.

As a consequence, one could observe variations in gene expression levels. It was found that the retinoic acid and the estrogen receptors are phosphorylated by TFIIH and that this phosphorylation parallels an increase of target gene expression level [34,35]. Indeed phosphorylation might regulate either the binding of the receptor to its responsive element and/or its dimerization. Upon interaction with TFIIH and further phosphorylation, these DNA binding proteins could stimulate some steps of the transcription reaction such as CTD phosphorylation, DNA opening, promoter clearance, or recruitment and/or stimulation of the elongation machinery.

Little is known concerning the role of CAK in DNA repair and there are some discrepancies in the literature. On the one hand, studies in yeast demonstrated that the kinase Kin28 (the counterpart of cdk7), is not required for in vitro NER [36]. On the other hand, inhibition of CAK activity was shown to restore DNA repair [23].

6. TFIIH: at the origin of transcription syndrome

Microinjection of either highly purified TFIIH or even TFIIH reconstituted from recombinant subunits expressed in a baculovirus system is able to complement the NER defect in XPD and XPB as well as TTD-A fibroblasts [10]. These syndromes are due to defects of TFIIH in one of its enzymatic or other structural functions, which might inhibit not only NER but also transcription. We thus demonstrated that mutations in the CTD of the XPD subunit (which represents more than 70% of the mutations found in XPD patients), disturb the architecture of TFIIH through a decrease in the interaction between the XPD and the p44 subunits [29]. This results in the abrogation of the stimulatory (or regulatory) function of p44 towards the XPD helicase within TFIIH which then cannot eliminate the DNA damage, thus explaining the NER defect observed in those patients. Moreover, the weakening of the p44/XPD interaction might have additional consequences on the cdk7 kinase activity which is associated with the core-TFIIH through XPD. Indeed, modification of the TFIIH architecture would not allow optimal transcription activity: modifications of the phosphorylation of either CTD or DNA binding proteins such as nuclear receptors (unpublished data) also decrease in vivo as well as in vitro transcription, thus explaining some phenotypes of the XPD patients.

We have also shown that TFIIH isolated from patients bearing a mutation in the 5′-end of XPB is deficient in its ability to open either the promoter or the DNA around the lesion [24]. Under these conditions, beside the NER defect, RNA pol II is unable to accurately synthesize RNA thus explaining the severity of the XPB phenotype and the low number of XPB patients.

The genetic defect in TTD-A is unknown, but caused by dysfunctioning TFIIH. Surprisingly none of the TFIIH subunits carries a mutation and immunopurified TFIIH from TTD-A cells is active in both transcription and repair [37]. Immunoblot and immunofluorescence analyses reveal a strong reduction in the TFIIH concentration. Thus, the phenotype of TTD-A appears to result from sublimiting amounts of TFIIH, probably due to a mutation in a gene determining the complex stability. Not only the identity of the gene but also its potential function remains obscure. It is likely that the molecular defect resides in either translation, complex assembly, post-translational modification (including conversion of TFIIH from transcription into NER mode and vice versa) or increased proteolytic degradation, all of which can influence steady-state levels of TFIIH. It cannot be excluded however that the TTD-A defect does not reside in TFIIH itself but in an endogeneous cellular factor causing rapid inactivation of the injected TFIIH.

There we are. Fig. 2 is incomplete. Several questions remain unanswered. How the enzymatic functions of TFIIH are regulated in transcription, DNA repair and cell cycle regulation? What the connection is between these three mechanisms? How TFIIH crosses from transcription to DNA repair? Is CAK a check point between DNA repair and cell cycle arrest? Answering these questions is our challenge to understand gene expression regulation and especially the connection between transcription and repair.

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References

[1] Semenza, G.L. (1998) Oxford University Press, Oxford.
[2] Svejstrup, J.Q., Vichi, P. and Egly, J.M. (1996) Trends Biochem. Sci. 20, 346–350.
[3] Coin, F. and Egly, J.M. (1998) Cold Spring Harbor, NY, in press.
[4] Frit, P., Bergmann, E. and Egly, J.M. (1999) Biochimie 81, 27–38.
[5] Bohr, V.A., Smith, C.A., Okumoto, D.S. and Hanawalt, P.C. (1985) Cell 40, 359–369.
[6] Mellon, I., Spivak, G. and Hanawalt, P.C. (1987) Cell 51, 241–249.
[7] Gerard, M., Fischer, L., Moncollin, V., Chipoulet, J.M., Chambon, P. and Egly, J.M. (1991) J. Biol. Chem. 266, 20940–20945.
[8] Schaeffer, L., Roy, R., Humbert, S., Moncollin, V., Vermeulen, W., Hoeijmakers, J.H.J., Chambon, P. and Egly, J.M. (1993) Science 260, 58–63.
[9] Weeda, G., van Ham, R.C.A., Vermeulen, W., Bootsma, D., van der Eb, A.J. and Hoeijmakers, J.H.J. (1990) Cell 62, 777–791.
[10] Vermeulen, W. (1994) Cold Spring Harbor Quant. Symp. LIX, pp. 317–329.
[11] Humbert, S., van Vuuren, H., Lutz, Y., Hoeijmakers, J.H.J., Egly, J.M. and Moncollin, V. (1994) EMBO J. 13, 2393–2398.
[12] Marinoni, J.C. et al. (1997) EMBO J. 16, 1093–1102.
[13] Schaeffer, L. et al. (1994) EMBO J. 13, 2388–2392.
[14] Roy, R., Adamczewski, J.P., Seroz, T., Vermeulen, W., Tassan, J.P., Schaeffer, L., Hoeijmakers, J.H.J. and Egly, J.M. (1994) Cell 79, 1093–1101.
[15] Adamczewski, J.P., Rossignol, M., Tassan, J.P., Nigg, E.A., Moncollin, V. and Egly, J.M. (1996) EMBO J. 15, 1877–1884.
[16] Rossignol, M., Kolb-Cheynel, I. and Egly, J.M. (1997) EMBO J. 16, 1628–1637.
[17] Fribourg, S. et al. (2000) J. Biol. Chem. 275, 31963–31971.
[18] Gervais, V.V., Busso, D., Wasielewski, E., Poterszman, A., Egly, J.M., Thierry, J.C. and Kieffer, B. (2001) J. Biol. Chem. 276, 7457–7464.
[19] Schultz, P., Fribourg, S., Poterszman, A., Mallouh, V., Moras, D. and Egly, J.M. (2000) Cell 102, 599–607.
[20] Chang, W.H. and Kornberg, R.D. (2000) Cell 102, 609–613.
[21] Feaver, W.J., Henry, N.L., Wang, Z., Wu, X., Svejstrup, J.Q., Bushnell, D.A., Friedberg, E.C. and Kornberg, R.D. (1997) J. Biol. Chem. 272, 19319–19327.
[22] Aboussekhra, A. et al. (1995) Cell 80, 859–868.
[23] Araujo, S.J. et al. (2000) Genes Dev. 14, 349–359.

[24] Coin, F., Bergmann, E., Tremeau-Bravard, A. and Egly, J.M. (1999) EMBO 18, 1357–1366.
[25] Tirode, F., Busso, D., Coin, F. and Egly, J.M. (1999) Mol. Cell 3, 87–95.
[26] Hawley, D.K. and Roeder, R.G. (1987) J. Biol. Chem. 262, 3452–3461.
[27] Ohkuma, Y. and Roeder, R.G. (1994) Nature 368, 160–163.
[28] Evans, E., Moggs, J.G., Hwang, J.R., Egly, J.-M. and Wood, R.D. (1997) EMBO J. 16, 6559–6573.
[29] Coin, F., Marinoni, J.C., Rodolfo, C., Fribourg, S., Pedrini, M.A. and Egly, J.M. (1998) Nature Gen. 20, 184–189.
[30] Seroz, T., Perez, C., Bergmann, E., Bradsher, J. and Egly, J.M. (2000) J. Biol. Chem. 275, 33260–33266.
[31] Akoulitchev, S., Chuikov, S. and Reinberg, D. (2000) Nature 407, 102–106.
[32] Dahmus, M.E. (1995) Biochim. Biophys. Acta 1261, 170–182.
[33] Makela, T.P., Parvin, J.D., Kim, J., Huber, L.J., Sharp, P.A. and Weinberg, R.A. (1995) Proc. Natl. Acad. Sci. USA 92, 5174–5178.
[34] Rochette-Egly, C., Adam, S., Rossignol, M., Egly, J.M. and Chambon, P. (1997) Cell 90, 97–107.
[35] Chen, D., Riedl, T., Washbrook, E., Pace, P.E., Coombes, R.C., Egly, J.M. and Ali, S. (2000) Mol. Cell 6, 127–137.
[36] Svejstrup, J.Q., Wang, Z., Feaver, W.J., Wu, X., Bushnell, D.A., Donahue, T.F., C, F.E. and Kornberg, R.D. (1995) Cell 80, 21–28.
[37] Vermeulen, W., Bergmann, E., Auriol, J., Rademakers, S., Frit, P., Appeldoorn, E., Hoeijmakers, J.H. and Egly, J.M. (2000) Nat. Genet. 26, 307–313.