3784–3788 *Nucleic Acids Research, 1998, Vol. 26, No. 16*

Targeting of CDK8 to a promoter-proximal RNA element demonstrates catalysis-dependent activation of gene expression

Moses O. Gold and Andrew P. Rice*

Division of Molecular Virology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, USA

Received April 9, 1998; Revised June 18, 1998; Accepted June 30, 1998

ABSTRACT

During transcription of mRNA genes, there is a correlation between the phosphorylation state of the C-terminal domain (CTD) of the large subunit of RNA polymerase II (RNAP II) and the ability of the RNAP II complex to processively transcribe the gene. To examine the involvement of CTD phosphorylation in modulation of RNAP II function, we have analyzed the ability of a known CTD kinase, human Cdk8, to modulate HIV-1 LTR-driven gene expression upon directed targeting to a promoter-proximal nascent RNA element. The results indicated that Cdk8, when localized to an RNA element, activates gene expression in a catalysis-dependent manner. Also, Cdk8 targeted to RNA was observed to act in a synergystic manner with DNA-targeted Sp1 but not with DNA-targeted HIV-1 Tat, suggesting that RNA-targeted Cdk8 acts on similar rate limiting post-initiation events as Tat. As recent observations suggest that Tat/TAR-mediated transcription of the proviral genome of HIV depends on specific phosphorylation of RNAP II in its CTD by the Tat-associated kinase (TAK/p-TEFb/Cdk9), our results indicate that Cdk8 shares with Cdk9 the ability to modulate transcription upon targeting to a nascent RNA element.

INTRODUCTION

For RNA polymerase II (RNAP II) to read an mRNA gene, it must first localize to the gene promotor and then migrate along the entire path to be transcribed. While promotor localization appears to be defined by networks of protein–DNA and protein–protein interactions, pre-mRNA synthesis is additionally characterized by enzyme-driven events that co-ordinate translocation of RNAP II along the DNA template. As the mechanisms involved in promotor localization differ from the enzymatic mechanisms that enable pre-mRNA synthesis, a layer of transcription regulation appears to facilitate the functional transition between promoter localization and pre-mRNA synthesis (1,2). Biochemical and genetic studies (3–5) indicate that the events associated with the transition of RNAP II from promotor localization to subsequent productive transcription include hyperphosphorylation of the large subunit of RNAP II in its C-terminal domain (CTD). The CTD consists of a repeating heptad unit (52 repeats in humans) containing phosphorylatable serine, threonine and tyrosine residues and is structurally and functionally conserved in eukaryotes (6).

Some cyclin-dependent kinases (CDKs) have been observed to hyperphosphorylate the CTD of RNAP II in a manner that is thought to activate transitions between transcription initiation and elongation. Observations to date have identified three human CDKs (Cdk7, Cdk8 and Cdk9) that appear to regulate transcription by phosphorylating the CTD. Cdk7 is a component of the general transcription factor TFIIH (7–9), Cdk8 is a component of the RNAP II holoenzyme (10–13) and Cdk9 is a component of the elongation factor p-TEFb and the related TAK, the human immunodeficiency virus (HIV) Tat-associated kinase (14–18).

Biochemical data indicate that, whereas Cdk7 modulates the earliest events in pre-mRNA synthesis (19,20), Cdk9 operates at a later step and modulates the processivity of transcriptionally engaged RNAP II (17,21). Direct demonstration of the involvement of human Cdk8 in transcription has not been established, however, the *Saccharomyces cerevisiae* homolog of Cdk8, Srb10, affects transcription in a catalysis-dependent manner in either a positive or negative manner (22–24). For further understanding of the roles of CDKs in transcriptional regulation, it is of interest to determine to what extent human Cdk8 may be functionally equivalent to yeast Srb10.

We have previously found Cdk8 to be a component of a complex, most likely a form of RNAP II holoenzyme, that can interact specifically with functional activation domains of viral transcription factors adenovirus E1A and herpes simplex virus VP16 and which can hyperphosphorylate the CTD of RNAP II (25,26). Consistent with observations that Cdk8 is associated with the RNAP II holoenzyme, we observed that localization of a GAL4–Cdk8 fusion protein to a promotor containing GAL4 DNA-binding sites activates gene expression (26). These experiments also demonstrated that gene activation by GAL4–Cdk8 was independent of Cdk8 catalytic function, suggesting that Cdk8 does not require enzymatic function to associate with RNAP II holoenzyme.

In the study presented here, we have exploited recent findings on mechanisms involved in transcriptional activation by the HIV Tat protein to identify a biological role associated with the catalytic function of human Cdk8. It has been demonstrated that

*To whom correspondence should be addressed. Tel: +1 713 798 5774; Fax: +1 713 798 3490; Email: arice@bcm.tmc.edu

Tat, via interaction with TAR RNA, activates HIV transcription by localizing a Cdk9-containing complex to elongating RNAP II (14,16–18,27). This finding suggests that Tat acts as an interface between a Cdk9-containing complex and TAR RNA, enabling transcribing RNAP II to be hyperphosphorylated by TAK/Cdk9, generating a highly processive RNAP II complex. We therefore reasoned that targeting of Cdk8 to a similarly localized RNA element might modulate gene expression in a catalysis-dependent manner.

In this report we demonstrate that a fusion of Cdk8 to the HIV-1 Rev protein activates expression of an HIV-1 long terminal repeat (LTR) containing the Rev-response element (RRE) in place of TAR. Furthermore, we show that such activation depends on the catalytic function of Cdk8. Additionally, Cdk8 targeted to RNA was observed to act in a synergistic manner with DNA-targeted Sp1 but not with DNA-targeted Tat, suggesting that RNA-targeted Cdk8 acts on similar rate limiting post-initiation events as Tat.

MATERIALS AND METHODS

Plasmids

Plasmid pBC12-Rev(+MCS), used in constructing Rev fusion proteins, was generated by PCR amplification of an HXB2 Rev coding sequence (amino acids 1–116) and ligation into the pBC12 vector (obtained from B.Cullen). A polylinker region (5′-*Hin*dIII–*Sph*I–*Eco*RV–*Hin*dIII–*Bgl*II-3′) was incorporated into the 5′ primer for subsequent generation of fusion proteins. Plasmid pCdk8-Rev was constructed by the PCR methodology, such that the full-length Cdk8 coding sequence was placed 5′ of the Rev coding sequence, employing the *Eco*RV and *Bgl*II sites of the polylinker in pBC12-Rev(+MCS). Plasmid pD173A-Rev, for expression of the catalytically inactive Cdk8–Rev fusion protein, was similarly constructed, using the mutant D173A sequence (obtained from J.P.Tassan) as PCR template. Other Rev fusions were similarly constructed by ligation of PCR products into appropriate polylinker sites.

Plasmids pSLIIB-CAT and pΔSLIIB-CAT were obtained from B.Cullen (28). Plasmid pG5-CMVIE-SLIIB-CAT was constructed by replacing the TAR element of plasmid pG5-CMVIE-TAR-CAT (obtained from B.Cullen) with the SLIIB Rev-binding element. Plasmid pSG424-Sp1 for expression of GAL4–Sp1 was constructed by PCR amplification of the sequence coding for amino acids 83–262 of human Sp1 and insertion into the *Eco*RI and *Xba*I sites of pSG424. Plasmid pGal4-Tat (29) was constructed by insertion of HXB2 Tat-1 coding sequences (amino acids 1–72) into pSG424. Plasmid pDM128 was obtained from T.Hope (30).

Transfections

For transfection experiments with Rev fusions, 200 ng pSLIIB-CAT or pΔSLIIB-CAT reporter, 500 ng Rev fusion plasmid, 500 ng pCH110 (as β-galactosidase control) and 800 ng salmon sperm DNA were transfected into HeLa cells using Lipofectamine reagent (Life Technologies) according to the manufacturer's protocol. Cells were harvested 48 h post-transfection and assayed for chloramphenicol acetyltransferase (CAT) and β-galactosidase activities. For co-transfections of GAL4 fusions with Rev fusions, 500 ng pG5-CMVIE-SLIIB-CAT, 250 ng GAL4 fusion plasmids, 500 ng Rev fusion plasmid and 500 ng pCH110 (β-galactosidase control) were transfected into HeLa cells using Lipofectamine reagent and assayed as above. For transfections to measure Rev function of Rev fusions, 1 μg pDM128 (Rev CAT reporter), 500 ng Rev fusion plasmid and 500 ng pCH110 (β-galactosidase control) were transfected into HeLa cells using Lipofectamine Plus. In each experiment, the total amount of transfected DNA was the same for all samples.

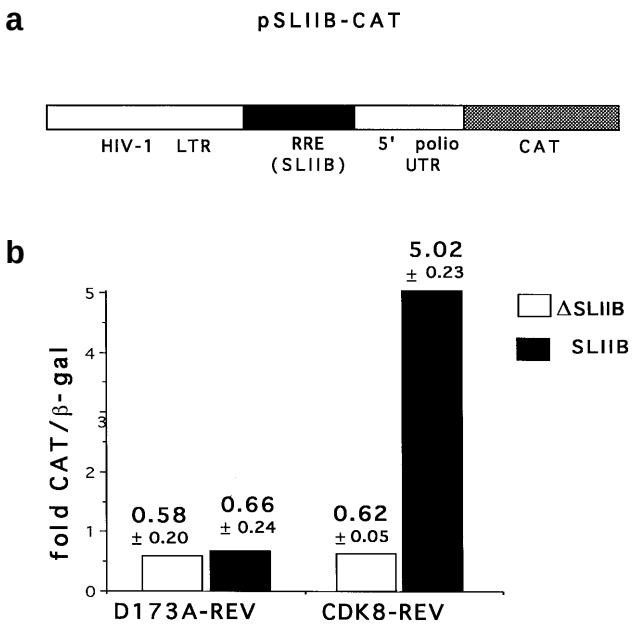

Figure 1. Activation of gene expression by Cdk8–Rev. (**a**) Reporter plasmid. pSLIIB-CAT contains an HIV-1 LTR, an SLIIB Rev-binding element, a poliovirus 5′-UTR and a CAT reporter gene. (**b**) Reporter activation by Cdk8–Rev. A plasmid expressing Cdk8–Rev or catalytic mutant D173A–Rev was co-transfected in HeLa cells with the pΔSLIIB-CAT or pSLIIB-CAT reporter. Cells were harvested 48 h post-transfection and assayed for CAT and β-galactosidase activities. Fold activity is relative to the Rev-expressing parental plasmid, with values averaged from duplicate samples. Results shown are representative of multiple independent experiments.

RESULTS

Cdk8 targeted to a promotor-proximal RNA element activates gene expression in a manner dependent on Cdk8 catalytic function

As our previous observations with a GAL4–Cdk8 fusion protein demonstrated that targeting to DNA activates gene expression independently of Cdk8 catalytic function, we were interested in identifying a mode of action that would be dependent on Cdk8 catalysis. Because Cdk8 can hyperphosphorylate the CTD and such phosphorylation is indicative of an elongation-competent transcription complex, we reasoned that targeting of Cdk8 to RNAP II at a point subsequent to transcription initiation might demonstrate a Cdk8 catalysis-dependent function. To this end, we constructed plasmids that express HIV-1 Rev fusion proteins for targeting to the pSLIIB-CAT reporter plasmid (28); pSLIIB-CAT is derived from the HIV-1 LTR and contains the minimal RRE in place of the TAR RNA element (Fig. 1A). As a control reporter plasmid we used pΔSLIIB-CAT, which contains mutations within SLIIB that greatly reduce binding by Rev or Rev fusion proteins (28).

Co-transfection of HeLa cells with the reporter plasmids and plasmids expressing Rev fusions of wild-type Cdk8–Rev or Cdk8

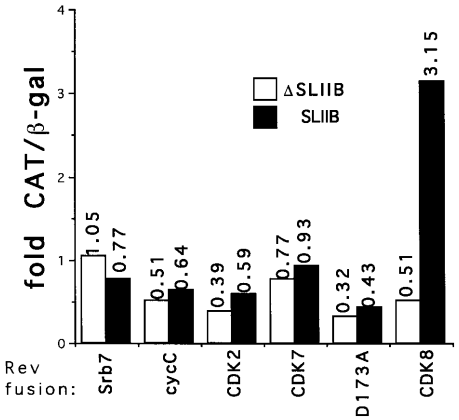

Figure 2. Effects of Rev fusion proteins on gene expression. Plasmids expressing the indicated Rev fusion proteins were co-transfected in HeLa cells with pΔSLIIB-CAT or pSLIIB-CAT. Cells were harvested 48 h post-transfection and assayed for CAT and β-galactosidase activities.

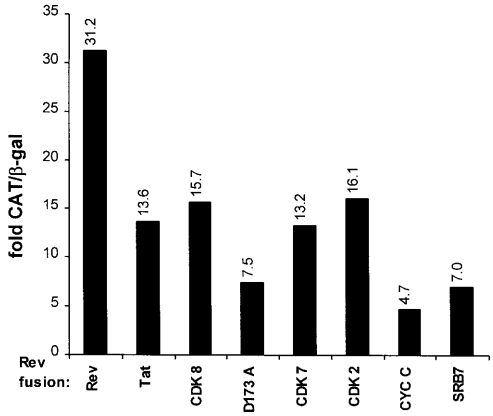

Figure 3. Effects of Rev fusion proteins on the Rev reporter plasmid. A Rev-responsive CAT reporter plasmid, pDM128, was co-transfected with plasmids expressing the indicated Rev fusion and a β-galactosidase internal reference plasmid. Fold activity is relative to the Rev-expressing parental plasmid, with values averaged from duplicate samples that varied <10%.

catalytic mutant D173A–Rev demonstrated that targeting wild-type Cdk8 to a promotor-proximal RNA element activated gene expression 5-fold (Fig. 1B). This activation was dependent upon binding to the RRE, as ΔSLIIB–CAT was not responsive to the Cdk8–Rev fusion. Additionally, the D173A catalytic mutant of Cdk8 did not activate the SLIIB–CAT reporter, indicating that Cdk8 targeted to an RNA element uses a catalytic function to modulate gene expression.

To examine the specificity of Cdk8 in activation of the SLIIB–CAT reporter, we constructed and analyzed additional plasmids that express Rev fusions of four human proteins: Cdk2, Cdk7, cyclin C and SRB7. Cdk2 and Cdk7 were examined because they are additional CDK family members and Cdk7 is known to be involved in transcriptional regulation; additionally, Cdk2 can hyperphosphorylate the CTD (31). Cyclin C and SRB7 were examined because, like Cdk8, they are components of the RNAP II holoenzyme and are human homologs of components in the yeast mediator complex; additionally, cyclin C is the cyclin partner of Cdk8. Analysis of these four additional Rev fusion proteins indicated that unlike Cdk8, these other similarly constructed fusion proteins were not able to activate expression of the SLIIB–CAT reporter (Fig. 2). Immunoprecipitation analyses with a Rev antiserum demonstrated that all Rev fusion proteins used in this study were expressed as stable proteins (data not shown). To assay whether the Rev fusion proteins possessed Rev function, plasmids expressing Rev fusion proteins were co-transfected with pDM128, a Rev-responsive CAT reporter (30). The results demonstrated that all Rev fusion proteins were functional for Rev activity (Fig. 3).

We note that conclusions are limited regarding the inability of Rev fusion proteins to activate the SLIIB–CAT reporter, as it cannot be ruled out that these proteins, when expressed as Rev fusions, are structurally and/or functionally perturbed. However, the observation that these four additional Rev fusions did not activate gene expression suggests a degree of specificity in the ability of Cdk8 to function in this system.

Cdk8–Rev synergizes with Sp1 but not with Tat

Because CTD hyperphosphorylation is thought to facilitate elongation of transcription, we performed experiments that would enable inference at the rate limiting step in the transcription cycle at which the Cdk8–Rev fusion protein might be acting. In this regard, we constructed a CAT reporter plasmid containing a minimal cytomegalovirus immediate-early (CMV IE) promoter linked to five GAL4 sites and an SLIIB element (Fig. 4A). This reporter was used to analyze co-transfections of plasmids expressing GAL4–Sp1 and GAL4–Tat fusion proteins with Cdk8–Rev. Sp1 has been proposed to primarily affect transcriptional initiation, whereas GAL4–Tat has been proposed to affect transcriptional elongation (29,32,33). If Cdk8–Rev acts primarily to activate elongation, it is predicted to be able to function synergistically with GAL4–Sp1 but not GAL4–Tat. Conversely, if Cdk8–Rev acts primarily to activate initiation, it is predicted to be able to act synergistically with GAL4–Tat but not GAL4–Sp1. We also analyzed a Tat–Rev fusion protein which is predicted to function synergistically with GAL4–Sp1 but not GAL4–Tat.

When transfected with the control plasmid expressing the GAL4 DNA-binding domain alone, the wild-type Cdk8–Rev plasmid was unable to activate the G5–CMVIE–SLIIB–CAT reporter (Fig. 4B). This inability to activate this reporter is likely due to low basal expression from this minimal promotor and the consequent low level of SLIIB RNA targets for the Cdk8–Rev fusion protein. Consistent with this, the Tat–Rev fusion protein was only able to activate the reporter ~2.5-fold, although Tat–Rev activates the SLIIB–CAT reporter used in Figure 1 from 60- to 90-fold (27,28). When the GAL4–Sp1 plasmid was co-transfected with the control plasmid that expresses Rev alone, a 2.5-fold activation of the reporter was observed. When GAL4–Sp1 and Cdk8–Rev plasmids were co-transfected, an ~8-fold activation of the reporter was observed. Because Cdk8–Rev alone is unable to activate this reporter, these results suggest that GAL4–Sp1 and Cdk8–Rev act in a synergistic manner and not an additive manner to activate the reporter. Co-transfection of the GAL4–Sp1 and

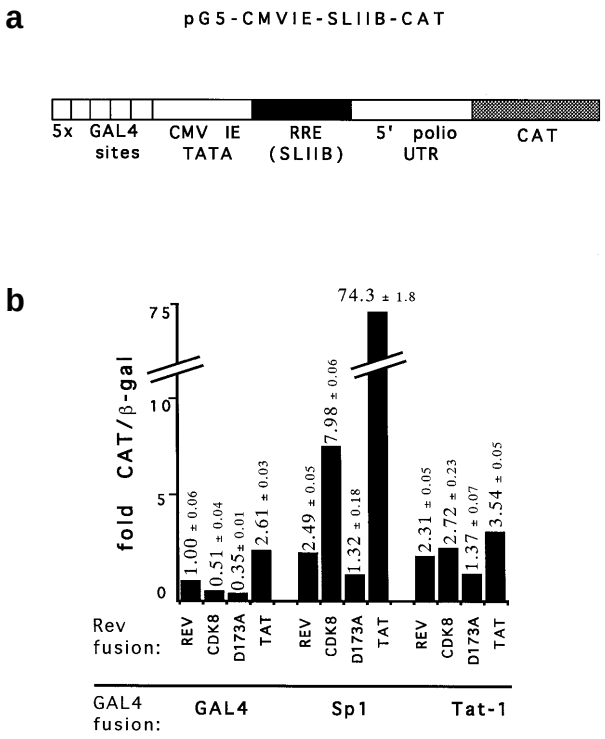

Figure 4. Synergy between Cdk8–Rev and GAL4 fusions. (**a**) Reporter for synergy experiments. The plasmid pG5-CMVIE-SLIIB-CAT contains five GAL4 sites, a minimal cytomegalovirus (CMV) immediate-early (IE) promotor, the SLIIB Rev-binding element, the poliovirus 5′-UTR and a CAT reporter gene. (**b**) Reporter activation. Plasmids expressing Rev, Cdk8–Rev catalytic mutant D173A–Rev or Tat–Rev were co-transfected into HeLa cells with GAL4–Sp1 or GAL4–Tat1, using pG5-CMVIE-SLIIB-CAT as reporter. Fold activity is relative to the Rev-expressing parental plasmid, with values averaged from duplicate samples. The results are representative of multiple independent experiments.

Tat–Rev plasmids resulted in a 74-fold synergistic activation of the reporter.

Co-transfection of the GAL4–Tat and Rev alone plasmids resulted in a 2.3-fold activation of the reporter, indicating a weak activation by GAL4–Tat that is comparable with that of GAL4–Sp1. Co-transfection of GAL4–Tat with Cdk8–Rev or Tat–Rev plasmids demonstrated 2.7- and 3.5-fold activation, respectively, of the reporter. These results indicate that unlike GAL4–Sp1, GAL4–Tat is unable to act in a synergistic fashion with either Cdk8–Rev or Tat–Rev fusion proteins. These results are therefore consistent with the interpretation that Cdk8–Rev primarily affects transcriptional elongation. Additionally, as observed in previous experiments, the activating effect of Cdk8–Rev on the reporter was dependent on intact Cdk8 catalytic function.

In summary, these results suggest that Cdk8–Rev modulates gene expression, in a manner dependent on Cdk8 catalytic function, by affecting a step subsequent to the initiation of transcription. Further, these results suggest that the Cdk8–Rev fusion protein works on a similar rate limiting step to HIV-1 Tat protein.

DISCUSSION

We have shown here that when targeted to a promotor-proximal RNA element, Cdk8 activates gene expression in a manner dependent on Cdk8 catalytic function. This result is consistent with the proposal that phosphorylation of the CTD of RNAP II is a regulatory event that can facilitate transcription. The observation that Cdk8–Rev can synergize with Sp1 but not Tat suggests further that Cdk8 targeted to RNA operates on a similar rate limiting step of the transcription cycle as Tat. We infer from this that Cdk8 can operate on post-initiation events. Although we cannot provide direct evidence that the effect of Cdk8 in these experiments is mediated by CTD phosphorylation, it seems the most favorable hypothesis, given the functional characterizations of yeast Srb10 and vertebrate Cdk8 presented previously (11,22,34).

That Cdk8 localized to an RNA element modulates gene expression only when Cdk8 is catalytically functional, whereas Cdk8 localized to DNA works independently of its catalytic function, may have mechanistic relevance to the TAR RNA-dependent function of HIV Tat proteins. Although GAL4–Tat has been demonstrated to activate gene expression, Tat is generally more efficient when localized through the natural TAR RNA element (35,36). Our results with Cdk8 suggest that localization of a CTD kinase via an RNA element at the 5′-end of the transcript is kinetically compatible with kinase-dependent modulation of RNAP II function, consistent with the proposed mechanism of action of HIV Tat proteins.

Recently, we have shown that Cdk9, the catalytic subunit of the Tat-associated kinase, can also activate transcription upon directed targeting to an RNA element (27). Hence, the results presented here indicate that Cdk8 shares with Cdk9 the ability to modulate transcription upon targeting to a nascent RNA element. In this regard, it may be noteworthy that, like Cdk8, Cdk9 associates with a C-type cyclin (11,14,15). That both Cdk8 and Cdk9 can activate transcription upon direct targeting to a nascent RNA element may suggest that those CDKs that associate with C-type cyclins may regulate transcription elongation according to a common biochemical theme. Identification of additional C-type cyclin-containing complexes may enable further determination of the mechanistic commonalties which they share, as well as an understanding of the functional specificities imparted by each.

Two Cdks have now been identified, Cdk8 and Cdk9, that appear to be able to function after initiation to activate processivity of the RNAP II complex. The existence of these two and perhaps additional kinases therefore allows a layer of regulation to control productive transcription of mRNA genes. Cdk8 is a component of the RNAP II holoenzyme and is presumably present in most RNAP II complexes that have initiated transcription. In contrast, Cdk9 appears not to be a component of the RNAP II holoenzyme (J.Parvin, A.P.Rice and C.H.Herrmann, unpublished observation) and, therefore, Cdk9 may need to be specifically recruited to the holoenzyme to activate elongation. A major challenge for future understanding of control of transcriptional elongation will be to identify and elucidate mechanisms whereby Cdk8 function is regulated in the holoenzyme and how Cdk9 function is recruited to the holoenzyme.

ACKNOWLEDGEMENTS

We thank Bryan Cullen, Tom Hope, Erich Nigg and Jean-Pierre Tassan for plasmids. We thank Chris Herrmann and Paul Ling for critical comments on the manuscript. This work was supported by NIH grants AI35381 and Training Program T32 AI07483 for Basic Research and AIDS (M.G.).

REFERENCES

1 Bentley,D.L. (1995) *Curr. Opin. Genet. Dev.*, **5**, 210–216.
2 Corden,J.L. and Patturajan,M. (1997) *Trends Biochem. Sci.*, **22**, 413–416.
3 Payne,J.M., Laybourn,P.J. and Dahmus,M.E. (1989) *J. Biol. Chem.*, **264**, 19621–19629.
4 O'Brien,T., Hardin,S., Greenleaf,A. and Lis,J.T. (1994) *Nature*, **370**, 75–77.
5 Carlson,M. (1997) *Annu. Rev. Cell Dev. Biol.*, **13**, 1–23.
6 Barron-Casella,E. and Corden,J.L. (1992) *J. Mol. Evol.*, **35**, 405–410.
7 Akoulitchev,S., Makela,T.P., Weinberg,R.A. and Reinberg,D. (1995) *Nature*, **377**, 557–560.
8 Serizawa,H., Makela,T.P., Conaway,J.W., Conaway,R.C., Weinberg,R.A. and Young,R.A. (1995) *Nature*, **374**, 280–282.
9 Shiekhattar,R., Mermelstein,F., Fisher,R.P., Drapkin,R., Dynlacht,B., Wessling,H.C., Morgan,D.O. and Reinberg,D. (1995) *Nature*, **374**, 283–287.
10 Rickert,P., Seghezzi,W., Shanahan,F., Cho,H. and Lees,E. (1996) *Oncogene*, **12**, 2631–2640.
11 Tassan,J.P., Jaquenoud,M., Leopold,P., Schultz,S.J. and Nigg,E.A. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 8871–8875.
12 Chao,D.M., Gadbois,E.L., Murray,P.J., Anderson,S.F., Sonu,M.S., Parvin,J.D. and Young,R.A. (1996) *Nature*, **380**, 82–85.
13 Maldonado,E., Shiekhattar,R., Sheldon,M., Cho,H., Drapkin,R., Rickert,P., Lees,E., Anderson,C.W., Linn,S. and Reinberg,D. (1996) *Nature*, **381**, 86–89.
14 Wei,P., Garber,M.E., Fang,S.M., Fischer,W.H. and Jones,K.A. (1998) *Cell*, **92**, 451–462.
15 Peng,J., Zhu,Y., Milton,J.T. and Price,D.H. (1998) *Genes Dev.*, **12**, 755–762.
16 Zhu,Y., Pe′ery,T., Peng,J., Ramanathan,Y., Marshall,N.F., Marshall,T.A.B., Amendt,B., Mathews,M.B. and Price,D.H. (1997) *Genes Dev.*, **11**, 2622–2632.
17 Mancebo,H.S.Y., Lee,G., Flygare,J., Tomassini,J., Luu,P., Zhu,Y., Peng,J., Blau,C., Hazuda,D., Price,D.H. and Flores,O. (1997) *Genes Dev.*, **11**, 2633–2644.
18 Yang,X., Gold,M.O., Tang,D.N., Lewis,D.E., Aguilar-Cordova,E., Rice,A.P. and Herrmann,C.H. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12331–12336.
19 Goodrich,J.A. and Tjian,R. (1994) *Cell*, **77**, 145–156.
20 Dvir,A., Conaway,R.C. and Conaway,J.W. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 9006–9010.
21 Marshall,N.F., Peng,J., Xie,Z. and Price,D.H. (1996) *J. Biol. Chem.*, **271**, 27176–27183.
22 Liao,S.M., Zhang,J., Jeffery,D.A., Koleske,A.J., Thompson,C.M., Chao,D.M., Viljoen,M., van Vuuren,H.J. and Young,R.A. (1995) *Nature*, **374**, 193–196.
23 Akhtar,A., Faye,G. and Bentley,D.L. (1996) *EMBO J.*, **15**, 4654–4664.
24 Kuchin,S. and Carlson,M. (1998) *Mol. Cell. Biol.*, **18**, 1163–1171.
25 Herrmann,C.H., Gold,M.O. and Rice,A.P. (1996) *Nucleic Acids Res.*, **24**, 501–509.
26 Gold,M.O., Tassan,J.-P., Nigg,E.A., Rice,A.P. and Herrmann,C.H. (1996) *Nucleic Acids Res.*, **24**, 3771–3777.
27 Gold,M.O., Yang,X., Herrmann,C.H. and Rice,A.P. (1998) *J. Virol.*, **72**, 4448–4453.Gold,M.O., Yang,X., Herrmann,C.H. and Rice,A.P. (1998) *J. Virol.*, **72**, 4448–4453.
28 Tiley,L.S., Madore,S.J., Malim,M.H. and Cullen,B.R. (1992) *Genes Dev.*, **6**, 2077–2087.
29 Rhim,H. and Rice,A.P. (1994) *Nucleic Acids Res.*, **22**, 4405–4413.
30 Hope,T.J., Huang,X.J., McDonald,D. and Parslow,T.G. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 7787–7791.
31 Herrmann,C.H. and Rice,A.P. (1995) *J. Virol.*, **69**, 1612–1620.
32 Kao,S.Y., Calman,A.F., Luciw,P.A. and Peterlin,B.M. (1987) *Nature*, **330**, 489–493.
33 Blau,J., Xiao,H., McCracken,S., O'Hare,P., Greenblatt,J. and Bentley,D. (1996) *Mol. Cell. Biol.*, **16**, 2044–2055.
34 Hengartner,C.J., Thompson,C.M., Zhang,J., Chao,D.M., Liao,S.-M., Koleske,A.J., Okamura,S. and Young,R.A. (1995) *Genes Dev.*, **9**, 897–910.
35 Southgate,C.D. and Green,M.R. (1991) *Genes Dev.*, **5**, 2496–2507.
36 Kamine,J., Subramanian,T. and Chinnadurai,G. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 8510–8514.