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Differential association of linker histones H1 and H5 with telomeric nucleosomes in chicken erythrocytes

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ABSTRACT

Rat liver telomeric DNA is organised into nucleosomes characterised by a shorter and more homogeneous average nucleosomal repeat than bulk chromatin as shown by Makarov *et al.* (1). The latter authors were unable to detect the association of any linker histone with the telomeric DNA. We have confirmed these observations but show that in sharp contrast chicken erythrocyte telomeric DNA is organised into nucleosomes whose spacing length and heterogeneity are indistinguishable from those of bulk chromatin. We further show that chicken erythrocyte telomeric chromatin contains chromatosomes which are preferentially associated with histone H1 relative to histone H5. This contrasts with bulk chromatin where histone H5 is the more abundant species. This observation strongly suggests that telomeric DNA condensed into nucleosome core particles has a higher affinity for H1 than H5. We discuss the origin of the discrimination of the lysine rich histones in terms of DNA sequence preferences, telomere nucleosome preferences and particular constraints of the higher order chromatin structure of telomeres.

INTRODUCTION

Telomeres, the physical ends of the chromosomes, are essential for the stability of the linear chromosomes and for the maintenance of the chromosome integrity (2). The telomeric DNA sequence consists of simple tandem GGGTTA-related repeats with the total length varying substantially between different species and cells, but it is the G-rich strand which always forms the 3′ end of the chromosome. Recently a nucleoprotein, called telomerase, has been identified which is responsible for the enzymatic addition or elongation of telomeric repeats at the 3′ end (reviewed in ref. 3). Under the appropriate conditions, the telomeric DNA sequence is able to adopt an intra- or intermolecular quadruplex structure (reviewed in 4). Such a non-B DNA structure is promoted or recognized by specialized proteins (5,6), a role which is in accord with their proposed biological function.

The bulk DNA inside the nucleus of eukaryotes is, however, not naked, but is normally compacted in a repeated structure containing nucleosomes. Each nucleosome contains on average 200 bp of DNA and comprises a linker and a distinct entity, the chromatosome, which contains 168 bp DNA associated with an octamer of core histones and one linker histone molecule. In the absence of the linker histone, only 146 bp of DNA is tightly associated with the histone octamer as the nucleosome core particle.

The telomeric DNA sequences of lower eukaryotes apparently lack a typical nucleosomal organization (7,8). By contrast Makarov *et al.* (1) recently demonstrated that the long telomeric tracts of rat liver nuclei (20 to 100 kb in length) are organized in uniformly spaced nucleosomes with an unusually short repeat length of 157 bp. The size of this nucleosomal repeat is shorter than the DNA length present in one chromatosome and consequently the authors suggested that the linker histones are absent in telomeres of higher eukaryotes. Indeed, they were unable to observe the linker histone H1 binding in rat liver mononucleosomes containing telomeric sequences and hypothesized that the telomeric GGGTTA motif itself might establish the short nucleosomal repeat. However, from the experiments of Tommerup *et al.* (9) we learned that the mammalian cells with relatively short telomeres (average size of 4 kb) possess an unusual nucleosomal organization. This was indicated by the absence of longer nucleosomal arrays on telomeric sequences. Also, the telomeric mononucleosomes appeared to be hypersensitive towards micrococcal nuclease (9).

The central question that remains is how general this special and specific telomeric nucleosomal organization is for higher eukaryotes, and whether it is maintained in other tissues or other eukaryotic species. To investigate this we analysed the nucleosomal organization of telomeric chromatin in chicken erythrocytes. Chicken erythrocytes were chosen for study, mainly because of the abundant presence of histone H5 in this tissue. H5 is a replacement variant of the linker histone H1. It is known that H5 both binds more tightly and confers greater stability on chromatin than H1 (10).

In a shotgun cloning experiment of chicken erythrocyte chromatosomal DNA we obtained a $(TTAGGG)_{28}$ DNA

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fragment (EMBL accession no. X 63499). We confirmed by Bal 31 digestion that this cloned DNA originates from a telomeric location as already suggested by its sequence. Using this clone as a probe for the chromatin organization of chicken erythrocyte telomeres we found remarkable differences with the rat liver chromatin organization. First, the nucleosomal repeat is identical to the bulk nucleosomal repeat and secondly the telomeric sequences are associated with linker histones into chromatosomes. In addition, we observed that the stoichiometry of the linker histone in these chromatosomes deviates from the value from the H1/H5 ratio present in chicken erythrocytes. This suggests that, besides the octamer histones showing a preferential interaction with particular DNA sequences to form a core particle, the linker histones also confer a differential binding with nucleosome particles containing different DNA sequences.

MATERIALS AND METHODS

The chicken erythrocyte and rat liver nuclei were prepared and digested with micrococcal nuclease to generate soluble fragmented chromatin as described previously (11). The chicken erythrocyte chromatin (2 mg DNA) was separated by ultracentrifugation through a linear 10−30 % sucrose gradient containing 0.2 mM EDTA, 1 mM Na-phosphate pH 6.8, 80 mM NaCl and 0.1 mM PMSF in an SW27 rotor at 25000 rpm for 4 h. The gradient was fractionated and the different fractions were treated with proteinase K in the presence of 0.5 % SDS and 400 mM NaCl. DNA was extracted twice with phenol and ethanol precipitated.

The chicken erythrocyte chromatosome sample was prepared according to Lambert *et al.* (12). The H1 and H5 containing chromatosomes were separated on a 5% polyacrylamide gel (29/1 acryl/bisacrylamide) in 0.5×TBE. After photography of the ethidium bromide stained gel, the DNA was denatured and used for Southern blotting and hybridization following standard protocols.

RESULTS

In the course of a statistical analysis to determine the DNA sequence organization in chromatosomes (13) we cloned chicken chromatosomal DNA fragments and noted the isolation of a clone containing a repeated DNA sequence (TTAGGG)28. This observation raises two interesting points. First, its sequence is identical to the sequence of telomeres in human, or many other eukaryotes (3) and consequently we inferred that this particular clone originated from a telomeric region of the chicken chromosomes. An exonucleolytic degradation by Bal 31 of chicken erythrocyte genomic DNA readily removed most of the TTAGGG-hybridizing material and consequently we concluded that our clone originated from a telomeric location. From a calibration of the digestion conditions using the linear bacteriophage λ DNA as an internal control, we estimated that the telomeric sequence has an average length of some 5 kb in chicken erythrocytes. This relatively short length for telomeric sequences is in agreement with the hypothesis that shorter telomeres are present in terminal differentiated cells or during cell ageing (14,15). Second, the length of our cloned fragment (28 repeats of TTAGGG = 168 bp) suggested that the telomeres in this tissue might be organized in nucleosomes containing linker histones (chromatosomes) in which the telomeric nucleosomal organization in chicken erythrocytes would differ substantially from the rat liver.

Chicken erythrocyte telomeric sequences are compacted in nucleosomes

We compared the telomere chromatin organization in chicken erythrocyte nuclei and rat liver nuclei. The DNA fragments generated after micrococcal nuclease digestions of the chromatin from these nuclei were separated on agarose gels. Normally only up to 6−7 nucleosomal bands can be clearly distinguished due to the DNA length heterogeneity of nucleosomes. To increase the resolution for the chicken erythrocyte sample, we first separated the chromatin fragments according to their size by sedimentation through a sucrose gradient (16). Ethidium bromide staining of the agarose gels revealed the characteristic nucleosomal ladder (Figure 1) from which rat liver and chicken erythrocyte nucleosomal repeats of respectively 190 and 210 bp were calculated, in accordance with previous published data (11). The autoradiograph of the Southern blot using the telomeric sequence as a probe shows a 210 bp repeat for the chicken erythrocyte telomeric chromatin in precise register with the ethidium bromide stained bands (Figure 1A). This is in sharp contrast with the more uniform telomere-specific rat liver nucleosomal repeat of 157 bp reported by Makarov *et al.* (1) and confirmed in figure 1B. The clear presence of oligonucleosomal bands of more than 3 nucleosomes within the chicken erythrocyte nuclei, a cell type with short telomeres (average of 5 kb in lenght), is also in contrast with the statement that short telomeres lack the canonical extensive nucleosomal arrays (9). Since no nucleosomal DNA ladder is seen when naked DNA was digested with micrococcal nuclease, we are confident that the DNA sequence specificity of micrococcal nuclease is not responsible for the observed pattern. These results confirm the conclusion that telomeres in higher eukaryotes are organized in nucleosomes but are at variance with the assumption that the telomeric-specific rat liver chromatin organization is imposed by the telomeric DNA sequence itself as previously hypothesized (1).

Telomeric sequences are contained in chromatosomes, preferred by the H1 linker histone

The exact mechanism responsible for the nucleosome spacing is still unclear. However, the involvement of the amount and type of linker histone has been recognized (11, 17). Therefore, it might be expected that the different nucleosomal repeats of the telomeres in chicken erythrocytes and rat liver results from the H5 binding to the chromatin of the former tissue. The most sensitive and revealing test to analyse the binding of the linker histones to the chicken erythrocyte telomeric chromatin is by looking at the level of the mononucleosomes. In case where the linker histone is stably associated with the nucleosome, the micrococcal nuclease will pause at 168 bp, corresponding to a chromatosome. It is also important to realize that the chromatosomes containing an H1 molecule and chromatosomes with an H5 linker histone can easily be distinguished on a nucleoprotein gel (18). On the other hand, if the linker histone is dissociated, a nucleosomal core particle band of 146 bp should be obtained readily upon micrococcal nuclease digestion. Makarov *et al.* (1) observed the latter situation in their experiments with rat liver telomeric chromatin, while Tommerup *et al.* (9) failed to observe any clear core particle band due to the hypersensitivity of the telomeric mononucleosomes for the nuclease.

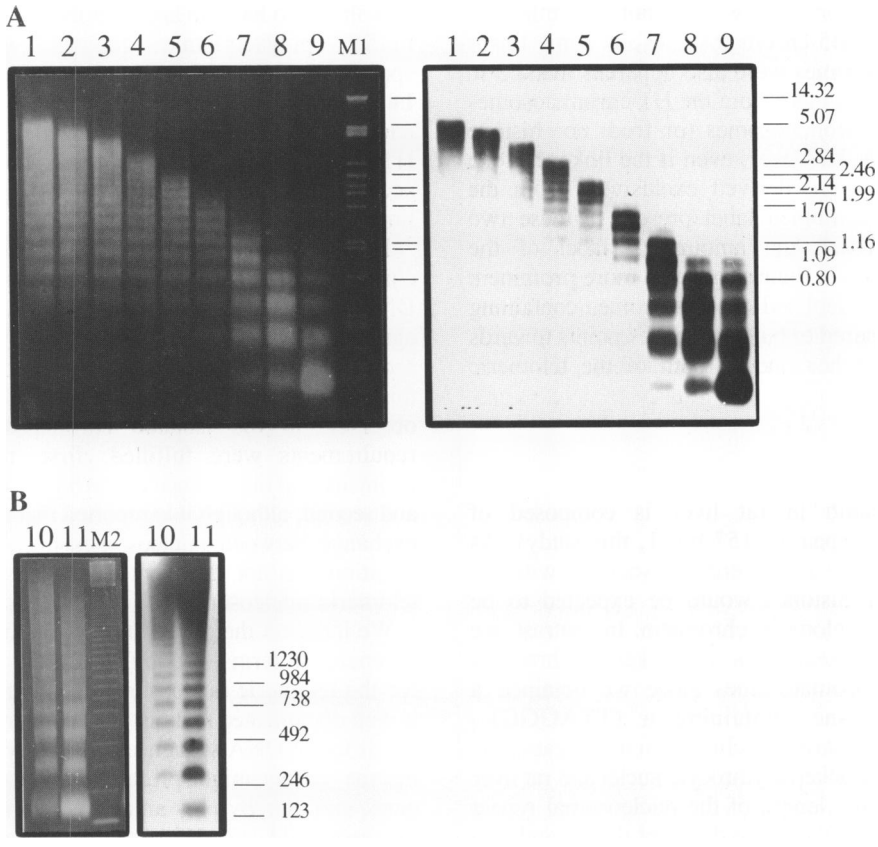

Figure 1. Length of the nucleosomal repeat of bulk and telomeric chromatin from chicken erythrocytes (**A**) and rat liver (**B**). A, left part, lanes 1 to 9: Chicken erythrocyte nuclei were incubated with micrococcal nuclease. The fragmented chromatin was extracted and size fractionated by sucrose gradient sedimentation (17). The different fractions were deproteinized and the DNA applied on agarose gel (lane 1 bottom fraction, lane 9 top fraction), and visualized by ethidium bromide staining. Lane M1 contains λ DNA digested with PstI, used as a size marker; the lengths in kb of these fragments are indicated at the right end of the figure. A, right part, lanes 1 to 9: Southern blot of the gel shown in the left part. The clone containing the telomeric sequences was used as a probe. B, left part, lanes 10 and 11: Rat liver nuclei were incubated with micrococcal nuclease for 30 and 60 seconds respectively and the extracted DNA applied on agarose gel. M2 is the 123 bp ladder (GIBCO/BRL) used as a DNA size standard; the exact length in bp of some marker fragments are indicated at the outer right end of the figure. B, right part, lanes 10 and 11. Southern blot of the gel shown in the left part. The clone containing the telomeric sequences was used as a probe.

We prepared chicken erythrocyte chromatosomes containing H1 and H5 as described by Lambert *et al.* (12). These H1 and H5 containing chromatosomes and core particles were then separated on a nucleoprotein gel. Staining with ethidium bromide reveals an apparent twofold excess of the H5 chromatosomes compared to the H1 chromatosomes (Figure 2A), totally in agreement with the 2 to 1 ratio of H5 and H1 in chicken erythrocyte nuclei (19). After removing the proteins, and electroblotting the DNA from this gel to nylon membranes, we hybridized the DNA with labelled bulk chicken genomic DNA as a probe. This Southern blot confirmed the proper transfer of the bands as the 2 to 1 ratio for the H5 chromatosomes to H1-chromatosomes was maintained (Figure 2B). Removal of the probe and rehybridization of the blot with the prelabelled telomeric sequence reveals an interesting picture (Figure 2C). First, the presence of the label at the position of the H1 and H5 chromatosomes proves that the mononucleosomes of chicken erythrocyte telomeres are compacted in both H1 and H5 containing chromatosomes. This is in sharp contrast to the rat, mouse or human telomeric chromatin (1,9) where the monomer subunit apparently does not contain histone H1. Our result means that the lack of the H1 binding to mammalian telomeric chromatin

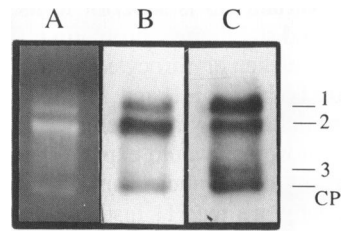

Figure 2. Nucleoprotein gel analysis of chicken erythrocyte chromatosomes. (**A**) Fluorogram of the ethidium bromide stained chromatosomes. (**B**) Autoradiogram obtained after hybridization with labelled total genomic chicken DNA to the DNA electrotransferred from the gel in (A). (**C**) Autoradiogram obtained after hybridization with labelled telomeric probe to the DNA electrotransferred from the gel in (A). The bands numbered 1, 2, 3 and CP mark the position of the H1 chromatosomes, the H5 chromatosomes, the linker histone depleted chromatosomes and core particles respectively.

(1,9) is certainly not a consequence of the inherent inability of this histone to interact with the telomeric DNA sequence. The second and probably most important conclusion from this blot is that chicken erythrocyte telomeres are preferentially packed

in H1 containing chromatosomes. We could not determine the exact ratio of the H1 and H5-chromatosomes, as some linker histone depleted chromatosomes were also apparent (band 3 in Figure 2C). These could originate from the H1-chromatosomes as well as from the H5-chromatosomes (or from non-histone bound mononucleosomes). However, even if the linker histone depleted chromatosomes were derived exclusively from the H5-chromatosomes, the combined label present in these two bands would only approach the amount of label of the H1-chromatosomes. Whatever their origin, the more prominent presence of linker histone depleted chromatosomes containing telomeric sequences compared to bulk sequences points towards the reduced stability of either one or both of the telomeric chromatosomes.

DISCUSSION

Telomere-specific chromatin in rat liver is composed of nucleosomes with a short repeat of 157 bp (1, this study). As this repeat is shorter than the DNA length associated within a chromatosome, no linker histones would be expected to be associated with the rat liver telomeric chromatin. In contrast, we surmised that the telomeric sequences in chicken erythrocytes could be organised in chromatosomes since we obtained a chromatosomal DNA clone containing a $(TTAGGG)_{28}$ sequence. We therefore compared the chromatin organization on the telomeric sequences in chicken erythrocyte nuclei and rat liver nuclei. It was found that the length of the nucleosomal repeat differed substantially between these two different tissues and also that the telomeric nucleosomal spacing of the chicken erythrocytes appeared to be less uniform than that of rat liver. It remains to be established whether these differences reflect the transcriptional and replication inactivity of the erythrocyte cells.

Whatever the origin of these differences, the size of the nucleosomal repeat in erythrocytes is consistent with chromatosome formation on telomeric DNA. In contrast to our expectation that telomeres would be packaged preferentially in H5 containing chromatosomes—since H1 chromatosomes were not observed in rat liver (1), mouse J558 or HeLa cells (9), and the linker histone variant H5 is superior to its homologue H1, in both binding to and stabilizing chromatin (10)—our Southern blot analysis of the chicken erythrocyte chromatosomes revealed the association of both the H1 and H5 linker histones with chromatosome particles harboring telomeric sequences. The presence of chromatosomes would explain the difference in the length and uniformity of the nucleosomal repeat between these chromatins. But, why are the H1 histones of rat liver, mouse J558 or HeLa cells not bound to the telomeric nucleosomes? From the data of Makarov *et al.* (1) and Tommerup *et al.* (9), it appears that there is no pause at 168 bp upon micrococcal nuclease digestion of the telomeric DNA, and therefore there are no chromatosomes as conventionally defined. However, on sedimentation, the rat liver telomeric nucleosomes behave like condensed chromatin which can be converted to the 145 bp core particles (1). We envisage two possibilities: either these mammalian telomeres have a modified core particle to which their linker histone subtypes cannot bind, or the histone octamers are associated with another protein which is distinct from the bulk H1, but which imposes for example the homogeneous 157 bp repeat in case of rat liver telomeric chromatin. An obvious candidate of such a protein would be the mammalian factor—TRF—which binds to the telomeric repeats (20). This protein was shown to have an apparant molecular weigth around 50 kDa in SDS polyacrylamide gels and was reported to interact specifically with TTAGGG repeats larger than 6 units (20). Interestingly, it has been noted that the β subunit of the Oxytricha telomere binding protein bears a sequence similarity to the histone H1 (21). This part of the Oxytricha nova protein was shown to accelerate the G-quartet formation, a property shared with the linker histone (5).

However, most remarkable was the stoichiometry of the H1/H5 chromatosomes on telomeres. Although two thirds of the bulk DNA organised in chromatosomes are associated with H5, in agreement with their twofold excess (19), the telomeric DNA sequences are preferentially associated with H1. The fact that this preferential occurrence of H1 on telomeric nucleosomes was observed in the isolated chromatosomes means that two requirements were fulfilled: first, the H1 preference was maintained in the absence of the higher order chromatin structure and second, although it is reported that the linker histones readily exchange between nucleosomes (22), any such exchange on isolation was not sufficient to abolish the selectivity of H1 for telomeric nucleosomes.

We interpret the observation of the increased H1/H5 ratio on telomeres in terms of a higher affinity of the H1 linker histone for the telomeric nucleosomes compared to the histone H5. It is well documented that histone octamers have different affinities for different DNA sequences (23). Also, a particular lysine-rich histone variant might preferentially associate with a particular gene, and thereby play an active role in the regulation of gene expression (24, 25). However, to the best of our knowledge, this is the first report that the linker histone variants possess a differential affinity for nucleosomes containing different DNA sequences.

The origin of the H1, H5 difference in binding to the telomeric sequences is likely to reside within the globular part. This central region of the lysine rich histones is essential for the chromatosome binding (26,27) and although the overall structure of the GH5 and GH1 is very similar (28, 29), several important differences were apparent both in the loop between the helices II and III, the binding and recognition helix respectively, and in the location of the highest positive electrostatic potential surfaces (29).

What could be the role of the telomeric sequence itself in this preferential H1 association? We suggest that the preferential binding with H1 might be the result of a selection at one of three levels: the DNA sequence itself, the nucleosomal architecture, or the higher order chromatin structure of the telomeres. With respect to the first possibility, many reports support the preferential binding of the lysine rich histones to A/T rich sequences or to particular DNA modifications (30, 31). In a recent report a purified lysine-rich H1e variant had a preference for G/C rich DNA indicating that some subfractions might behave differently (32). So, it is plausible that at least one of the H1 subtypes of the chicken erythrocyte prefers the telomeric DNA sequence. As discussed before, this H1 subtype would be absent in rat liver chromatin.

For the second possibility, we refer to the reports that the linker histones have an affinity in decreasing order for linker histone depleted chromatosomes, for core particles and for protein-free DNA respectively (33). Also, a higher affinity was observed for a four-way junction DNA molecule compared to B-DNA (34). All these evidences favor the idea of a differential affinity of the linker histones for different DNA structures. The telomere sequence has a sequence repeat of 6 basepairs which deviates

from the 10.2 average helical repeat preferred by histone octamers (13, 35). Therefore, it is conceivable that the geometry of telomeric sequence wrapped on a nucleosome is different from the average nucleosomal structure, and that these differences are sensed differently by H1 and H5.

The third possibility suggests that the higher order chromatin structure, the 30 nm fibre, imposes the binding of a particular H1 subtype for proper telomere folding or functioning. It is tempting to assign a functional role to formation of such a domain containing (nearly) exclusively a particular H1 subtype. The correlation between the appearance of specific lysine rich subtypes and cell differentiation or differences in chromatin function has been witnessed on several occasions (24, 25, 36). Again Mohr *et al.* (25) showed convincingly that the appearance of the H1 I-1 variant was restricted to the centromeres and to a limited number of other bands in the salivary gland chromosomes of Chironomus thummi. Likewise, the H1 histone of the chicken erythrocyte (or at least one of its subtypes) might be confined to a distinct subfraction of chromatin including or perhaps restricted to telomeres. Such a preferential association would be wholly consistent for functionally distinct roles for different linker histones within a single nucleus.

Finally, it still could be envisaged that the more abundant presence of H1 on telomeres is a consequence of the late replication of these sequences. Because H5 has an intrinsically higher affinity than H1 for chromatin, the pool of lysine rich histones could be largely depleted of H5 by the time the telomeres replicate.

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REFERENCES

1. Makarov V.L., Lejnine S., Bedoyan J. and Langmore J.P. (1993) *Cell* **73**, 775–787.
2. Blackburn E.H. (1994) *Cell* **77**, 621–623.
3. Blackburn E.H. (1992) *Ann.Rev.Biochem.* **61**, 113–129.
4. Schultze P., Smith F.W. and Feigon J. (1994) *Structure* **2**, 221–233.
5. Fang G. and Cech T.R. (1993) *Cell* **74**, 875–885.
6. Giraldo R. and Rhodes D. (1994) *EMBO J.* **13**, 2411–2420.
7. Blackburn E.H. and Chiou S.S. (1981) *Proc.Natl.Acad.Sci.USA* **78**, 2263–2267.
8. Wright J.H. Gottschling D.E. and Zakian V.A. (1992) *Genes Dev.* **6**, 197–210.
9. Tommerup H., Dousmanis A. and de Lange T. (1994) *Mol.Cell.Biol.* **14**, 5777–5785.
10. Sun J., Ali Z., Lurz R. and Ruiz-Carrillo A. (1990) *EMBO J.* **9**, 1651–1658.
11. Muyldermans S., Lasters I., Wyns L. and Hamers R. (1981) *Nucl.Acids Res.* **9**, 3671–3680.
12. Lambert S., Muyldermans,S. Baldwin J.P., Kilner,J., Ibel K. and Wyns L. (1992) *Biochem.Biophys.Res.Comm.* **179**, 810–816.
13. Muyldermans S. and Travers A.A. (1994) *J.Mol.Biol.* **235**, 855–870.
14. Harley C.B, Futcher A.B. and Greider C.W. (1990) *Nature* **345**, 458–460.
15. Greider, C.W. (1992) *Curr. Opinion Gen. Dev.* **4**, 203–211.
16. Muyldermans S. Lasters I. Hamers R. and Wyns L. (1985) *Eur.J.Biochem.* **150**, 441–446.
17. Lauderdale J.D. and Stein A. (1993) *Biochemistry* **32**, 489–499.
18. Huang S.Y. and Garrard W.T. (1989) *Meth.Enzymol.* **170**, 116–142.
19. Bates D.L. and Thomas J.O. (1981) *Nucl.Acids Res.* **8**, 5883–5894.
20. Zhong Z., Shiue L., Kaplan S. and de Lange T. (1992) *Mol.Cell.Biol.* **12**, 4834–4843.
21. Hicke B.J., Celander D.W., MacDonald G.H., Price C.M. and Cech, T.R. (1990) *Proc.Natl.Acad.Sci.USA* **87**, 1481–1485.
22. Caron F. and Thomas J.O. (1981) *J.Mol.Biol.* **146**, 513–537.
23. Shrader T.E. and Crothers D.M. (1990) *EMBO J.* **9**, 1651–1658.
24. Roche J., Gorka C., Goeltz P. and Lawrence J.J. (1985) *Nature* **314**, 197–198.
25. Mohr E., Trieschmann L. and Grossbach U. (1989) *Proc.Natl.Acad.Sci.USA* **86**, 9308–9312.
26. Allan J., Hartman P.G. Crane-Robinson C. and Aviles F.X. (1980) *Nature* **288**, 675–679.
27. Buckle R.S., Maman J.D. and Allan J. (1992) *J.Mol.Biol.* **223**, 651–659.
28. Ramakrishnan V., Finch J.T., Graziano V., Lee P.L. and Sweet R.M. (1993) *Nature* **362**, 219–223.
29. Cerf C., Lippens G., Ramakrishnan V., Muyldermans S., Segers A., Wyns L., Wodak S.J and Hallenga, K. (1994) *Biochemistry* **33**, 11079–11086.
30. Izaurralde E., Käs E. and Laemmli U.K. (1989) *J.Mol.Biol.* **210**, 573–585.
31. Jost J.P. and Hofsteenge J. (1992) *Proc.Natl.Acad.Sci.USA* **89**, 9499–9503.
32. Wellman S.E., Sittman D.B. and Chaires J.B. (1994) *Biochemistry* **33**, 384–388.
33. Pennings S., Muyldermans S. and Wyns L. (1989) *Mol.Biol.Rep.* **13**, 191–196.
34. Varga-Weisz P., Zlatanova J., Leuba S.H., Schroth G.P. and van Holde K. (1994) *Biochemistry* **91**, 3525–3529.
35. Satchwell S.C., Drew H.R. and Travers A.A. (1986) *J.Mol.Biol.* **191**, 659–675.
36. Ner S. and Travers A. *EMBO J.* **13**, 1817–1822.