Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 322 (2004) 705–711
BBRC
www.elsevier.com/locate/ybbrc

Breakthroughs and Views

# Detecting DNA-binding of proteins in vivo by UV-crosslinking and immunoprecipitation ☆

Lemin Zhang[a,*], Keqin Zhang[a], Ralf Prändl[b,1], Fritz Schöffl[b]

[a] Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming, Yunnan 650091, PR China
[b] Zentrum für Molekularbiologie der Pflanzen—Allgemeine Genetik, Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany

Received 29 July 2004

**Abstract**

The temporal and spatial binding of proteins on DNA is important to the regulation of genome expression and maintenance. However, examining how the protein–DNA complexes assemble in living cells is challenging. The development of UV-crosslinking/immunoprecipitation (UV-X-ChIP) technique and the progress of its applications show the powerful potential of this method in detecting such binding behavior in vivo. UV light is a zero length crosslinker and is believed to produce less perturbation of the complex than chemical crosslinker. The use of UV laser as UV light source allows the number of photons required for crosslinking to be delivered in nano- or pico- or femtosecond intervals, extremely shortening the irradiation time and achieving higher crosslinking efficiency than conventional UV lamp, thus being well suitable for kinetic studies. UV-X-ChIP technique has been successfully applied on the study of DNA replication, transcription, chromatin structure, and genome-wide location of DNA-binding proteins.


*Keywords:* UV-crosslinking; Immunoprecipitation; Protein–DNA interactions

The binding of proteins on recognition DNA sites plays a crucial role in regulation of genome expression and maintenance. This interest has motivated the development of a number of methods for investigating protein–DNA interactions in vivo. One of the most widely used methods is formaldehyde-induced crosslinking/immunoprecipitation. However, formaldehyde also forms chemical bridges, creating artifacts; and induces protein–protein crosslinking, generating indirect results. These drawbacks can be overcome, in principle, by the use of UV irradiation as crosslinker. In the early 1960s [2], it was shown that ultraviolet (UV) light irradiation induced stable crosslinking between protein and DNA. Later this technique was used to crosslink a specific protein to DNA. Now, UV-crosslinking combined with immunoprecipitation (UV-X-ChIP) has been developed as an effective tool for detecting DNA-binding of specific proteins in vivo and detailed protocols have been established in many laboratories [10,11,19]. Irradiation of living cells with UV light of wavelength near 260 nm produces covalent bonds between contact points of nucleic acid and protein. UV light is a zero length crosslinker and is believed to produce less perturbation of the complex than chemical crosslinker. The new development of UV laser as UV light source allows the number of photons required for crosslinking to be delivered in nano- or pico- or femtosecond intervals,

☆ *Abbreviations:* Eve, homeodomain protein even-skipped; Ftz, homeodomain protein fushi taraza; HSF, heat shock transcription factor; hOrc, human homolog of origin recognition complex; hCdc6p, human homolog of the replication initiator protein; hMcm, human homologs of minichromosome maintenance proteins; RNA pol II, RNA polymerase II; *Ubx*, *Ultrabithorax*; USF, upstream stimulating factor.

* Corresponding author. Fax: +86 871 5034838.
*E-mail address:* zhanglm@ynu.edu.cn (L. Zhang).
[1] Present address: SerCon GmbH, Heinrich-von-Brentano-Sre. 2, D-55130 Mainz, Germany.

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.07.202

extremely shortening the irradiation time and achieving higher crosslinking efficiency than conventional UV lamp. For example, irradiation of a single UV laser pulse of 5-ns, 50 mJ on yeast crude extract allows detectable complex formation of specific protein and DNA [21]. As concerning that the protein–DNA interactions are dynamic in vivo, for example, many proteins involved in regulating gene activity move and exchange quickly with the target DNA [20], the rapid freezing of the interaction at a particular step during the assembly of protein–DNA complex is essentially important. To meet this requirement, UV laser crosslinking shows its powerful potential especially in kinetic studies.

After chromatin isolation, the protein–DNA complex is immunoprecipitated with the antibody against the protein of interest, the bound DNA is reversed, and analyzed as outlined in Fig. 1. UV-X-ChIP technique has been successfully used for studying DNA replication, transcription, chromatin structure, and genome-wide location of DNA-binding proteins, and in vivo DNA binding of many proteins including RNA pol II, topoisomerase I, various transcription factors, and the components of DNA replicative complex have been examined (Table 1). In this review, we summarize the characterization of this technique and the progress of its application on studying protein–DNA interactions in vivo.

## Photochemistry of UV-crosslinking

Formation of a covalent bond(s) between nuclei acid and protein results from excitation of nucleotide bases and reaction with amino acid resides by UV light. The optimum excitation wavelength for the nucleic bases is between 250 and 270 nm. When a nucleic base is excited by a single photon, it is promoted to the first excited singlet state (S1) or the first excited triplet state (T1). The photo-crosslinking of a nucleic base to an amino acid can occur from either S1 or T1. The absorption of an additional photon by the excited nucleic base promotes the base from S1 (life time = 10 ps) or T1 (life time = 1 μs) to a higher excited state, Hs and Ht, respectively, and is achieved by high intensity of pulsed lasers. This kind of excitation is called biphotonic excitation (reviewed in [29]).

The rate of crosslink formation depends on the time required for the base excitation and the duration of chemical reaction. Base excitation takes several nano- or picoseconds. The life times of free radicals exceed microseconds. The chemical reaction of crosslinking is completed in less than one microsecond [22]. Because the microconformational transitions of macromolecules take more than 100 μs it appears that the UV light can freeze molecules undergoing movements in one particular conformation [8]. On the other hand, because the life

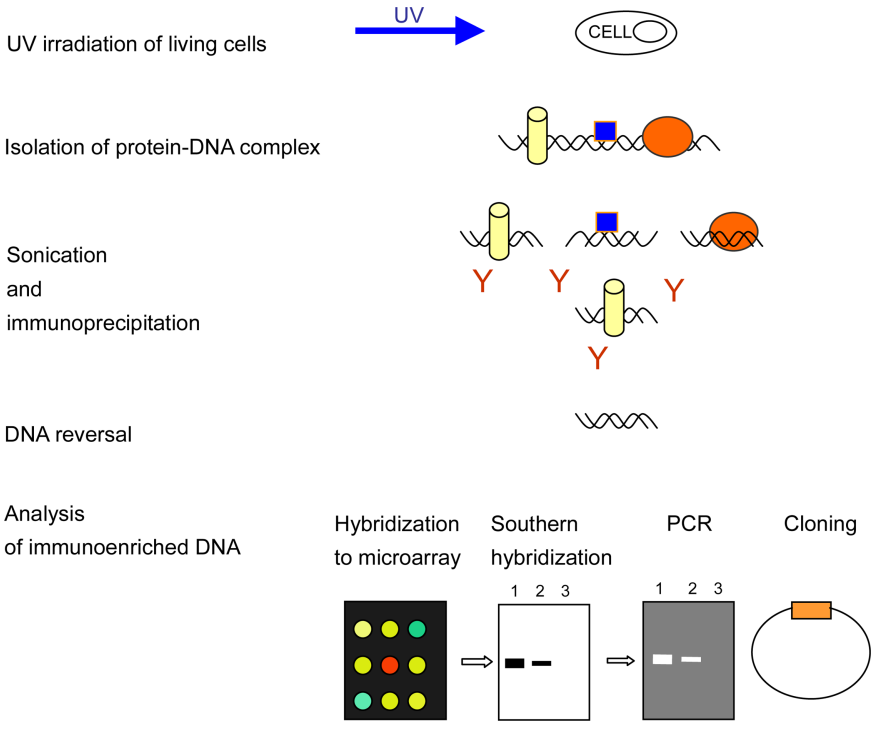


Fig. 1. Outline of the experimental protocol of UV-X-ChiP. For detailed descriptions, see analysis of immunoprecipitated DNA in text. The following samples were exemplified in DNA analysis: (1) Total genomic DNA used for positive control. (2) iDNA containing antibody immunoenriched target. (3) Background control sample which was processed without antibody. Arrow indicates the position of signals in Southern hybridization and PCR analysis. (▭) In orange color, target DNA. (○) In red color, IP-enriched DNA. (○) In green color, unenriched DNA. (○) In yellow color, merged. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Table 1
Proteins which binding sites are investigated using UV-X-ChIP technique in various organisms

| Protein | Organism | Year | References |
|---|---|---|---|
| RNA polymerase | Bacterium | 1984 | [18] |
| RNA polymerase II | *Drosophila*, rat | 1985,1987,1993, 1997,1998 | [13–17,26,32] |
| Paire | *Drosophila* | 1999 | [9] |
| Bicoid | *Drosophila* | 1999 | [9] |
| Eve | *Drosophila* | 1994, 1999 | [9,44,45] |
| Ftz | *Drosophila* | 1994, 1999 | [9,44,45] |
| Engrailed | *Drosophila* | 1995,2003 | [39,41] |
| GAGA | *Drosophila* | 1995 | [33] |
| Zeste | *Drosophila* | 1994, 1996, 1997 | [24,25,44] |
| Lamins | *Drosophila* | 1998 | [37] |
| B52 | *Drosophila* | 1991,1994 | [6,7] |
| Topoisomerase I | *Drosophila* | 1986, 1987 | [14,15] |
| Max | Rat | 1997 | [4] |
| Myc | Rat | 1997 | [4] |
| USF | Rat | 1997 | [4] |
| c-Jun | Rat | 1999 | [38] |
| Acetylated histones | Rat | 1996 | [31] |
| Histone1, 2A, 4 | Frog | 1990 | [12] |
| Histone3 | Human | 1999 | [27] |
| p300/CBP | Human | 2002 | [28] |
| hOrc1p | Human | 2003 | [1] |
| hOrc2p | Human | 2003 | [1] |
| hCdc6p | Human | 2003 | [1] |
| hMcm3p | Human | 2003 | [1] |
| HSF1 | *Arabidopsis* | 2001,2003 | [46–48] |
| HSF | Yeast | 2003 | [48] |

time of S1 state is very short, crosslinking from this state must involve groups in contact, assuming there is no intermediate reaction.

In principle, any amino acid residue can be induced to form covalent crosslinking with any nucleotide residue. The base specificity of crosslinking studied by in vitro assay shows that pyrimidine-containing oligonucleotides are most effective, thymidine being the most [22,23]. The amino acids most reactive toward the DNA are Cys, Lys, Phe, Trp, and Tyr [40].

Despite the fact that reaction mechanism is not fully understood, the introduction of covalent bonds between proteins and nucleic acids by UV irradiation represents a useful and reproducible technique.

## The efficiency of crosslinking

The practical use of UV-crosslinking depends on crosslinking efficiency defined as yield of crosslinked product. Properties, such as binding affinity, the geometry of the interaction, and abundance of protein, affect the efficiency. On the other hand, the sources of UV light also affect the efficiency. Using conventional UV light, model systems such as the interactions of DNA and histones, RNA Pol II, Q50 homeodomain proteins have been studied [9,12,13,15–17,26,28,31,32,41,44]. However, very little succeeded about specific regulatory proteins and their target DNA sequences. The amount of immunoprecipitated DNA targets of specific regulatory proteins obtained using conventional UV light is very low, below the detection level. To achieve higher efficiency, UV laser has been employed. Besides the short time of irradiation, the laser-induced reactions proceed via biphotonic excitation, which sharply increases the efficiency of crosslinking. According to the data obtained by in vitro experiments, 15–20% of protein–DNA complexes are often crosslinked. UV laser increased the yield up to two orders of magnitude compared with conventional light [30]. We successfully detected DNA-binding of specific transcription factor, heat shock factor 1 (HSF1), in vivo by UV laser irradiation of living *Arabidopsis* suspension culture cells for 60 s, while conventional UV light failed [46].

On the other hand, however, UV laser irradiation also causes mainly high DNA damage, such as pyrimidine dimer formation, breaks of sugar phosphate backbone of DNA, inter-strand DNA–DNA crosslinks, local denatured sites in DNA, and single-strand breaks, which hamper the analysis of crosslinked DNA. For example, PCR techniques require the DNA integrity for primer extension. Therefore, high efficiency and low DNA damage (effective crosslinking) are essential. To improve effective crosslink yields, different laser parameters have been studied in vitro. The yield obtained with nano-, pico-, and femtosecond pulses shows that the shorter pulses were more effective. A significant improvement from values of effective crosslinking <1% with nanosecond pulses to values

close to 5% with femtosecond pulses is observed using progesterone receptor (PR) as model protein [36]. A comparison of the effective crosslink yield obtained with UV pulses alone and with an optimized combination of two wavelength fs laser crosslinking (UV and blue pulses) shows that the latter technique minimized the damage and led to a significant improvement of effective crosslinking. In this strategy, the UV pulses for the excitation to the singlet S1 can be kept low, thus reducing DNA damage. The high crosslinking efficiency can still be attained by applying blue pulses which are too long to excite bases from the ground state, but provide additional energy to cross the ionization of the excited DNA bases and promote the bases from S1 or T1 to Hs and Ht [35]. It is anticipated that with the optimization of laser parameters, effective efficiency of UV-crosslinking will be achieved for broad applications.

## Immunoprecipitation conditions

UV induced covalent linkage is stable in general lysis or immunoprecipitation buffer, such as chromatin extraction buffer, antibody buffer [30,46], RiPA [1], containing a combination of denaturing and non-denaturing detergents (Triton, sodiumdeoxycholate, and sodium dodecyl sulfate). But the crosslinked complex is not stable under acidic conditions [11]. After crude chromatin isolation, ultracentrifugation in CsCl gradients was used to separate free protein, the peaks containing enriched fraction of protein–DNA complexes were harvested. However, ultracentrifugation is time consuming, up to 72 h are required, and some crosslinked material is lost. To increase the yield of the immunoprecipitated material we skipped the pre-purification step. Instead, crude chromatin extracts solubilized by sonication were used for immunoprecipitation. Free protein does not interfere with the immunoprecipitation procedure and we achieved significant signals over background. Background was reduced by the use of anionic (strong) detergents in chromatin extraction buffer or antibody buffer. It is essential to use protein A bound to magnetic particles instead of protein-A–Sepharose. This strongly reduced the background in our hands. Possible reasons are centrifugation sediment particles present in crude chromatin extracts with unspecifically bound DNA, and Dynabeads protein A may lower unspecific adsorption to the carrier material compared to protein-A–Sepharose. It is also important to optimize sonication time and power to obtain good signal/background ratios. Longer time and higher power result in lower background. In our experiments, only strong sonication ($10 \times 120$ s using a Branson Sonifier B-12 at level 4) of *Arabidopsis* crude extract can abolish unspecific signals [46].

In most protocols, affinity-purified polyclonal antibodies were used to achieve sufficient immunoprecipitate. But, when the protein under investigation contains a domain that is also present in other proteins, it is important to ensure that the DNA-binding sequences are associated with the protein of interest, not with others. For example, there are 21 homologs of HSF in *Arabidopsis*, which share common structural domains for DNA-binding and multimerization. Polyclonal antibodies against full length HSF1 cross-react with other HSFs. Therefore, specific antibodies that recognize unique regions of the protein of interest must be obtained and specific immunoprecipitation has to be assessed. This was achieved by using antibodies directed against the less conserved C-terminal region of HSF1. The specific antibodies showed no crossreaction with another HSF of *Arabidopsis* and allowed to analyse the binding behavior of HSF1 in vivo, which is different from the result obtained with antibodies against full length HSF1 [47].

## Analysis of immunoprecipitated DNA

UV-crosslinked DNA is fragmented by sonication and/or enzyme digestion to reduce DNA size, usually to a few of hundred base pairs, allowing the analysis of binding sites at high resolution. Antibody immunoprecipitated DNA (iDNA) contains virtually all in vivo binding sites of a given protein. Conventionally, southern hybridization is employed to determine the enrichment of target DNA. The iDNA can be radiolabeled and used as a probe for various investigations. Comparison of the hybridization profile by iDNA with that of a control DNA (obtained by mock precipitation using preimmunserum) identifies fragments, which are bound by the protein [17,31]. Or alternatively, immobilized iDNA, bound to a membrane, can be probed with a specific genomic fragment that may be known as a potential genomic target of the respective protein [12,44]. In this approach, the intensity of the signal can also be compared with a series of dilutions of input (amount of chromatin DNA used for the immunoprecipitation) to evaluate the efficiency of the immunoprecipitation of the target sequence. However, the sensitivity of southern hybridization is limited in many cases and requires an intermediate PCR amplification. Such amplification may misrepresent the real levels of protein–DNA crosslinking. PCR amplification is an excellent choice when the genomic target (DNA-binding site of the respective protein) is known [28,46]. In such analyses a pair of primers covering the contiguous region of a protein-binding site is designed. The PCRs are performed using iDNA and control DNA (obtained by mock precipitation using preimmunserum). The amplification of a specific fragment within the iDNA indicates protein binding. To minimize the effect of DNA damage on primer extension, the space between primers is kept short

and by this way we successfully detected the enrichment of binding sites of *Arabidopsis* HSF1 in vivo. For identification of genome-wide location of a protein, some papers describe the construction of a library of iDNA [26,38,41]. To obtain sufficient amount of iDNA for performing cloning, antibody immunoprecipitated DNA is amplified by an adaptor-mediated PCR. Clones of iDNA libraries have to be verified by additional investigations. Only the fragments for which the hybridization signal intensity of iDNA probe versus background probe is significantly higher are considered. UV light or formaldehyde crosslinking/immunoprecipitation combined with DNA microarray assay is a new development for analysis of the genome-wide binding sites. Both immunoprecipitation enriched (IP-enriched) and unenriched pools of labeled DNA were hybridized to a DNA microarray which contains several thousand DNA fragments throughout genome. The IP-enriched/unenriched ratio of signal intensity was used to calculate the relative binding of the protein of interest to each sequence represented on the assay and genome-wide locations of the protein are detected [3,5].

## Application and perspective

Applications of UV-X-ChIP have led to the identification of genomic binding sites of a variety of proteins at high resolution in vivo (Table 1), thus providing valuable insights into how these proteins function in chromatin context.

One of the model systems is the study on the regulation of heat shock gene expression. Considerable progress has been made in kinetics of RNA pol II binding to *hsp70* gene in *Drosophila* by John, T. Lis's lab. At un-induced condition, RNA pol II poises on the promoter region of *hsp70*, which may be critical for rapid transcription activation upon heat shock. At induced condition, it binds on entire gene [13], and its density on the *hsp70* gene is rapidly increased after an instantaneous heat shock [13,14,17,32]. This work and other achievements obtained in John, T. Lis's laboratory, such as the characterizations of GAGA factor binding to *hsp70* and *hsp26* genes with its coincident with that of RNA pol II [33]; topoisomerase I interaction with the transcribed region of *hsp70* gene, independently with RNA pol II [14]; association of B52 with boundaries of transcriptionally active *hsp* gene [6,7] in *Drosophila*; and the detection of heat shock-dependent in vivo binding of HSF1 in *Arabidopsis* using UV laser crosslinking by Schöffl's Lab [46,47], allow deepening the understanding of molecular mechanism of heat shock gene regulation.

Recently, a significant progress was gained on the understanding of DNA replication mechanism by G. Abdurashidova et al. [1]. They applied simultaneous pulse irradiation of HeLa cells with two wavelengths of laser light at 266 and 355 nm for 150 s and successfully detected the presence of specific proteins on the human lamin B2 ori sequence. Their detailed investigation on dynamic binding behavior and precise binding sites of different components of DNA replicative complex in different phases of the cell cycle elucidated elaborate modulations of DNA replicative activity.

The temporal and spatial binding of proteins on DNA is critical for understanding the mechanism of DNA replication and transcription. Above works show the potential of UV-X-ChIP in detecting such binding behavior especially on the kinetic following of binding events of complex assemblies, such as the transcription initiation complex and DNA replication complex. In such multiprotein–DNA complex, to exclude the formation of protein–protein adducts and to follow the sequential binding of different components, induction of only protein–DNA adducts, and rapid fixation of the contact are essential. The development of UV laser crosslinking well meets these requirements.

Genome-wide identification of target genes that are directly regulated by transcription factors rather than merely in the downstream pathways remains essential for understanding of gene regulation. One important application of UV-X-ChIP is detecting the genomic binding sites of transcriptional regulators across the entire genome, combined with cloning technique. To this purpose, the libraries of binding DNA fragments by RNA pol II, transcription factor c-Jun, and homeodomain protein engrailed were successfully established [26,38,41]. The sequencing of the inserts of engrailed recognition DNA fragments and comparison with database leads to the identification of 203 engrailed target genes which involve in different developmental pathways [41]. With availability of DNA microarrays in a broad variety of organisms, its combined use with UV-X-ChIP will provide new approach for genome-wide identification of in vivo targets. Such a method has been established with formaldehyde crosslinking and successfully applied in yeast and mammalian systems. Many individual transcription factors, as well as protein components for DNA replication, recombination, and chromatin structure have been studied on genome-wide maps of the interaction between protein and DNA [5,34]. A genome-wide survey of HSF1 binding sites in mammalian cells found that 94 promoters are bound by HSF1. Most of them are heat-inducible and consistent with the model that HSF binding results in heat shock gene expression. However, among them 48 genes are not heat-inducible. It is also surprising that many heat-inducible genes are not bound by HSF1. This evidence suggests that the regulation of heat shock response is more complex than previously thought [43].

Other significant achievements obtained with UV-X-ChIP include studies on nucleosome structure, for

instance, distributions of acetylated histones on the active *rRNA* genes [31] and distributions of histones on the enhancers and promoters of ribosomal spacer upon active transcription of the ribosomal genes [12]. The work from Biggin's group on detection of the binding of Q50 homeodomain proteins shows that unlike transcription factor, Zeste, which only binds on a target promoter of *Ultrabithorax* (*Ubx*) gene and not on non-target genes; Eve, Ftz, bicoid, and paired bind to a wide range of genes. These results significantly improve our understanding of how the homeodomain proteins function in controlling *Drosophila* development [9,44,45].

Conventional UV light has been mainly applied in *Drosophila* cells or embryos. With the improvement of efficiency, UV laser technique has shown its success in other model organisms, such as *Arabidopsis*, yeast, and human [1,46,48]. However, studies indicate that different crosslinking reagents have different preferences for the proteins. Neither method is likely to be universally applicable. For example, formaldehyde detects Zeste binding to a fragment just 5′ of the *Ubx* target element, which is not detected by the UV technique. UV crosslinking detects DNA binding of Zeste as efficiently as it detects binding by a second protein eve, whereas formaldehyde crosslinking detects Zeste at least 50-fold more efficiently than it detects Eve. Formaldehyde may be useful for examining one set of proteins, and the same is true for UV-crosslinking [42]. It is important to determine a range of crosslinking methods so that all classes of DNA-binding proteins can be studied.

Our knowledge on the chromatin composition of genes is still scarce, especially at high resolution. Many protein–DNA interactions remain to be investigated. Yet, the achievement of UV-X-ChIP technique in molecular biology has emerged, and the power of this technique is shown to be exciting.

## Acknowledgments

This work was supported by grants from Natural Science Foundation of Yunnan Province (2003C0012R), open research program of Key Laboratory, Yunnan, PR China, and grants from Deutsche Forschungsgemeinschaft (PR511/1-1; SFB446-A2).

## References

[1] G. Abdurashidova, M.B. Danailov, A. Ochem, G. Triolo, V. Djeliova, S. Radulescu, A. Vindigni, S. Riva, A. Falaschi, Localization of proteins bound to a replication origin of human DNA along the cell cycle, EMBO J. 22 (2003) 4294–4303.

[2] P. Alexander, H. Moroson, Cross-linking of deoxyribonucleic acid to protein following ultra-violet irradiation different cells, Nauchni. Tr. Vissh. Med. Inst. Sofiia 194 (1962) 882–883.

[3] M.D. Biggin, To bind or not to bind, Nat. Genet. 28 (2001) 303–304.

[4] K.E. Boyd, P.J. Farnham, Myc versus USF: discrimination at the *cad* gene is determined by core promoter elements, Mol. Cell. Biol. 17 (1997) 2529–2537.

[5] M.J. Buck, J.D. Lieb, ChIP-chip: considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments, Genomics 83 (2004) 349–360.

[6] D.T. Champlin, J.T. Lis, Distribution of B52 within a chromosomal locus depends on the level of transcription, Mol. Biol. Cell 5 (1994) 71–79.

[7] D.T. Champlin, M. Frasch, H. Saumweber, J.T. Lis, Characterization of a *Drosophila* protein associated with boundaries of transcriptionally active chromatin, Genes Dev. 5 (1991) 1611–1621.

[8] G. Careri, P. Fasella, E. Gratton, Statistical time events in enzymes: a physical assessment, CRC Crit. Rev. Biochem. 3 (1975) 141–164.

[9] A. Carr, M.D. Biggin, A comparison of in vivo and in vitro DNA-binding specificities suggests a new model for homeoprotein DNA binding in *Drosophila* embryos, EMBO J. 18 (1999) 1598–1608.

[10] A. Carr, M.B. Biggin, An in vivo UV crosslinking assay that detects DNA binding by sequence-specific transcription factors, Methods Mol. Biol. 119 (1999) 497–508.

[11] S.I. Dimitrov, T. Moss, UV laser-induced protein–DNA cross-linking, Methods Mol. Biol. 148 (2001) 395–402.

[12] S.T. Dimitrov, V.Y. Stefanovsky, L. Karagyozovl, D. Angelov, I.G. Pashev, The enhancers and promoters of the *Xenopus laevis* ribosomal spacer are associated with histones upon active transcription of the ribosomal genes, Nucleic Acids Res. 18 (1990) 6393–6397.

[13] C. Giardina, J.T. Lis, Polymerase processivity and termination on *Drosophila* heat shock genes, J. Biol. Chem. 268 (1993) 23806–23811.

[14] D.S. Gilmour, G. Pflugfelder, J.C. Wang, J.T. Lis, Topoisomerase I interacts with transcribed regions in *Drosophila* cells, Cell 44 (1986) 401–407.

[15] D.S. Gilmour, J.T. Lis, Protein–DNA cross-linking reveals dramatic variation in RNA polymerase II density on different histone repeats of *Drosophila melanogaster*, Mol. Cell. Biol. 7 (1987) 3341–3344.

[16] D.S. Gilmour, J.T. Lis, RNA polymerase II interacts with the promoter region of the noninduced *hsp70* gene in *Drosophila melanogaster* cells, Mol. Cell. Biol. 6 (1986) 3984–3989.

[17] D,S. Gilmour, J.T. Lis, In vivo interactions of RNA polymerase II with genes of *Drosophila melanogaster*, Mol. Cell. Biol. 5 (1985) 52009–52018.

[18] D.S. Gilmour, J.T. Lis, Detecting protein–DNA interactions in vivo: distribution of RNA polymerase on specific bacterial genes, Proc. Natl. Acad. Sci. USA 81 (1984) 4275–4279.

[19] D.S. Gilmour, A.E. Rougvie, J.T. Lis, Protein–DNA cross-linking as a means to determine the distribution of proteins on DNA in vivo, Methods Cell. Biol. 35 (1991) 369–381.

[20] G.L. Hager, C. Elbi, M. Becker, Protein dynamics in the nuclear compartment, Curr. Opin. Genet. Dev. 12 (2002) 137–141.

[21] D.T. Ho, D.M. Sauve, M. Roberge, Detection and isolation of DNA-binding proteins using single-pulse ultraviolet laser cross-linking, Anal. Biochem. 218 (1994) 248–252.

[22] J.W. Hockensmith, W.L. Kubasek, W.R. Vorachek, E.M. Evertsz, P.H. von Hippel, Laser cross-linking of protein–nucleic acid complexes, Methods Enzymol. 208 (1991) 211–236.

[23] J.W. Hockensmith, W.L. Kubasek, W.R. Vorachek, P.H. von Hippel, Laser cross-linking of nucleic acids to proteins. Methodology and first applications to the phage T4 DNA replication system, J. Biol. Chem. 261 (1986) 3512–3518.

[24] J.D. Laney, M.D. Biggin, Zeste-mediated activation by an enhancer is independent of cooperative DNA binding in vivo, Proc. Natl. Acad. Sci. USA 94 (1997) 3602–3604.

[25] J.D. Laney, M.D. Biggin, Redundant control of *Ultrabithorax* by zeste involves functional levels of zeste protein binding at the *Ultrabithorax* promoter, Development 122 (1996) 2303–2311.
[26] A.K. Law, Hirayoshi, T. O'Brien, J.T. Lis, Direct cloning of DNA that interacts in vivo with a specific protein: application to RNA polymerase II and sites of pausing in *Drosophila*, Nucleic Acids Res. 26 (1998) 919–924.
[27] S.G. Lejnine, G. Durfee, M. Murnane, H.C. Kapteyn, V.L. Makarov, J.P. Langmore, Crosslinking of proteins to DNA in human nuclei using a 60 femtosecond 266 nm laser, Nucleic Acids Res. 27 (1999) 3676–3684.
[28] H. Lu, C.A. Pise-Masison, T.M. Fletcher, R.L. Schiltz, A.K. Nagaich, M. Radonovich, G. Hager, P.A. Cole, J.N. Brady, Acetylation of nucleosomal histones by p300 facilitates transcription from tax-responsive human T-cell leukemia virus type 1 chromatin template, Methods Mol. Biol. 22 (2002) 4450–4462.
[29] K.M. Meisenheimer, T.H. Koch, Photocross-linking of nucleic acids to associated proteins, Crit. Rev. Biochem. Mol. Biol. 32 (1997) 101–140.
[30] T.S. Moss, I. Dimitrov, D. Houde, UV-laser crosslinking of proteins to DNA, Methods: A Companion to Methods in Enzymology 11 (1997) 225–234.
[31] V.J. Mutskov, V.R. Russanova, S.I. Dimitrov, I.G. Pashev, Histones associated with non-nucleosomal rat ribosomal genes are acetylated while those bound to nucleosome-organized gene copies are not, J. Biol. Chem. 271 (1996) 11852–11857.
[32] T. O'Brien, J.T. Lis, Rapid changes in *Drosophila* transcription after an instantaneous heat shock, Mol. Cell. Biol. 13 (1993) 3456–3463.
[33] T. O'Brien, R.C. Wilkins, C. Giardina, J.T. Lis, Distribution of GAGA protein on *Drosophila* genes in vivo, Genes Dev. 9 (1995) 1098–1110.
[34] B. Ren, F. Robert, J.J. Wyrick, O. Aparicio, E.G. Jennings, I. Simon, J. Zeitlinger, J. Schreiber, N. Hannett, E. Kanin, T.L. Volkert, C.J. Wilson, S.P. Bell, P.A. Young, Genome-wide location and function of DNA binding proteins, Science 290 (2000) 2306–2309.
[35] C.H. Russmann, J. Stollhof, C. Weiss, R. Beigang, M. Beato, Two wavelength femtosecond laser induced DNA–protein crosslinking, Nucleic Acids Res. 26 (1998) 3967–3970.
[36] C. Russmann, M. Truss, A. Fix, C. Naumer, T. Herrmann, J. Schmitt, J. Stollhof, R. Beigang, M. Beato, Crosslinking of progesterone receptor to DNA using tuneable nanosecond, picosecond and femtosecond UV laser pulses, Nucleic Acids Res. 25 (1997) 2478–2484.
[37] R. Rzepecki, S.S. Bogachev, E. Kokoza, N. Stuurman, P.A. Fisher, In vivo association of lamins with nucleic acids in *Drosophila melanogaster*, J. Cell Sci. 111 (1998) 121–129.
[38] R. Santoro, S. Wolfl, H.P. Saluz, UV-Laser induced protein/DNA crosslinking reveals sequence variations of DNA elements bound by c-Jun in vivo, Biochem. Biophys. Res. Commun. 256 (1999) 68–74.
[39] N. Serrano, H.W. Brock, C. Demeret, J.-M. Dura, N.B. Randsholt, T.B. Kornberg, F. Maschat, *Polyhomeotic* appears to be a target of engrailed regulation in *Drosophila*, Development 121 (1995) 1691–1703.
[40] M.D. Shetlar, J. Christensen, K. Hom, Photochemical addition of amino acids and peptides to DNA, Photochem. Photobiol. 39 (1984) 125–133.
[41] P.J. Solano, B. Mugat, D. Martin, F. Girard, J.M. Huibant, C. Ferraz, B. Jacq, J. Demaille, F. Maschat, Genome-wide identification of in vivo *Drosophila* engrailed-binding DNA fragments and related target genes, Development 130 (2003) 1243–1254.
[42] J. Toth, M.D. Biggin, The specificity of protein–DNA crosslinking by formaldehyde: in vitro and in *Drosophila* embryo, Nucleic Acids Res. 28 (2000) E4–e4.
[43] N.D. Trinklein, J.I. Murray, S.J. Hartman, D. Botstein, R.M. Myers, The role of heat shock transcription factor1 in the genome-wide regulation of the mammalian heat shock response, Mol. Biol. Cell 15 (2003) 1254–1261.
[44] J. Walter, C.A. Dever, M.D. Biggin, Two homeodomain proteins bind with similar specificity to a wide range of DNA sites in *Drosophila* embryos, Genes Dev. 8 (1994) 1678–1692.
[45] J. Walter, M.D. Biggin, DNA binding specificity of two homeodomain proteins in vitro and in *Drosophila* embryos, Dev. Biol. 93 (1996) 2680–2685.
[46] L. Zhang, G. Eggers-Schumacher, F. Schöffl, R. Prändl, Analysis of heat-shock transcription factor–DNA binding in *Arabidopsis* suspension cultures by UV laser crosslinking, Plant J. 28 (2001) 217–223.
[47] L. Zhang, C. Lohmann, R. Prändl, F. Schöffl, Heat stress-dependent DNA binding of *Arabidopsis* heat shock transcription factor HSF1 to heat shock gene promoters in *Arabidopsis* suspension culture cells in vivo, Biol. Chem. 384 (2003) 959–963.
[48] L. Zhang, Analysis of in vivo binding of HSF to DNA using UV laser crosslinking in suspension culture cells of yeast and *Arabidopsis*, Dissertation, Der Fakultat fur Biologie der Eberhard Karls Universitat Tubingen. Zur Erlangung des Grades eines Doctors der Naturwissenschaften, 2003.