

# Emergence of the *ZNF91* Krüppel-associated box-containing zinc finger gene family in the last common ancestor of Anthroidea

(zinc finger proteins/gene amplification/primate evolution/gene clusters)

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**ABSTRACT** The *ZNF91* gene family, a subset of the Krüppel-associated box (KRAB)-containing group of zinc finger genes, comprises more than 40 loci; most reside on human chromosome 19p12–p13.1. We have examined the emergence and evolutionary conservation of the *ZNF91* family. *ZNF91* family members were detected in all species of great apes, gibbons, Old World monkeys, and New World monkeys examined but were not found in prosimians or rodents. In each species containing the *ZNF91* family, the genes were clustered at one major site, on the chromosome(s) syntenic to human chromosome 19. To identify a putative “founder” gene, >20 murine KRAB-containing zinc finger protein (ZFP) cDNAs were randomly cloned, but none showed sequence similarity to the *ZNF91* genes. These observations suggest that the *ZNF91* gene cluster is a derived character specific to Anthroidea, resulting from a duplication and amplification event some 55 million years ago in the common ancestor of simians. Although the *ZNF91* gene cluster is present in all simian species, the sequences of the human *ZNF91* gene that confer DNA-binding specificity were conserved only in great apes, suggesting that there is not a high selective pressure to maintain the DNA targets of these proteins during evolution.

The zinc finger nucleic-acid binding motif (Cys<sub>2</sub>–His<sub>2</sub>) occurs in hundreds to thousands of vertebrate proteins and is traceable throughout eukaryotes (1). Although structural features common to various subsets, including the 7-amino acid “H/C link” between adjacent fingers (2, 3), and various conserved N-terminal modules (4–8) have been described, functions are known only for the few zinc finger proteins (ZFPs) that act as transcription regulators in differentiation and development; the functions of most mammalian ZFPs are unknown (refs. 9–15; reviewed in ref. 16).

Several related ZFP genes in human and *Xenopus* are arranged in clusters (17–21). In humans, the *ZNF91* family, containing >40 highly related Krüppel-associated box (KRAB)-containing ZFP genes or pseudogenes (KRAB-ZFP), has recently been identified on chromosome 19p12–p13.1 (22). *ZNF91* family members share a common exon-intron organization, with all zinc finger repeats in a single exon and the KRAB domain encoded by two separate exons. The linker region between the N-terminal KRAB domain and the first zinc finger is conserved in length and sequence. *ZNF91* family members are transcribed in many tissues but most abundantly in T-lymphoid cells.

Here we have studied the emergence and evolutionary conservation of the *ZNF91* family in search of clues to its function. We sought homologous genes in rodents and in a

panel of primate species. We also attempted to clone orthologs of the *ZNF91* gene by PCR with primers corresponding to the zinc finger amino acids determining DNA-binding specificity.\*\*

## MATERIALS AND METHODS

**Genomic DNAs.** Genomic DNAs were isolated as described (23) from the following cell lines or tissue samples: human genomic DNA from HeLa cells; *Pan troglodytes* (chimpanzee), *Gorilla gorilla* (gorilla), and *Pongo pygmaeus* (orangutan) DNA from lymphoblastoid cell lines provided by J. Kidd (New Haven, CT), *Hylobates syndactylus* (gibbon) DNA from the lymphoblastoid cell line provided by J. Wienberg (Munich); genomic DNAs from *Lemur fulvus fulvus* (brown lemur), *Propithecus verreauxi* (sifaka), *Loris tardigradus* (slender loris), *Galago moholi* (lesser bushbaby), and *Tarsius syrichta* (Philippine tarsier) from tissue samples from Duke University Primate Center, (Durham, NC); genomic DNAs from *Nasalis larvalis* (proboscis monkey), *Presbytis entellus* (Indian langur), and *Cebus apella* (capuchin) from peripheral blood lymphocyte samples provided by J. Powell (New Haven, CT). *Symphalangus syndactylus* (siamang), *Theropithecus gelada* (gelada baboon), *Colobus polykomos* (colobus monkey), *Erythrocebus patas* (patas monkey), and *Aotus trivirgatus* (owl monkey) DNAs were a gift from S. J. O'Brien (National Institutes of Health, Bethesda). *Mandrillus sphinx* (mandrill) and *Saimiri boliviensis* (squirrel monkey) DNAs were a gift from J. Rodgers (San Antonio, TX). *Callithrix jacchus* (marmoset) DNA was a gift from G. Miller (New Haven, CT).

**RNA Isolation and Reverse Transcription-PCR.** Total RNA was extracted by the guanidinium isothiocyanate method (24) and was purified by centrifugation through cesium chloride. Single-stranded cDNA was prepared with oligo(dT) primers and the cDNA cycle kit (Invitrogen). Twenty-five nanograms of the cDNA mixture was diluted in 50  $\mu$ l of PCR mixture and amplified as described below.

**PCR.** PCR primers and cycling conditions are described in the figure legends. PCRs were performed in 100- $\mu$ l reaction mixtures containing 0.2–1  $\mu$ g of genomic DNA, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP, 0.5  $\mu$ M primers, and 2.5 units of *Taq* poly-

Abbreviations: ZFP, zinc finger protein; KRAB, Krüppel-associated box; YAC, yeast artificial chromosome.

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\*\*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U29495–29514 and U29524–U29527).

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merase (Perkin-Elmer/Cetus or Promega) using the supplier's buffer.

**Cloning and Sequencing of PCR Products.** PCR products were made blunt-ended with Klenow enzyme and cloned into the pGEM3 (Promega) vector. Alternatively, PCR products were cloned in the TA vector from Invitrogen. Inserts were sequenced with the Sequenase kit (United States Biochemical). Sequence analysis utilized the software package of the University of Wisconsin Genetics Computer Group (Madison, WI).

**Nucleic Acid Hybridization.** Southern blots were done exactly as described using Biotrans membrane (ICN) (22). Final washes were at 50°C in 2× SSC/0.1% SDS. cDNA probes were <sup>32</sup>P-labeled using the Prime-It kit (Stratagene). Oligonucleotides were <sup>32</sup>P-end-labeled with [<sup>32</sup>P]ATP and T4 polynucleotide kinase. Membranes were exposed to Kodak XRP-5 films at -70°C for 2–24 h.

**In Situ Hybridization.** Metaphase chromosome spreads were prepared by standard methods (25). Human chromosomes were obtained from peripheral blood lymphocytes; great ape and gibbon chromosomes were from lymphoblastoid cell lines; *Tarsius* and *Galago* chromosomes were from fibroblasts cultured from biopsies obtained from the Duke University Primate Center (Durham, NC). The chromosome-specific library pBS19 (27) and DNA from the yeast artificial chromosome (YAC) clones 71D9, 179B7, 245F12, 143B11, 414H7, 60D10, 138D1, 332C9, 158C9, 335H4, 84A10, 131F11, 88C4, 96A1, 140G4, 143B6, 143D6, and 16B10 of the CEPH library, encompassing the ZNF91 KRAB-ZFP gene cluster on chromosome 19p12–p13.1, was labeled with either biotin or digoxigenin by standard nick-translation methods. *In situ* suppression hybridization, detection, counterstaining, microscopy, image processing, and photography were done as described (22). The programs used for image capture (CCD IMAGE CAPTURE) and merging and pseudocoloring (GENE JOIN MAX PIX), written by T. Rand, are available for a fee from the Office of Cooperative Research at Yale University.

## RESULTS

**Appearance of KRAB-ZFP Genes of the ZNF91 Family in Higher Primates.** The ZNF91 KRAB-ZFP genes have been identified in humans but are undetectable by hybridization in mouse, rat, and hamster genomic DNAs, suggesting that the ZNF91 gene family evolved later than Rodentia (22). To further investigate the emergence time of this KRAB gene subfamily, we screened a panel of primate genomic DNAs by PCR. Since the sequence of the link region between the KRAB box and the first finger domain is specific to KRAB-ZFP genes of the ZNF91 family, we designed degenerate primers to the most conserved regions of this ZNF91-specific element. PCR products were electrophoresed, blotted, and hybridized with a <sup>32</sup>P-labeled ZNF91 cDNA spacer probe to confirm the identity of the amplified DNA. PCR products of the expected size (250 bp) were observed in all ape, New World monkey, and Old World monkey samples (Fig. 1), but none was detected in prosimian species (tarsier, loris, galago, lemur, and sifaka). To test the efficiency of the PCR amplifications, we used degenerate primers directed against the more widely conserved KRAB domain and hybridized the PCR products with a ZNF91 KRAB oligonucleotide probe. The expected 91-bp PCR products were observed in all species examined, including prosimians and rodents. We estimated the number of ZNF91-related sequences by slot-blot analysis of primates' genomic DNA using the ZNF91 spacer probe, with a c-myc probe as loading control (data not shown). ZNF-related genes in all simians show copy numbers similar to humans' (Fig. 1B): 25–35 per haploid genome (as estimated by comparing signal intensity to serial dilutions of ZNF91 DNA).

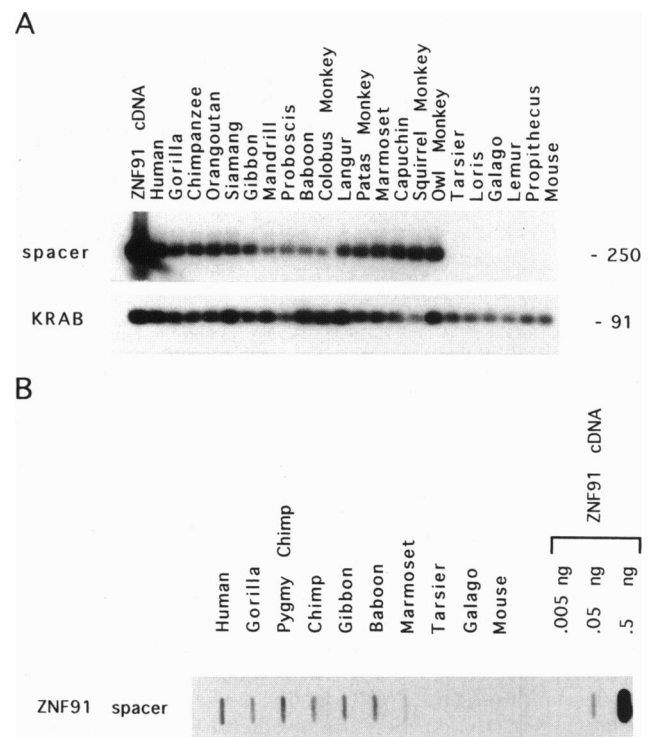


FIG. 1. Detection by PCR and dot blot analysis of ZNF91 homologous genes in primates. (A) PCR products separated by electrophoresis and analyzed by Southern blotting. PCR conditions: 94°C for 3 min, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C. The last cycle was followed by a final elongation step (72°C for 10 min). Coordinates (22) and sequences of the degenerate primers: (Upper) nt 359–386, 5'-ATT TT(G/C) C(C/T)C (A/G)AG A(T/C)(T/C) TTT G(G/T)C C(A/G)G AGC-3'; nt 585–609, 5'-TGT CT(G/A) TTT GAA TTT (G/A)AA A(T/A)T (T/A)TA T-3'; nt 215–234, 5'-(C/T)T(G/A) (T/A)AG TTC TC(C/T) A(G/A)C AT(C/T) AC-3'. The KRAB and spacer probe were ZNF91 cDNA, nt 168–188 and 359–609, respectively. (B) Dot blot analysis of 2 µg of genomic DNA from each species and serial dilutions of ZNF91 cDNA using a ZNF91 spacer probe (nt 372–693). Membranes were exposed to autoradiographic films for 4 h.

We explored the chromosomal organization of the primate ZNF91 genes by double-label fluorescence *in situ* hybridization, hybridizing simultaneously a human chromosome 19-specific library (to "paint" regions syntenic to human chromosome 19), and a pool of YAC clones containing the human ZNF91 cluster from chromosome 19p12–p13.1 (Fig. 2). In human, orangutan, and chimpanzee, the chromosome 19 and the YAC probes cohybridized and labeled only one chromosome (PTR20 and PPY20) (28). In gibbon (*H. syndactylus*), the human chromosome 19 library painted two chromosome pairs, HL13 and HL17 (26), and the YAC probe gave a unique signal on chromosome HL13. In galago (*Galago crassicaudatus*) and tarsier (*T. syrichta*), the human chromosome 19 library painted part of one, as yet unidentified, chromosome, but the 19 synteny region had no ZNF91 signal, in agreement with our PCR and hybridization analysis (see above). However, multiple ZNF91 signals were detected on the telomeric regions of other chromosomes; these might represent cross-hybridization with other ZFP-enriched loci or other conserved sequences represented within the pool of YAC DNA.

In humans, the ZNF91 gene family represents about one-third of the KRAB-ZFP genes and is expressed predominantly in T-lymphoid cells (22). To identify the founder gene(s) of the ZNF91 family, which might have had insufficient sequence similarity in the spacer region to be detected, we performed PCR on cDNA from a mouse T-cell line (DA-2), using primers in the conserved KRAB domain (element A) and in the H/C



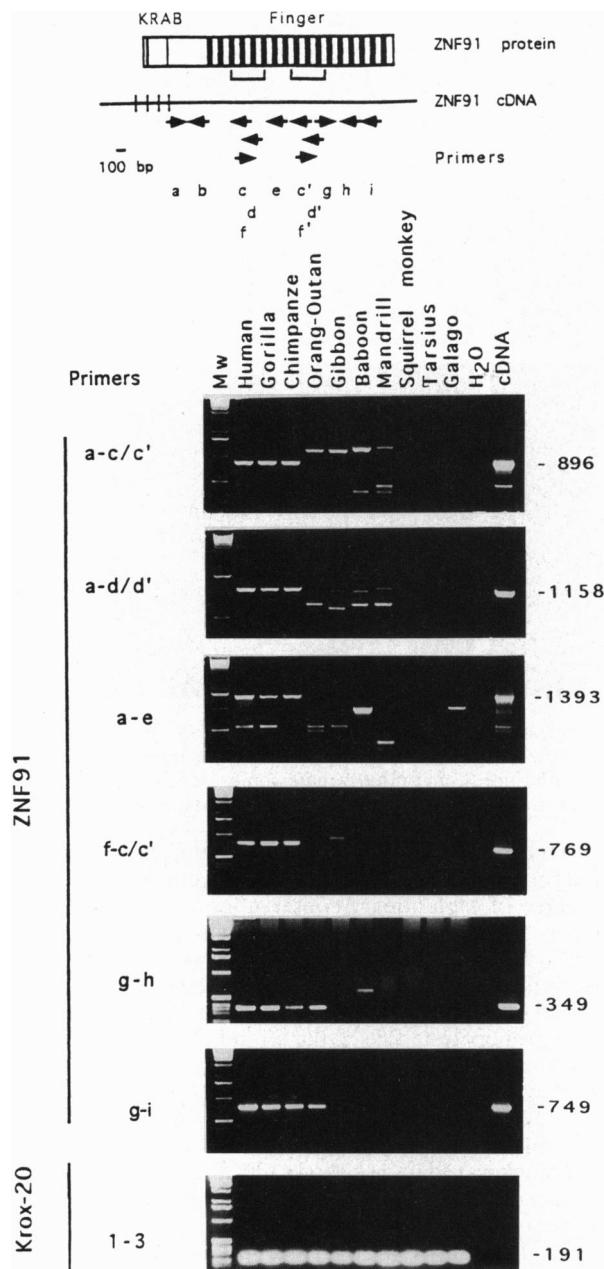


Fig. 4. PCR analysis of orthologous *ZNF91* genes in primates. Electrophoretic separation of PCR products: PCR conditions were the same as in Fig. 2, except for primer pairs a-c/c' (annealing, 58°C; elongation, 1 min), a-d/d' (annealing, 56°C; elongation, 1 min 30 sec), a-e (annealing, 52°C; elongation, 1 min), g-i (annealing, 56°C; elongation, 1 min). Coordinates and sequences (22) of the *ZNF91* primers: A (nt 373-396), 5'-CTTT TGG CCA GAG CAG AGC ATG TA-3'; B (nt 666-692), 5'-GTT GGG TTT TGT GTA AAC GGA TGC AA-3'; c (nt 1244-1269 and 2168-2193), 5'-GTT GAG AGT CGC TTA AAA GTT TTG TC-3'; D (nt 1508-1531 and 2432-2455), 5'-TCT AGT TAG GGT TGA AGA CCA TAT-3'; E (nt 1743-1766), 5'-TGT AGA AAG ACT TGA GGA ATG ATT-3'; F (nt 1424-1447 and 2248-2271), 5'-AAC TGG TCC TCA AGC CTT ACT AAA-3'; G (nt 2526-2539), 5'-AGC CGT TCC TCA ACC CTT ACT AAG-3'; H (nt 2852-2875), 5'-TGT AGT AAG GTG TGA AGG CTG GCT-3'; I (nt 3272-3275), 5'-TCC ATT TAG GGT TGA GGA TGA TAT-3'. Coordinates and sequences (30) of the human *EGR2* (Krox-20) primers: nt 1107-1130, 5'-TCC CGC TCT GAC GAG CTG ACA CGG-3' (finger 1); nt 1275-1298, 5'-GCG CTT CCT CTC ATC ACT CCG GGC-3' (finger 3).

chin orthologs encode proteins with only 75-78% sequence identity (Fig. 5).

Since the best criterion for orthology among ZFPs may be the conservation of the amino acids preceding the histidine residues conferring DNA-binding specificity (31), we next chose primers from the regions preceding the histidine residues of different units of the *ZNF91* finger domain (primers b-i, not primer a). Primers c, d, and f are present twice in the *ZNF91* cDNA sequence because it contains two groups of nearly identical tandemly repeated finger units (fingers 6-12 and 17-23). These primer pairs amplified products of the expected size only in humans and great apes (Fig. 4). With primer pairs a-c/c', a-e, and f-c/c', PCR products of the expected size could not be detected in orangutan DNA. With the primer pairs used for the *ZNF85* gene, another member of the *ZNF91* family, PCR products were also detected only in human and great apes (data not shown). This suggests that the conservation of the DNA-binding specificity of *ZNF91* genes is not under a high selective pressure.

## DISCUSSION

The human *ZNF91* KRAB-ZFP family contains  $\approx 40$  highly related genes, most of which are clustered on chromosome region 19p12-p13.1 and are highly expressed in T-lymphoid cells (22). Our present studies reveal that the *ZNF91* family emerged after separation of the lineage Anthropeoidea and that in the species containing the *ZNF91* family, the *ZNF91*-related genes cluster at one major site on the chromosomes syntenic to human chromosome 19. The presence and apparently similar copy number of the *ZNF91* gene family in all simian primates suggest that the duplication events producing this gene family occurred early in simian primate evolution, before the catarrhine primates (human, apes, and Old World monkeys) diverged from platyrrhines (New World monkeys). This correlates with the nucleotide divergence (26/258 nt) observed between the spacer-region sequences of the putative *ZNF91* ortholog genes from human and capuchin, the most distantly related species in which orthology has been identified. Using a rate of nucleotide substitution of  $1.56 \times 10^{-9}$  per site per year (32), the *ZNF91* gene is estimated to have appeared some 64 million years ago. *Tarsius*, whose relationship to other primates has been much disputed (33, 34), has no detectable *ZNF91* genes. The apparent absence of the *ZNF91* subfamily of KRAB-ZFP genes in rodents and strepsirrhine prosimians (lemur, loris, galago, and sifaka) suggests that the mammalian common ancestor lacked this subfamily; its presence in anthropoids but not in *Tarsius* suggests that it arose in the most recent common ancestor of Anthropeoidea.

The *ZNF91* family, with its recent origin, contrasts sharply with the conserved ZFPs that serve as developmental or differentiation-regulating transcription factors (16, 35-37). These ZFPs tightly conserve their finger domains (particularly the residues that determine binding specificity), whereas *ZNF91* finger domains are poorly conserved. PCR primers from DNA-binding domains failed to identify *ZNF91* orthologs in prosimians, Old World monkeys, or New World monkeys. Since *ZNF91* DNA-binding domains diverge even among primates, their target sequences may also be divergent. In both *Drosophila* (38) and *Xenopus* (39), ZFPs that interact with rapidly evolving, species-specific repetitive or transposable nucleic acid sequences have been described.

Genes encoding proteins important in the control of embryonic development are highly conserved, as exemplified by the homeobox genes (40). The recent evolutionary appearance of the *ZNF91* family suggests that its ZFPs do not regulate fundamental developmental processes. What then might be the function of genes of the *ZNF91* family? Although many possibilities exist, it is interesting to note that several ZFPs [i.e., su(Hw), Suvar] may participate in formation of chromatin structure (41). An examination of the intracellular distribution

Human KVLLLRKYEKCGHENLQLRKGCKSVDECKVHKEGYNKLNQCLTTAQSQKVFQCGKYLKVFYKFLNSNRHTIRHTGKKCFKCKKCVKSF  
 Chimpanzee -----  
 O. Outan -----  
 Baboon --I--RF--Y-P-----Y-----N-----AY--F-----SD-----LF-----K-----E--SS-G-----P-  
 Capuchin ----R-K-----YR-----N-----GH--F-----RSE-----F-----K-----E--SS-G-----FP-

FIG. 5. Sequence alignment of the spacer fragments obtained from the *ZNF91* orthologous genes of different primates. Dashes represent amino acid residues identical to the human *ZNF91* sequence.

of the *ZNF91* family proteins across the cell cycle might illuminate their potential biological function(s).

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1. Bellefroid, E. J., Lecocq, P. J., Benhida, A., Poncelet, D. A., Belayew, A. & Martial, J. A. (1989) *DNA* **8**, 377–387.
2. Rosenberg, U. B., Schröder, C., Preiss, A., Kienlin, A., Cote, S., Riede, I. & Jäckle, H. (1986) *Nature (London)* **319**, 336–339.
3. Schuh, R., Aicher, W., Gaul, J., Cote, S., Preiss, A., Maier, D., Seifert, E., Nauber, U., Schroder, C., Kemler, R. & Jackle, H. (1986) *Cell* **47**, 1025–1032.
4. Bellefroid, E. J., Poncelet, D. A., Lecocq, P. J., Revelant, O. & Martial, J. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3608–3612.
5. Rosati, M., Marino, M., Franze, A., Tramontano, A. & Grimaldi, G. (1991) *Nucleic Acids Res.* **19**, 5661–5667.
6. Knöchel, W., Pötting, A., Koster, M., El-Baradi, T., Nietfeld, W., Bouwmeester, T. & Pieler, T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6097–6100.
7. Klocke, B., Köster, M., Hille, S., Bouwmeester, T., Böhm, S., Pieler, T. & Knöchel, W. (1993) *Nucleic Acids Res.* **21**, 3767–3775.
8. Numoto, M., Niwa, O., Kaplan, J., Wong, K. K., Merrel, K., Yanagihara, K. & Calame, K. (1993) *Nucleic Acids Res.* **21**, 3767–3775.
9. Schneider-Maunoury, S., Topilko, P., Seitanidou, T., Levi, G., Cohen-Tannoudji, M., Pournin, S., Babinet, C. & Charnay, P. (1993) *Cell* **75**, 1199–1214.
10. Kinzler, K. W., Ruppert, J. M., Bigner, S. H. & Vogelstein, B. (1988) *Nature (London)* **332**, 371–374.
11. Schimmang, T., Lemaistre, M., Vorkamp, A. & Rüther, U. (1992) *Development (Cambridge, U.K.)* **116**, 799–804.
12. Kreidberg, J., Sariola, H., Loring, J., Maeda, M., Pelletier, J., Housman, D. & Jaenisch, R. (1993) *Cell* **74**, 679–691.
13. Morishita, K., Parker, D. S., Mucenski, M. L., Jenkins, N. A., Copeland, N. G. & Ihle, J. N. (1988) *Cell* **54**, 831–840.
14. Kreider, B. L., Orkin, S. T. & Ihle, J. N. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6454–6458.
15. Wimmer, E. A., Jäckle, H., Pfeife, C. & Cohen, S. M. (1993) *Nature (London)* **366**, 690–694.
16. Pieler, T. & Bellefroid, E. J. (1994) *Mol. Biol. Rep.* **20**, 1–8.

17. Huebner, K., Druck, T., Croce, C. M. & Thiesen, H.-J. (1991) *Am. J. Hum. Genet.* **48**, 726–740.
18. Hoovers, J. M. N., Mannens, M., John, R., Blik, J., Van Heyningen, V., Porteous, D. J., Leschot, N. J., Westerveld, A. & Little, P. F. R. (1992) *Genomics* **12**, 254–263.
19. Lichter, P., Bray, P., Ried, T., Dawid, I. B. & Ward, D. C. (1992) *Genomics* **13**, 999–1007.
20. Rousseau-Merck, M. F., Tunnacliffe, A., Berger, R., Ponder, B. A. J. & Thiesen, H. J. (1992) *Genomics* **13**, 845–848.
21. Nietfeld, W., Conrad, S., van Wijk, I., Giltay, R., Bouwmeester, T., Knöchel, W. & Pieler, T. (1993) *J. Mol. Biol.* **230**, 400–412.
22. Bellefroid, E. J., Marine, J.-C., Ried, T., Lecocq, P. J., Rivière, M., Amemiya, C., Poncelet, D. A., Coulie, P. G., de Jong, P., Szpirer, C., Ward, D. C. & Martial, J. A. (1993) *EMBO J.* **12**, 1363–1374.
23. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
24. Chirgwin, J., Przybyla, R., MacDonald, R. & Rutter, W. (1979) *Biochemistry* **18**, 5294–5299.
25. Lichter, P., Tang, C. C., Call, K., Hermanson, G., Evans, G. A., Housman, D. & Ward, D. C. (1990) *Science* **247**, 64–69.
26. Koehler, U., Arnold, N., Weinberg, J., Tofaneli, S. & Stanyon, R. (1995) *Am. J. Phys. Anthropol.* **97**, 37–47.
27. Collins, C., Kuo, W., Seagraves, R., Fuscoe, J., Pinkel, D. & Gray, J. W. (1991) *Genomics* **11**, 997–1006.
28. Jauch, A., Weinberg, J., Stanyon, R., Arnold, N., Tofaneli, S., Ishida, T. & Cremer, T. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 8611–8615.
29. Ihle, J. N., Smith-White, B., Sisson, B., Parker, D., Blair, D. G., Schultz, A., Kozak, C., Lunsford, R. D., Adkew, D., Weinstein, Y. & Isfort, R. J. (1989) *J. Virol.* **63**, 2959–2966.
30. Joseph, L. J., Le Beau, M. M., Jamieson, G. A., Acharya, S., Shows, T. B., Rowly, J. D. & Sukhatme, V. P. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7164–7168.
31. Sommer, R. J., Retzlaff, M., Goerlich, K., Saunder, K. & Tautz, D. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10782–10786.
32. Sakoyama, Y., Hong, K.-J., Byun, S., Hisajima, H., Ueda, S., Yaoita, Y., Hayashida, H., Miyata, T. & Honjo, T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1080–1084.
33. Dutrillaux, B. (1979) *Ann. Genet.* **48**, 251–314.
34. Healy, K. C. (1992) Ph.D. dissertation (Duke Univ., Durham, NC).
35. Wimmer, E. A., Jackle, H., Pfeife, C. & Cohen, S. (1993) *Nature (London)* **366**, 690–694.
36. Zarkower, D. & Hodgkin, J. (1992) *Cell* **70**, 237–249.
37. Garriga, G., Guenther, C. & Horvitz, H. R. (1993) *Genes Dev.* **7**, 2097–2109.
38. Harrison, D. A., Gdula, D. A., Coyne, R. S. & Corces, V. G. (1993) *Genes Dev.* **7**, 1966–1978.
39. Schafer, U., Rausch, O., Bouwmeester, T. & Pieler, T. (1994) *Eur. J. Biochem.* **226**, 567–576.
40. Pendleton, J. W., Nagai, B. K., Murtha, M. T. & Ruddle, F. H. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6300–6304.
41. Reuter, G., Giarre, M., Farah, J., Gansz, J., Spierer, A. & Spierer, P. (1990) *Nature (London)* **344**, 219–223.