7. Bardiya, M. C., Ph.D. thesis, IARI, New Delhi, 1970.
8. Gaur, A. C. and Sachar, S., *Curr. Sci.*, 1980, **49**, 553.
9. Gaur, A. C. and Gaind, S., *Sci. Cult.*, 1983, **49**, 110.

HETEROCHROMATIN OF RYE AND TRITICALE—REPETITIVE SEQUENCES

V. R. K. REDDY
Department of Botany, Bharathiar University, Coimbatore 641 046, India

BANDING techniques have provided valuable information about organizational aspects of chromosomal heterochromatin in rye, wheat and triticale. The chromosomes of the three species show clear structural differences. Rye chromosomes have large blocks of heterochromatin located at the telomeric ends (identifiable by C-banding, figure 1), while in wheat, heterochromatin is present in intercalary positions.

The molecular structure of heterochromatin in rye and wheat has been studied[1-3]. The studies revealed that the DNA density in heterochromatic regions[4] is 1.5 to 4 times[5] higher than that in euchromatic regions. Heterochromatin composed of late-replicating repetitive DNA sequences[6] has a direct relationship with it[7]. The proportions of single-copy and repetitive DNA in wheat, rye and triticale are presented in table 1. Kinetic analysis (renaturation of denatured DNA) of genome organization in rye and wheat has revealed that repeated sequences are interspersed with unique sequences. The repetitive sequences of heterochromatin of rye and wheat are classified into three subfractions: (i) low repeat (consisting of about ten copies), (ii) intermediate repeat (a hundred to a few thousand copies), and (iii) high repeat (over a million copies). It has also been demonstrated that most of the highly repeated sequences represent constitutive heterochromatin[8,9] and are species-specific[10,11].

The repetitive DNA makes up about 50% of the total DNA in triticale; the highly repeated fraction contains two families, of 3×10^7 and 5×10^6 repeats, which constitute 8.3 and 2.4% of the genome. In rye the repetitive DNA makes up 75–90% of the total DNA and is grouped into four repetitive DNA fami-

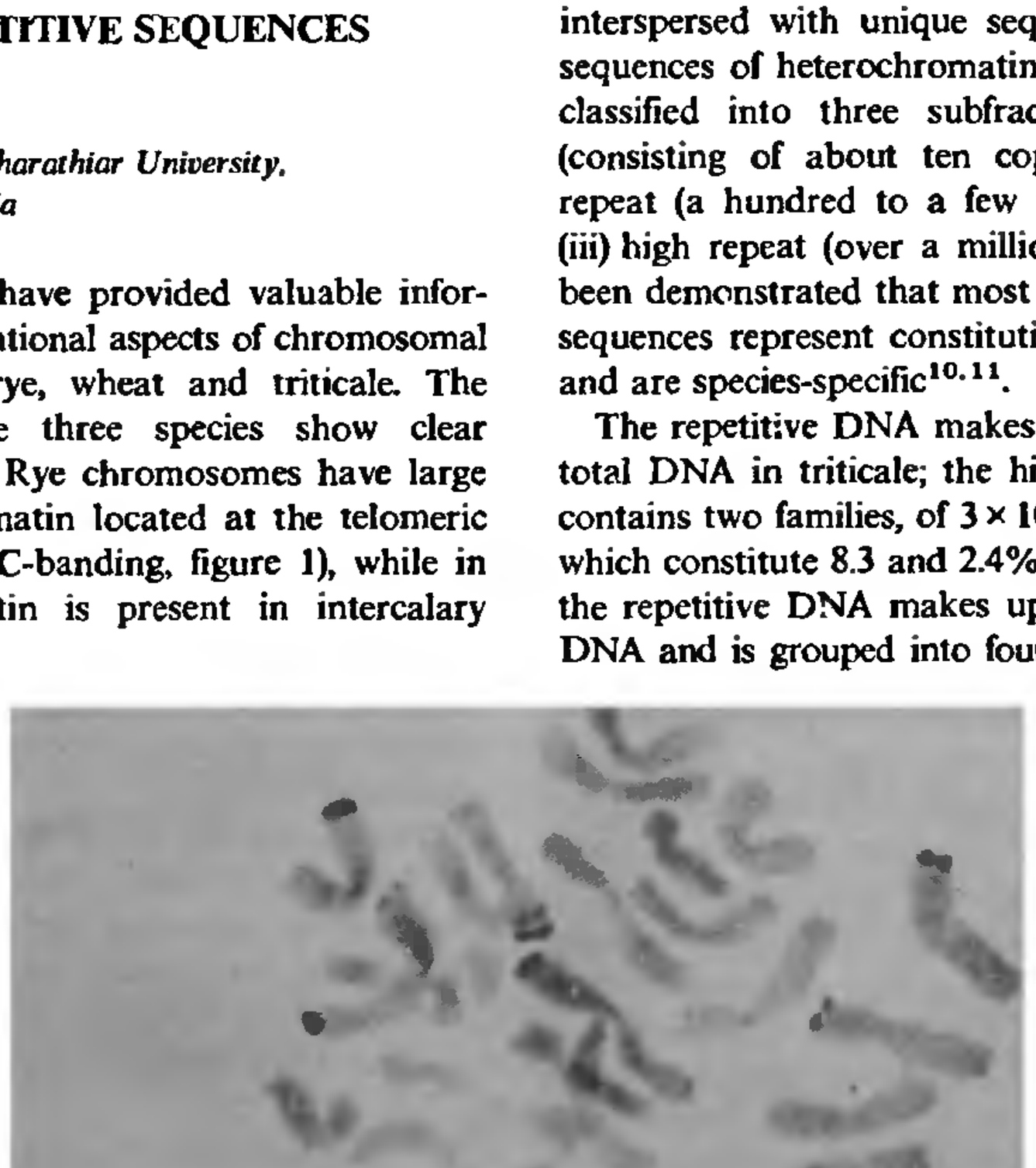

Figure 1. C-banding of rye chromosomes, showing heterochromatin at the telomeric ends.

Table 1 *Repetitive sequence organization pattern in wheat, rye and triticale*

Sequence	Wheat	Rye	Triticale
Short non-repeats (single-copy sequences) interspersed with short repeated sequences (%)	30–40	25–35	45–55
Interspersed repeats or tandem repeats (%)	60–75	65–75	45–55
Long non-repeats (%)	2	5	7

lies[6,12]. Two repetitive families were confined to telomeric heterochromatin identifiable by Giemsa C-banding, while the remaining two are present in constitutive heterochromatin. Of these four, three sequences were rye-specific (480 base pairs, 610 bp and 630 bp) and one (120 bp) was common to rye and wheat genomes[7]. All the four families have been identified and studied in different species related to rye (*Secale cereale* (rye), *S. montanum*, *S. vavilovii*, *S. silvestre*, *S. africanam* and *S. iranicum*). Each species was unique with respect to the chromosomal distribution and amount of the repeated sequence families. Species other than *S. cereale* have a lower percentage of repeated sequences and heterochromatin. In most of the families the repeated sequences are similar, as indicated by restriction analysis using *Eco*RI, *Hin*dIII and *Hae*III. As many as 12 different DNA sequences specific to rye chromosomes have been characterized and described[13]. *In situ* hybridization with a cDNA probe demonstrated that the highly repetitive DNA sequence common to both rye and wheat genomes is distributed either on B chromosomes or on 4A and 7A (ref. 14) chromosomes. Recently, Metzlaff *et al.*[15] discovered wheat-specific repetitive DNA sequences (pTa 1, pTa 2, pTa 7, pTa 8) that are GC-rich, as against rye repetitive sequences that are AT-rich[9]. In triticale these sequences were shown to affect chromosome movement[16] and pairing[8], which consequently influence meiotic stability. Selective deletion of some of the repetitive sequence families specific to rye has been shown to improve meiotic stability in the triticale cultivar Rosner[7], thereby indicating that the heterochromatin of rye chromosomes in triticale does undergo alterations.

A study of DNA and heterochromatin content within the genus *Secale* indicated that the amount of heterochromatin increased during evolution[4]. Flavell *et al.*[17] showed that the magnitude of the DNA/heterochromatin differences in the repeated sequences of various species is related to the extent of evolutionary divergence between the species. Further, the amount of telomeric heterochromatin in *S. cereale* varies between different populations, between different individuals within a population and even between different cells in a single root-tip[18]. It was hypothesized that these variations in repetitive DNA originate by the sudden disproportionate replication (saltatory replication) of specific nucleotide sequences in heterochromatin. These were regarded as the major event in the evolution of a species. Variation in these sequences occurs by mutations, deletions, translocations and rearrangements[19]. Chromosomal DNA content is therefore maintained by a balance between sequence amplification and deletion (termed 'turning over')[18] of the genome.

Unequal chromatid exchanges are believed to have been a source of quantitative change in an array of repeated DNA sequences during evolution[20]. Unequal meiotic exchange within the repeated sequences near the euchromatin–heterochromatin junction may be a source of gradual quantitative variation in the repeated sequences[21]. The B chromosomes present in many species of rye have been considered to be another source of variation in the heterochromatin of normal chromosomes, through translocation events, incorporating segments from the B chromosomes. Special cases such as transposable elements, gene conversion and aberrations in mitotic divisions[22] are some of the rare events that are also responsible for changes in the genome.

Thanks are due to Dr P. K. Gupta, Department of Agricultural Botany, Meerut University, Meerut, for help in improving the manuscript and, ICAR, New Delhi, for financial help.

19 May 1988; Revised 27 January 1989

1. Ranjekar, P. K., Lafontaine, J. G. and Pallotta, D., *Chromosoma*, 1974, **48**, 427.
2. Bendich, A. F. and Anderson, R. S., *Biochemistry*, 1977, **16**, 4655.
3. Smith, G. P. and Flavell, R. B., *Biochem. Biophys. Acta*, 1977, **474**, 82.
4. Bennett, M. D., Gustafson, J. P. and Smith, J. B., *Chromosoma*, 1977, **61**, 149.
5. Lukaszewski, A. J., Wozna, J., Apolinarska, B. and Naganowska, B., *Theor. Appl. Genet.*, 1982, **62**, 145.
6. Bedbrook, J. R., O'Dell, M. and Flavell, R. B., *Nature* (*London*), 1980, **288**, 133.
7. Jones, J. D. G. and Flavell, R. B., *Chromosoma*, 1982, **86**, 595.

8. Appels, R., Driscoll, C. and Peacock, W. J., *Chromosoma*, 1978, **70**, 67.
9. Gerlach, W. L. and Peacock, W. J., *Heredity*, 1980, **44**, 269.
10. Appels, R., *Int. Rev. Cytol.*, 1982, **80**, 93.
11. Sanchez, D. J. F. and Meyer, W. A. O., *Can. J. Bot.*, 1978, **56**, 1291.
12. Flavell, R. B., Rimpau, J. and Smith, D., *Chromosoma*, 1977, **63**, 205.
13. Hutchinson, J., Chapman, V. and Miller, T. E., *Heredity*, 1980, **45**, 245.
14. Dennis, E. S., Gerlach, W. L. and Peacock, W. J., *Heredity*, 1980, **44**, 349.
15. Metzlaff, M., Troebner, W., Baldauf, F., Schlegel, R. and Cullum, J., *Theor. Appl. Genet.*, 1986, **72**, 207.
16. Bennett, M. D., In: *Plant Biosystematics*, (ed.) R. K. Vickery, Academic Press, Toronto, Canada, 1984.
17. Flavell, R. B., O'Dell, M. and Smith, D., *Heredity*, 1979, **42**, 309.
18. Britten, R. J. and Kohne, D. E., *Science*, 1968, **161**, 529.
19. Vedel, F. and Delseny, M., *Plant Physiol. Biochem.*, 1987, **25**, 191.
20. Smith, G. P., *Science*, 1976, **191**, 528.
21. Appels, R. and Peacock, W. J., *Int. Rev. Cytol.*, 1978, **8**, 69.
22. Egel, R., *Nature* (*London*), 1981, **290**, 191.

MULTIPLE FORMS OF HUMAN URINARY γ-GLUTAMYLTRANSPEPTIDASE

K. BASHEER AHAMAD, S. B. HINCHIGERI and K. S. DATTA*
Department of Chemistry, Karnatak University, Dharwad 580 003, India
**The Karnatak Cancer Therapy and Research Institute, Navanagar, Hubli 580 025, India*

OF several enzymes excreted in human urine, γ-glutamyltranspeptidase (GGTP) is considered as one of the important enzyme markers that can be used to assess the uro-renal system. Among different body fluids, urine was found to be a rich source of the transpeptidase. In cancer patients, though urinary GGTP activity was found to be increased, there was no significant change in serum transpeptidase activity. The work described here is part of a study of transpeptidase isozymes in urine of normal individuals and cancer patients.

Urine samples collected from normal and diseased individuals were dialysed extensively against 10 mM Tris–HCl buffer, pH 8.0, for about 24 h. The dialysed urine samples were centrifuged at 3,500 rpm for 15 min, and supernatants were freeze-dried for chromatographic and electrophoretic experiments.

A known aliquot of a solution of desalted concentrated urine samples in 10 mM Tris–HCl buffer, pH 8.0, was passed through a DEAE-cellulose column pre-equilibrated with the sample buffer. The column was developed with the sample loading buffer. The unbound protein fraction contained transpeptidase activity, and was called unbound isozyme. After washing the column with 2–3 bed volumes of the sample buffer, bound transpeptidase activity was desorbed into two peaks, bound-I and bound-II, by applying a linear salt gradient (0 to ,0.5 M NaCl) in the buffer. The enzymatically active fractions were dialysed and concentrated for polyacrylamide disc gel electrophoresis (PAGE)[1]. The concentrated samples were suspended in 1% Triton X-100 and electrophoresed in 7.5% acrylamide gels containing 0.01% Triton X-100. After the electrophoresis, transpeptidase activity was localized by the method of Selvaraj and Balasubramanian[2]. Each of the three enzymatically active fractions yielded one activity band.

Gel filtration chromatography of the desalted freeze-dried samples through Sephadex G-200 gave two enzymatically active peaks. Electrophoretic (PAGE) analysis of the first enzymatically active peak (high mol. wt form) showed one transpeptidase isozyme while that of the second (low mol. wt form(s)) peak gave two enzymatically active bands.

Direct electrophoresis of the desalted concentrated urine samples in 1% Triton X-100 gave three enzymatically active bands in the zymogram (figure 1). The pattern of transpeptidase isozyme distribution in the electrophoretogram was observed to be comparable to that of detergent-solubilized human kidney transpeptidase[3] preparation.

Rambabu *et al.*[4] showed the existence of the two transpeptidase isozymes and that the transpeptidase activity is increased in urine of leukaemia patients. But we have detected three transpeptidase isozymes in human urine. Grossly speaking, we also observed an increased level of the total transpeptidase activity in urine of leukaemia patients, but detailed investigations on several leukaemia patients have indicated that the activity of the unbound transpeptidase