SHORT COMMUNICATIONS

β′-SUBUNIT OF *ESCHERICHIA COLI* RNA POLYMERASE MOST PROBABLY HAS ONE "ZINC FINGER" PROTEIN MOTIF

DIPANKAR CHATTERJI and K. GURUPRASAD
Centre for Cellular & Molecular Biology, Uppal Road, Hyderabad 500 007, India.

In the last a few years a novel protein conformational motif has emerged that appears to be used for the binding of proteins to DNA and RNA; this form is called the "Zn-finger"[1]. It was originally shown to be present in the xenopus transcription factor IIIA in tandem repeat. Klug and his group have postulated that several such fingers act in concert, where each finger recognizes half a turn of the DNA A or B – helix enriched in "G"-nucleotides[2,3]. As most of these proteins act at the level of the regulation of transcription, it is fairly easy to visualize the necessity for such a specific interaction between DNA and these proteins. So far, the protein structure used for DNA binding, which is best understood, is the helix-turn-helix motif seen in the crystal structure of several regulatory proteins of bacterial origin[4,5]. However, the involvement of metal atoms was not shown in these cases, although many of the regulatory proteins are Zn-metalloproteins. In "Zn-finger", Zn is an essential part of the interaction, primarily acting as a co-factor. Miller *et al*[2] showed that the xenopus TF IIIA is enriched in the dipeptide sequence Cys-Cys, and the His-His sequences occur in tandem and that this linear arrangement can be folded around a Zn-atom tetrahedrally co-ordinated with two Cys and two His residues at the bottom of a finger while the tip of the finger is enriched in basic amino acids required for DNA binding. Several such finger proteins have now come into light upon sequence analysis and are listed in a recent review[1].

Escherichia RNA polymerase ($\alpha_2\beta\beta'\sigma$) is a Zn-metalloenzyme, in which Zn is essential for the regulation of transcription and for DNA binding[6]. The Zn atom is usually very tightly bound with RNA polymerase, one Zn atom is present with the β subunit or the substrate binding site of the enzyme and the other one is present with the β' subunit or the template binding site of the enzyme. The factor σ is mainly responsible for the promoter search and the regulation of transcription. However, σ contains neither a Zn-atom, nor the strech of Cys-Cys or His-His residues. In fact, a sequence analysis of σ has actually predicted the presence of helix-turn-helix motif in this protein[9].

The amino acid sequence of all the subunits of *E. coli* RNA polymerase has been reported[10–12]. We have carried out a computer search of these sequences for potential Zn-binding sites and looked for the occurrence of Cys-Cys or His-His sequences in close proximity with a spacing of about 10–20 amino acids. In the cases of putative Zn-binding domains of prokaryotic regulatory proteins, a sequence of Cys-Cys ..... Cys-Cys has been observed instead of the Cys-Cys ..... His-His pattern as in the eukaryotic counterpart. Moreover, they do not contain the conserved hydrophobic residues e.g. Tyr, Phe, Leu, as in xenopus TF IIIA, but instead contain acidic residues to form salt bridges with the basic residues[1]. Therefore, we searched for the repetitive occurrence of Cys-Cys residues with basic amino acid residues in the middle to form the tip of the finger. Surprisingly, no such sequence was found in the case of the α and β subunits. However, the β' sequence shows the presence of two such regions, one at the N-terminal from the 68th residue to 88th residue and the other from residue[12] 865 to 898. The second stretch contains very few basic residues, and has a mixture of acidic and hydrophobic amino acids. The N-terminal Cys-rich domain runs as follows; -^{68}YE(C)L(C)-GKYKRLKHRGVI(C)EK(C)^{88}-, which is enriched in basic amino acids. Moreover, the β' -subunit binds one Zn-atom and also it is a DNA binding subunit; therefore it is tempting to propose that the Zn atom is probably co-ordinated with four -Cys-residues folded like a finger giving rise to the tip of the finger with many basic amino acids and a few hydrophobic ones.

A computer search of cysteine-rich sequences in DNA binding proteins has recently been used to predict that such sequences are metal binding sites[13]. Excepting for the periodic occurrence of -Cys-residues, no other consensus is easily discernable[14].

8 March 1988

1. Klug, A. and Rhodes, D., *Trends Biochem. Sci.*, 1987, **12**, 464.
2. Miller, J., McLachlan, A. D. and Klug. A., *EMBO J.*, 1985, **4**, 1609.
3. Rhodes, D. and Klug, A., *Cell*, 1986, **46**, 123.

4. Pabo, C. O. and Sauer, R. T., *Annu. Rev. Biochem.*, 1984, **53,** 293.
5. Anderson, J. E., Ptashne, M. and Harrison, S. C., *Nature (London)*, 1987, **326,** 846.
6. Chatterji, D. and Wu, F. Y. -H., *Biochemistry*, 1982, **21,** 4651.
7. Chatterji, D. and Wu, F. Y. -H., *Biochemistry*, 1982, **21,** 4657.
8. Chatterji, D. Wu, C. -W, and Wu, F. Y. -H., *J. Biol. Chem.*, 1984, **259,** 284.
9. Gribskov, M. and Burgess, R. R., *Nucl. Acid Res.*, 1986, **14,** 6745.
10. Ovchinnikov, Y. A., Lipkin, V. M., Modyanov, N. N., Chertov, O. Y. and Smirnov, Y. V., *FEBS Lett.*, 1977, **76,** 108.
11. Ovchinnikov, Y. A. *et al.*, *Eur. J. Biochem.*, 1981, **116,** 621.
12. Ovchinnikov, Y. A., *et al.*, *Nucl. Acid Res.*, 1982, **10,** 4035.
13. Berg, J. M., *Science*, 1986, **232,** 485.
14. Giedroc, D. P., Keating, K. M., Williams, K. R., Konigsberg, W. H. and Coleman, J. E., *Proc. Natl. Acad. Sci. (USA)*, 1986, **83,** 8452.

ANCIENT PLANT ECONOMY AT CHALCOLITHIC TULJAPUR GARHI, DISTRICT AMRAOTI, MAHARASHTRA

M. D. KAJALE
Archaeology Department, Deccan College, Postgraduate and Research Institute, Pune 411 006, India.

ANCIENT carbonized grains of seventeen wild and cultivated species of plants, some of them unique in the Indian subcontinent, are being reported from Tuljapur Garhi (21°10'N: 77°35'E), a Chalcolithic site in Amraoti district of Maharashtra. This is the only Chalcolithic site known and reported so far in the Vidarbha region of Maharashtra. It was excavated during 1985–86 by Sri B. P. Bopardikar, Superintending Archaeologist, Prehistory Branch of the Archaeological Survey of India. The site revealed 1.5 m thick habitational deposit belonging to the Chalcolithic culture. The entire range of pottery belongs to the wheel made category. Malwa and Jorwe ware are the main pottery assemblages in phase A and B respectively with signs of overlapping (personal communication from the excavator).

The plant remains have been critically investigated under a stereo-binocular microscope (Leitz-Wetzlar). The initial results (table 1) show that out of the assemblage of 17 plant species, 15 are botanically diagnosed whereas two species, in all probability wild, still remain to be determined. Of the 15 diagnosed species, 4 belong to the cultivated cereals, 8 to domesticated pulses, 1 to oil yielding plant and 1 to fibre cum vegetable plant. The remaining one can be assigned to a fodder plant species.

The excavator has furnished the following radiocarbon data on charcoal samples from habitational deposits with a half-life value of 5570 ± 30 years. (1) 2870 ± 100 years B.P. for a sample from Tr. A1 Qd.1, layer (3), depth 35 to 56 cm; (2) 340 ± 90 years B.P. for a sample from Qd.IV, layer (2). This date appears to be erratic; (3) 3310 ± 90 years B.P. for a sample from Tr. A4, Qd.II, layer (1), and (4) 2410 ± 100 years B.P. for a sample from Tr. C1 Qd.I, pit sealed by layer (1).

On the basis of viable radiocarbon dates and typology of the associated cultural materials like pottery and stone tools, it may be inferred that the levels yielding plant remains under study, belong to the first millenium B.C. rather than the late phase of the second millenium B.C. The plant remains are assignable to phase B (Jorwe period), except one sample Tr. A3 Qd.III, layer (3) which may belong to the transitional level between Malwa and Jorwe cultures.

The preliminary interpretation of the present investigations is based on an assumption that the domesticated species of various grains were actually cultivated by the Chalcolithic inhabitants in the vicinity of Tuljapur Garhi. It is clear that the ancient farmers around the site practiced agriculture in two seasons. They cultivated winter crops (wheat, barley, lentil, grass pea, gram) as well as summer (monsoon) crops (rice, great millet, hyacinth bean, horse gram, black gram, green gram). They also probably raised Deccan hemp (Kenaf/Roselle), perhaps for exploitation of fibres and as vegetables as can be conjectured from the ethnographic parallels. In view of the difficulties in separating seeds of these two related species under light microscope, the identification is tentative. Detailed examination of seed coat sculpturing of grains of *Brassica* type and *Hibiscus* sp. under scanning electron microscope will be undertaken for resolving them up to species level of diagnosis.

The presence of seeds of Indian gum arabic (babul) is interesting. This is the second occurrence of the species from any archaeological site in the Deccan. The author has noted seeds of babul in palaeoethnobotanical collections made from Malwa, Early Jorwe and Late Jorwe cultural deposits at Inamgaon (C. 1600–700 B.C.) in Pune district of