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Localisation of the human N-*ras* oncogene to chromosome 1cen - p21 by *in situ* hybridisation

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The N-*ras* gene is a transforming gene isolated from a variety of human tumour cell lines and is a member of a family of related *ras* genes. Somatic cell hybrids have previously shown that the N-*ras* gene is located on chromosome 1. We have confirmed this localisation by *in situ* hybridisation to metaphase preparations of lymphocytes and localised the gene to the region 1cen - p21. A survey has found 47 reported cases of malignancy involving deletions in the short arm of chromosome 1. Fifteen of the 47 involved a deletion in this region.

Key words: chromosomal localisation/chromosome 1/*in situ* hybridisation/N-*ras*/oncogene

Introduction

Many cellular oncogenes have now been mapped in the human genome and it has become apparent that there is a correlation between the position of some of the oncogenes and specific chromosomal breakpoints or deletions found in various cancers (Rowley, 1983). The best studied example of this has been the *c-myc* oncogene on chromosome 8 and its involvement in the reciprocal translocations, found in Burkitt's lymphoma, between chromosome 8 and any one of the chromosomes carrying an immunoglobulin locus (Taub *et al.*, 1982; Dalla-Favera *et al.*, 1982). Other examples include *c-mos* (Neel *et al.*, 1982) and *c-abl* (de Klein *et al.*, 1982) which have been located at, or close to, the breakpoints in 8;21 and 9;22 translocations associated with acute myeloblastic leukaemia and chronic myelogenous leukaemia.

The use of DNA transfection techniques to identify activated transforming genes in human tumours has led to much interest in a family of cellular oncogenes, the *ras* genes which are related to the oncogenes of the Harvey and Kirsten murine sarcoma viruses. The members of this gene family have been identified using either genomic library screening with the viral *ras* oncogenes or DNA transfection techniques. c-Ha-*ras* 1 and 2 are cellular homologues of v-Ha-*ras*, the oncogene carried by Harvey murine sarcoma virus and c-Ki-*ras* 1 and 2 are cellular homologues of v-Ki-*ras*, the oncogenes carried by Kirsten sarcoma virus (Chang *et al.*, 1982). Recently a new *ras* gene, N-*ras*, has been identified by DNA tranfection in 3T3 cells (Marshall *et al.*, 1982a; Hall *et al.*, 1983; Shimizu *et al.*, 1983a, 1983b; Murray *et al.*, 1983) and this gene has no known viral counterpart. N-*ras* was initially isolated from the human cell lines HT1080 (a fibrosarcoma line) (Hall *et al.*, 1983), SK-N-SH (a neuroblastoma line) (Shimizu *et al.*, 1983a, 1983b) and HL60 (a promyelocytic leukaemia line) (Murray *et al.*, 1983) and cross-hybridisation to the other cloned *ras* genes at low stringency showed it to be a member of the *ras* gene family (Hall *et al.*, 1983; Shimizu *et al.* 1983b; Murray *et al.*, 1983).

In situ hybridisation is the most powerful method available for a precise regional localisation of genes, with a resolution down to one or two chromosome bands or 0.5 – 1% of the genome. The technique has been used to map Ig heavy chain genes (Kirsch *et al.*, 1982) and Ig kappa light chain genes (Malcolm *et al.*, 1982) to the regions of chromosomes 14 and 2 involved in specific translocations in Burkitt's lymphoma and to map *c-myc* (Neel *et al.*, 1982; Taub *et al.*, 1982) and *c-mos* (Neel *et al.*, 1982) on chromosome 8. Studies with somatic cell hybrids have placed N-*ras* on chromosome 1 (Hall *et al.*, 1983) but further work with lines containing translocated chromosomes would be necessary to localise the gene more precisely using this technique. Here we report the use of *in situ* hybridisation to locate precisely the position of N-*ras* on chromosome 1.

Results

N-ras specific hybridisation probe

The 4-kb *Bgl*II fragment used as a hybridisation probe was isolated by gel fractionation from a molecular clone pAT 8.8 containing part of the transforming gene from the human sarcoma cell line HT1080 (Marshall *et al.*, 1982a; Hall *et al.*, 1983). The fragment contains no repeated sequences and the *Bgl*II site at the 3′ end has been maintained in all transfectants analysed so far and is therefore either within or very close to the transforming gene.

Mapping by in situ hybridisation

Chromosome spreads banded by 1% Lipsol were photographed prior to hybridisation. After exposure, the same cells were relocated and the grain distribution analysed. This procedure, although time consuming allows unequivocal identification of all chromosomes and avoids any possible observer bias. The grains from 16 cells were plotted as a histogram (see Figure 1) and a concentration of grains on the short arm of chromosome 1 close to the centromere was found (69% of all chromosome 1s were labelled in this region). This confirms the localisation of N-*ras* on chromosome 1 (Hall *et al.*, 1983). The distribution along chromosome 1 in 26 cells was examined carefully (see Figure 2) and the peak falls between the centromere and p21 with the major concentration at p13.

Discussion

We have confirmed the localisation of N-*ras* on chromosome 1 and localised it to the region centromere – p21, probably band p13. The region cen - p21 makes up 0.9% of the genome or 2.7 x 10^7 bp.

Four other members of the *ras* gene family have been localised, although none of them to the precision achieved in this study. The gene for c-Ha-*ras* 1 which has been shown to

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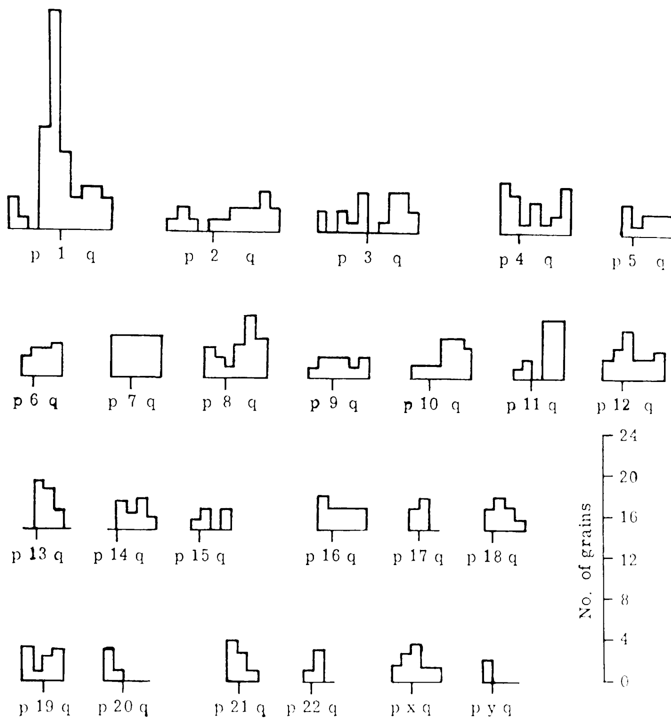

Fig. 1. Distribution of silver grains over metaphase chromosomes, obtained from an analysis of 16 cells. A significant grain accumulation is noted just above the centromere in the short arm of chromosome 1.

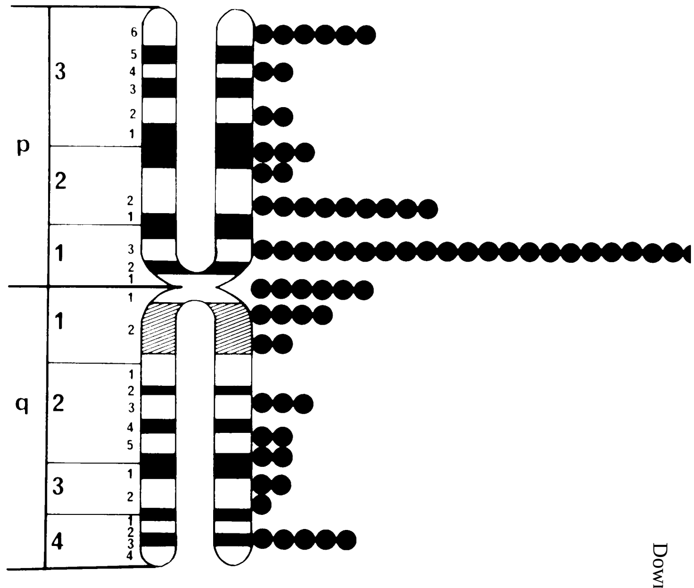

Fig. 2. Diagrammatic representation of chromosome 1 showing the distribution of silver grains. The peak of grains is found between the centromere and p21 with the greatest concentration at band p13. Twenty six cells were analysed.

be the transforming gene in the EJ/T24 human bladder carcinoma (McBride *et al.*, 1982), and the HS242 lung carcinoma (Yuasa *et al.*, 1983) has been mapped to chromosome 11 by somatic cell hybrids (O'Brien *et al.*, 1982; de Martinville *et al.*, 1983; McBride *et al.*, 1982). Synteny with the chromosome 11 short arm marker enzyme, lactate dehydrogenase A (11p 1203 - 11p 1208) and lack of synteny with the long arm marker, esterase A4 (11 cen - 11q22) excluded the distal portion of the chromosome 11 long arm as the locus of the c-Ha-*ras* 1 gene (McBride *et al.*, 1982). Hybrids made from cells carrying a 11;15 translocation confirmed the presence of the gene on the short arm and narrowed it down slightly to 11p11 to 11p15 (de Martinville *et al.*, 1983). As the short arm of chromosome 11 carries a number of well defined genetic loci, including insulin (Brown *et al.*, 1981) and beta-globin (Jeffreys *et al.*, 1979; Malcolm *et al.*, 1981) for which restriction fragment polymorphisms are common, it should be possible to establish linkage of c-Ha-*ras* 1 with these 11p markers and establish both a linear order and recombination distance.

The second cellular homologue of Harvey murine sarcoma virus c-Ha-*ras* 2 hybridises poorly to c-Ha-*ras* 1 and is more divergent than c-Ha-*ras* 1 from the viral sequence (v-Ha-*ras*). It has been mapped to the X chromosome (O'Brien *et al.*, 1982). As both the rat (Chang *et al.*, 1982) and human (O'Brien *et al.*, 1982) c-Ha-*ras* 2 genes have been shown to lack the intervening sequences present in c-Ha-*ras* 1, it has been suggested that it may represent a pseudogene. The two genes more closely related to Kirsten murine sarcoma virus, c-Ki-*ras* 1 and c-Ki-*ras* 2, have been mapped to chromosome 6 (O'Brien *et al.*, 1982) and chromosome 12 (O'Brien *et al.*, 1982; Sakaguchi *et al.*, 1983), respectively. The presence of a fifth member of the family on chromosome 1 shows a wide chromosomal dispersion of a related family of genes and although two chromosomal sites are commonly found for different members of a gene family (McKusick, 1983) this is probably the most dispersed so far studied.

Many possible mechanisms exist for the activation of a cellular oncogene. At least one of these appears to involve a specific chromosomal translocation causing the expression of the oncogene to be altered by its introduction into a new cellular environment. The mechanism of this could be to bring the oncogene under the influence of a new promoter, or of an enhancer or to change the chromatin structure in the vicinity of the gene. In the case of the EJ human bladder tumour line, the c-Ha-*ras* 1 gene has been activated by a single base change in the coding sequence giving rise to an alteration in the amino acid sequence of the cellular p21 protein (Reddy *et al.*, 1982) and therefore it is unlikely that this particular *ras* gene would be associated with specific chromosomal translocations. However, somatic cell hybrid studies (Spira *et al.*, 1981; Marshall *et al.*, 1982b; Stanbridge *et al.*, 1982) can be interpreted on the basis that an activated oncogene may act in a recessive fashion and can therefore only be expressed if the normal homologous chromosome is deleted, or possibly if the activated gene is duplicated. In the case of Wilm's tumour, for instance, the tumour only occurs when the genes on 11p causing predisposition are hemizygous because of a deletion of one allele. It remains to be seen whether c-Ha-*ras* 1 maps to this region. Furthermore, a normal c-Ha-*ras* 1 gene has never been recovered from genomic libraries of the EJ/T24 bladder carcinoma cell line (Capon *et al.*, 1983; Taparowsky *et al.*, 1982). Chromosome changes could therefore play a role in transformation of cells by activated *ras* genes.

Rowley (1977) correlated karyotype data from 34 patients with haematologic neoplastic disorders who showed any abnormality of chromosome 1. A trisomy for the bands q25 - q32 was found in all 34 patients. A more comprehensive survey of 248 human neoplasms of all types (Brito-Babapulle and Atkin, 1981) involving chromosome 1 aberrations found a variety of types of rearrangement, including translocations, trisomies and deletions. The survey showed that 49.9% of breaks that occurred in chromosome 1 abnormalities were

found within or immediately adjacent to the centric heterochromatin region (i.e., below the centromere). Short arm deletions were found 47 times with a cluster of 15 between the centromere and band p12. These deletions are very close to and may include the N-*ras* gene and are particularly interesting because they occur quite frequently in solid tumours. The middle of the short arm of chromosome 1 (p31) and the region distal to this are the sites of non-random breaks and rearrangements in neuroblastomas (Gilbert *et al.*, 1982), but the results presented here rule out the involvement of N-*ras* in these structural abnormalities. It remains to be seen whether chromosomal alterations have a role to play in the activation of N-*ras* in other human tumours. The chromosomal localisation of N-*ras* presented in this paper will help analysing this possibility.

Materials and methods

Chromosome preparation

Metaphase chromosomes, made from short-term lymphocyte cultures, were prepared, banded and prepared for hybridisation as previously described (Barton *et al.*, 1982).

Probe preparation and in situ hybridisation

The DNA hybridisation probe was nick-translated using [^{3}H]dCTP (52 Ci/mmol) and [^{3}H]TTP (94 Ci/mmol) (Amersham International) to a specific activity of 1.3×10^8 d.p.m./μg. Chromosomes were denatured in 60% formamide, 0.1 mM EDTA 5 mM Hepes, pH 7.0, at 55°C for 7 min. 20 ng of boiled probe in 20 μl hybridisation buffer (50% formamide, 0.6 M NaCl, 5 mM Hepes, 1 mM EDTA, 10% Dextran sulphate, pH 7.6) were applied to each slide and hybridised at 43°C for 18–20 h. Slides were washed in 2 x SSC (0.3 M NaCl, 0.03 M Na_3 citrate) at 55°C for 1 h, followed by four washes in 50% formamide, 2 x SSC at 43°C for 30 min each and an overnight wash in 2 x SSC at room temperature. Slides were coated with Ilford K2 nuclear emulsion and exposed for 18 days at 4°C. Chromosomes were stained in 5% Giemsa in phosphate buffer, pH 6.8 (Gurr) for 40 min.

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References

Barton,P., Malcolm,S., Murphy,C. and Ferguson-Smith,M.A. (1982) *J. Mol. Biol.*, **156**, 269-278.
Brito-Babapulle,V. and Atkin,N.B. (1981) *Cancer Genet. Cytogenet.*, **4**, 215-225.
Brown,J.A., Owerbach,D., Bell,G.I., Rutter,W.J. and Shows,T.B. (1981) *Diabetes*, 30, 267-270.
Capon,D.J., Chen,E.Y., Levinson,A.D., Seeburg,P.H. and Goeddel,D.V. (1983) *Nature*, **302**, 33-37.
Chang,E.H., London,M.W., Ellis,R.W. Scolnick,E.M. and Lowy,D.R. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4848-4852.
Dalla-Favera,R., Bregni,M., Erikson,J, Patterson,D., Gallo,R.C. and Croce, C.M. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 7824-7827.
de Klein,A., van Kessel,A.G., Grosveld,G., Bartram,C.R., Hagermeijer,A., Bootsma,D., Spurr,N.K., Heisterkamp,N., Groffen,J. and Stephenson, J.R. (1982) *Nature*, **300**, 765-767.
de Martinville,B., Giacalone,J., Shih,C., Weinberg,R.A. and Francke,U. (1983) *Science (Wash.)*, **219**, 498-501.
Gilbert,T., Balaban,G., Moorhead,P., Bianchi,D. and Schlesinger,H. (1982) *Cancer Genet. Cytogenet.*, **7**, 33-42.
Hall,A., Marshall,C.J., Spurr,N.K. and Weiss,R.A. (1983) *Nature*, **303**, 396-400.
Jeffreys,A.J., Craig,I.W. and Francke,U. (1979) *Nature*, **281**, 606-608.
Kirsch,I.R., Morton,C.C., Nakahara,K. and Leder,P. (1982) *Science (Wash.)*, **216**, 301-303.
McBride,O.W., Swan,D.C., Santos,E., Barbacid,M., Tronick,S.R. and Aaronsen,S.A. (1982) *Nature*, **300**, 773-774.
McKusick,V.A. (1983) *Clin. Genet.*, **23**, 359-390.
Malcolm,S., Barton,P., Murphy,C. and Ferguson-Smith,M.A. (1981) *Ann. Hum. Genet.*, **45**, 135-141.
Malcolm,S., Barton,P., Murphy,C., Ferguson-Smith,M.A., Bentley,D.L. and Rabbitts,T.H. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4957-4961.
Marshall,C.J., Hall,A. and Weiss,R.A. (1982a) *Nature*, **299**, 171-173.
Marshall,C.J., Kitchin,R. and Sager,R. (1982b) *Somatic Cell Genet.*, **8**, 709-721.
Murray,M.J., Cunningham,J.M., Parada,L.F., Dantry,F., Lebowitz,P. and Weinberg,R.A (1983) *Cell*, **33**, 749-757.
Neel,B.G., Jhanwar,S.C., Chaganti,R.S.K. and Hayward,W.S. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 7842-7846.
O'Brien,S.J., Nash,W.G., Goodwin,J.L., Lowry,D.R. and Chang,E.H. (1982) *Nature*, **302**, 839-842.
Reddy,E.P., Reynold,R.K., Santos,E. and Barbacid,M. (1982) *Nature*, **300**, 149-152.
Rowley,J.D. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5729-5733.
Rowley,J.D. (1983) *Nature*, **301**, 290-291.
Sakaguchi,A.Y., Naylor,S.L., Shows,T.B., Toole,J.J., McCoy,M. and Weinber,R.A. (1983) *Science (Wash.)*, **219**, 1081-1083.
Shimizu,K., Goldfarb,M., Perucho,M. and Wigler,M. (1983a) *Proc. Natl. Acad. Sci. USA*, **80**, 383-387.
Shimizu,K., Goldfarb,M., Snard,Y., Perucho,M., Li,Y., Kamata,T., Feramisoa,J., Stavnezer,E., Fogh,J. and Wigler,M.H. (1983b) *Proc. Natl. Acad. Sci. USA*, **80**, 2112-2116.
Spira,J., Wiener,F., Babonits,M., Gamble,J., Miller,J. and Klein,G. (1981) *Int. J. Cancer*, **28**, 785-798.
Stanbridge,E.J., Der,C.J., Dersen,C.-J., Nishimi,R.Y., Peehl,D.M., Weissman,B.E., Wilkinson,J.E. (1982) *Science (Wash.)*, **215**, 252-259.
Taub,R., Kirsch,K., Morton,C., Lenoir,G., Swan,D., Tronick,S., Aaronson,S. and Leder,P. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 7837-7841.
Taparowsky,E., Suard,Y., Fasano,O., Shimizu,K., Goldfarb,M.P. and Wigler,M.P. (1982) *Nature*, **300**, 762-765.
Yuasa,Y., Srivastava,S.K., Dunn,D.Y., Rhim,J.S., Reddy,E.P. and Aaronson,S.A. (1983) *Nature*, **303**, 775-779.