muscle differentiation and elaborate elastin fibrils. However, no active role in differentiation has previously been proposed for these cells. Our chick chimeras have a significant population of neural crest–derived quail cells located in the aorticopulmonary septum (Fig. 1).

Thompson and Fitzharris (*9*) have described in detail the normal development of the truncal septum. The mesenchyme in the truncus is derived from two distinct sources: endocardium and a population of cells which migrate caudally into the cardiac jelly from the aortic arch. Rychter (*10*) has also described the participation of the 6th aortic arch mesenchyme in the formation of the aorticopulmonary septum. We believe that this population of caudally migrating mesenchymal cells corresponds to the neural crest from the occipital region and provides the impetus for aorticopulmonary septum formation. With this view of truncal septation any teratogenic agent that affects occipital neural crest formation or migration could cause septal malformations. Agents already known to produce transposition of the great vessels are trypan blue, dextroamphetamine, x-rays, anoxia, and avitaminoses (*11*).

The finding that removal of occipital neural crest results in a large percentage (94 percent) of hearts with aorticopulmonary defects should play an important role in determining the pathogenesis of this and related defects in human embryos. One of the major drawbacks to studying this congenital malformation has been its low incidence in laboratory animals. Shaner (*12*) examined 20,000 pig embryos to find 48 abnormal hearts in the early stages of transposition. It is likely that as the microsurgical technique for neural crest extirpation is improved any of the aorticopulmonary septal defects can be produced by more precise manipulation of the area and number of cells removed.

MARGARET L. KIRBY
THOMAS F. GALE
DONALD E. STEWART
Department of Anatomy, Medical College of Georgia, Augusta 30912

References and Notes

1. J. Warkany, *Congenital Malformations* (Year Book Medical Publishers, Chicago, 1971), pp. 515–525; A. J. Moss, F. H. Adams, G. C. Emmanouilides, in *Heart Disease in Infants, Children and Adolescents* (Williams & Wilkins, Baltimore, ed. 2, 1977), pp. 301–380; J. D. Keith, R. D. Rowe, P. Vlad, in *Heart Disease in Infancy and Childhood* (Macmillan, New York, ed. 3, 1977), pp. 452–517 and 590–637.
2. M. L. Kirby and D. E. Stewart, *Dev. Biol.*, in press.
3. C. H. Narayanan, *BioScience* **20**, 868 (1970).
4. V. Hamburger and H. Hamilton, *J. Morphol.* **88**, 49 (1951).
5. C. H. Narayanan and Y. Narayanan, *J. Embryol. Exp. Morphol.* **47**, 137 (1978); B. S. Wenger, *BioScience* **18**, 226 (1968).
6. K. W. Tosney, *Dev. Biol.* **89**, 13 (1982); D. M. Noden, *ibid.* **42**, 106 (1975).
7. M. L. Kirby, T. F. Gale, D. E. Stewart, unpublished data.
8. C. S. LeLievre and N. M. Le Douarin, *J. Embryol. Exp. Morphol.* **34**, 125 (1975).
9. R. P. Thompson and T. P. Fitzharris, *Am. J. Anat.* **154**, 545 (1979).
10. Z. Rychter, *Birth Defects. Orig. Art. Ser.* **14**, 443 (1978).
11. G. S. Wilson, *Anat. Rec.* **123**, 313 (1955); M. H. Fox and C. M. Goss, *Am. J. Anat.* **102**, 65 (1958); I. W. Monie, E. Fakaes, J. Warkany, *Anat. Rec.* **156**, 175 (1966): J. J. Nora, *Pediatr. Clin. North Am.* **18**, 1059 (1971): Z. Rychter, *Adv. Morphol.* **2**, 333 (1962); G. Le Douarin, *J. Embryol. Exp. Morphol.* **9**, 5561 (1961).
12. R. B. Shaner, *Am. J. Anat.* **88**, 35 (1951).
13. Supported by NIH grant ND 17063. We thank J. Whittle and G. Forbes for technical assistance, S. Doetsch for artwork, and P. O'Meara for typing the manuscript.

24 March 1983

Nucleotide Sequence Analysis of the T24 Human Bladder Carcinoma Oncogene

Abstract. *The nucleotide sequence of the T24 human bladder carcinoma oncogene was determined, and the coding and noncoding sequences of the genome were identified. The amino acid sequence of p21, the translational product of the T24 oncogene, was predicted from the nucleotide sequence of the oncogene. Comparison of this sequence with that of the normal cellular homolog showed that a single point mutation in the coding sequences of the T24 oncogene resulted in the acquisition of transforming properties. Other differences between the T24 oncogene and its normal cellular homolog were found in the 5′ noncoding and 3′ noncoding sequences, but these differences appear to be due to polymorphism and do not play a significant role in the transformation process.*

The DNA's from certain naturally occurring human tumors can induce malignant transformation of NIH/3T3 cells (*1, 2*), a continuous mouse cell line that is contact-inhibited and is highly susceptible to DNA transfection. Although only about 10 percent of human tumor DNA's have been shown to be capable of transforming NIH/3T3 cells in transfection assays, oncogenes have been detected in tumors representative of each of the major forms of human cancer. An oncogene present in T24 and EJ bladder carcinoma cell lines was the first to be isolated by molecular cloning techniques (*3–5*). This bladder carcinoma oncogene is closely related to the *onc* genes of Harvey and BALB murine sarcoma viruses (MSV), termed v-*has* and v-*bas* (*6*), respectively. Use of cloned DNA's of bladder carcinoma oncogene as sequence probes showed that this oncogene is derived from a sequence of similar structure present in the normal human genome (*3–6*). Unlike the T24 bladder carcinoma oncogene, which exhibited a transforming activity of $> 10^4$ focus-forming units per microgram of DNA, its normal cellular analog did not exhibit transforming activity in transfection assays. It is thus clear that the bladder oncogene arose by the mutation of a normal cellular gene during the process of carcinogenesis. The mechanisms of activation of T24 and EJ oncogenes were studied by constructing several recombinants from parts of the T24 oncogene and its normal homolog (*7*). Analysis of such recombinants for transforming activity and a limited comparison of their sequences showed that the genetic change leading to the activation of this oncogene is a point mutation, with thymine replacing guanine. This substitution results in the incorporation of valine instead of glycine as the 12th amino acid residue of the T24 oncogene–encoded protein p21. These findings provided evidence for a molecular mechanism in carcinogenesis and demonstrated that small phenotypic changes in the structure of certain proteins can lead to dramatic changes in the biological activity of these molecules. Nucleotide sequence analysis of the T24 human bladder carcinoma oncogene was undertaken to elucidate the structural organization of these transforming genes and the primary structure of the proteins encoded by these genes. The sequence of the T24 oncogene (Fig. 1) was compared to the sequence of its normal human analog and those of two related retroviral *onc* genes, v-*bas* (*8*) and v-*has* (*9*). Molecular cloning and characterization of the T24 human bladder carcinoma oncogene has been described (*3*).

Heteroduplex analysis of the T24 oncogene and its normal allele with v-*bas* and v-*has* had indicated that the putative coding sequences of these human genes are distributed among four exons (*6*). The sequences of the entire coding region as well as flanking cellular sequences of the T24 oncogene were determined, and the four exons were identified by comparison of these sequences with those of v-*bas* and v-*has*. Within the nucleotide sequence, the following re-

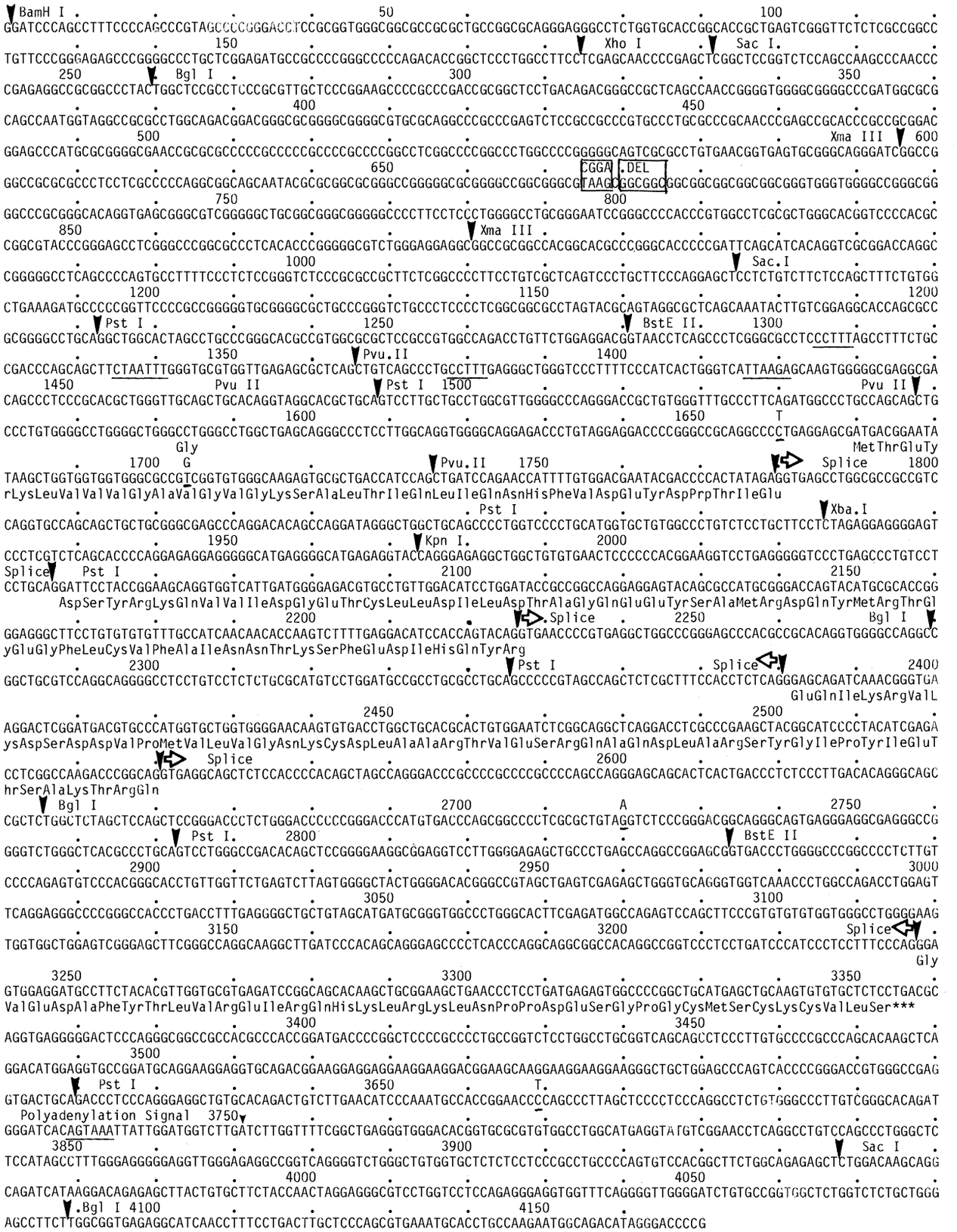

Fig. 1. Nucleotide sequence analysis of the T24 human bladder carcinoma oncogene. The nucleotide sequence analysis was performed according to the procedures of Maxam and Gilbert (*15*). The upper line shows the sequence of the T24 oncogene proceeding in 5′ to 3′ direction with respect to the polarity of the closely related v-*has* and v-*bas* retroviral messenger RNA's. The corresponding sequence of c-*has*/*bas* gene has also been determined and found to be identical to that of the T24 oncogene except at the six positions indicated. Only the change at position 1704 occurs within the coding region. Sequence analysis of a second c-*has*/*bas* clone revealed that this clone contained a C at position 1660, thereby retaining the Mst II site as described by Taparowsky *et al.* [in (*7*)]. Thus, there appears to be a C/T polymorphism at this position in various c-*has*/*bas* clones. The amino acid sequence deduced from the four exons are given. The coding regions are identified by comparison of these sequences with those of v-*has* and v-*bas*. The donor and acceptor splice signals are indicated, and the termination codon is designated by ★★★. Deletions are indicated by DEL.

gions can be identified: (i) the 5′ untranslated sequences from position 1 to 1669; (ii) the first exon from position 1670 to 1779, coding for the first 37 amino acids; (iii) the first intron extending between positions 1780 and 2046; (iv) the second exon between positions 2047 and 2226, which codes for 60 amino acids; (v) the second intron between positions 2227 and 2380; (vi) the third exon between positions 2381 and 2540, which codes for 53 amino acids; (vii) the third and largest intron between positions 2541 and 3237; (viii) the fourth exon extending between positions 3238 and 3354, coding for 39 amino acids at the carboxyl terminal; and (ix) the 3′ untranslated sequences extending from position 3355 to the end of the genome. This stretch of coding sequences would code for a protein of 189 amino acids with a molecular weight of approximately 22,000. This is in agreement with the estimated molecular weight of 21,000 to 23,000 for the translational product of T24 oncogene (*6, 7*).

Comparison of the nucleotide sequence of the entire coding region of the T24 oncogene with that of its normal homolog reveals that in the stretch of 1683 bases that constitute the four exons and three introns there are only two base changes at 1704 and 2720, only one of which is in the coding region. Experiments in which recombinants between the T24 oncogene and its normal homolog were used showed that the transforming property of the T24 oncogene and the mutation in the 12th codon segregated together (*7*). This point mutation results in the change of the glycine-coding triplet GGC to GTC (G, guanine; T, thymine; C, cytosine), which codes for valine. Thus, a single amino acid substitution seems to be sufficient to confer transforming properties to the gene product of the T24 human bladder carcinoma oncogene.

The amino acid sequences of Harvey- and BALB-MSV–encoded p21 proteins have been identified by nucleotide sequence analysis of their *onc* genes, v-*has* and v-*bas*, respectively (*8, 9*). Comparison of the amino acid sequence of the p21 protein coded for by the T24 oncogene with its viral counterparts showed complete identity except at amino acid positions 12 and 143 with respect to v-*bas* and at amino acid positions 12, 59, and 122 with respect to v-*has*. Two important points emerge from this comparison: (i) The c-*has*/*bas* gene family represents one that is highly conserved during evolution, with approximately 1 percent divergence in protein sequence from mouse to human. In β-globin, by contrast, there is an 18 percent divergence between mouse and human protein sequences. (ii) The only position where all three p21 protein sequences differ is 12. The normal homolog of rat c-*has*/*bas*, the gene that presumably recombined with Moloney murine leukemia virus to generate Harvey-MSV, also has glycine at position 12, as does the human homolog. It, therefore, appears that amino acid position 12 is a "hot spot" for point mutations. Multiple sites exist within the c-*has*/*bas* protooncogene at which mutations can lead to the activation of this gene. Thus, Yuasa *et al.* (*10*) cloned an oncogene of the c-*has*/*bas* family with a point mutation outside the first exon.

Goldfarb *et al.* (*4*), Santos *et al.* (*6*), and Tabin *et al.* (*7*) reported that T24 bladder carcinoma cells synthesize a 1.1- to 1.2-kb polyadenylated RNA that specifically hybridizes with the T24 oncogene. This transcript was also detected in normal cells, although at lower levels. Transcriptional control signals for the synthesis of such an RNA species are expected to be present in the 5′ noncoding sequences. We, therefore, analyzed the nucleotide sequence upstream from the initiator codon for sequences related to Pribnow (Goldberg-Hogness) box TATAAA (A, adenine) (*11*). There are two sets of such sequences at positions 1336–1341 and 1415–1421. Both of these sequences are preceded by sequences resembling the "cat" box CCAAT at positions 1305–1309, 1335–1341, and 1378–1382. The DNA sequence upstream from the promoter sequences plays an important role in the transcription of eukaryotic genes. The structural organization and mechanism of action of these sequences, which are often termed "enhancer sequences," are not well understood. The only systematic study of the role of these sequences has been conducted with the DNA tumor virus systems because a biological assay is available to monitor the effect of genetic manipulations on the phenotypic expression of these genes (*12, 13*). Because of its transforming activity, the T24 oncogene provides a powerful biological assay system for studying the effects of genetic manipulation of such sequences. Enhancer sequences appear to be present between the Xho I site at position 194–199 and the promoter region. This region, which is rich in G-C, exhibits certain polymorphic differences between various clones of c-*bas* and T24 oncogenes (see legend to Fig. 1). However, these changes do not seem to affect the biological activity of the molecule since the interchange of this set of sequences between the T24 oncogene and its normal homolog does not affect the biological properties of the molecules (*7*). However, deletion of sequences between the two Xma III sites at position 595 and 900 seems to drastically reduce the transforming activity of the T24 oncogene, indicating the crucial role played by these sequences. The availability of complete nucleotide sequence should allow further detailed analysis of the mechanism of action of these sequences.

In conclusion, a study of the structure and function of the T24 oncogene and its homologs may not only lead to understanding the mechanism by which the transforming gene products function, but also provide insights into the regulatory mechanisms that control the expression of mammalian genes.

Note added in proof: Capon *et al.* (*14*) reported molecular cloning and nucleotide sequence analysis of the 6.6-kbp Bam HI fragment of the T24 oncogene. Using the SV40 promoter system and recombinants between T24 and c-*has*/*bas* genes, they provided additional evidence that the mutation in the 12th codon is responsible for the activation of the c-*has*/*bas* protooncogene.

E. PREMKUMAR REDDY
Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205

References and Notes

1. G. M. Cooper, *Science* **217**, 801 (1982).
2. T. G. Krontiris and G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1181 (1981); C. Shih, C. Padhy, H. Murray, R. A. Weinberg, *Nature (London)* **290**, 261 (1981); M. A. Lane, A. Sainten, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5185 (1981); M. J. Murray, B.-Z. Shilo, C. Shih, D. Cowing, H. W. Hsu, R. A. Weinberg, *Cell* **25**, 355 (1981); M. Perucho, M. Goldfarb, K. Shimizu, C. Lama, J. Fogh, M. Wigler, *ibid.* **27**, 467 (1981); M. A. Lane, A. Sainten, G. M. Cooper, *ibid.* **28**, 873 (1982); S. Pulciani, E. Santos, A. V. Lauver, L. K. Long, S. A. Aaronson, M. Barbacid, *Nature (London)* **300**, 539 (1982).
3. S. Pulciani, E. Santos, A. V. Lauver, L. K. Long, K. C. Robbins, M. Barbacid, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2845 (1982).
4. M. Goldfarb, K. Shimizu, M. Perucho, M. Wigler, *Nature (London)* **296**, 404 (1982).
5. C. Shih and R. A. Weinberg, *Cell* **29**, 161 (1982).
6. C. J. Der, T. G. Krontiris, G. M. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3637 (1982); L. F. Parada, C. J. Tabin, C. Shih, R. A. Weinberg, *Nature (London)* **297**, 474 (1982); E. Santos, S. R. Tronick, S. A. Aaronson, S. Pulciani, M. Barbacid, *ibid.* **298**, 343 (1982).
7. C. J. Tabin *et al.*, *Nature (London)* **300**, 143 (1982); E. P. Reddy, R. K. Reynolds, E. Santos, M. Barbacid, *ibid.*, p. 149; E. Taparowsky, Y. Suard, O. Fasano, K. Shimizu, M. Goldfarb, M. Wigler, *ibid.*, p. 762.
8. E. P. Reddy *et al.*, in preparation.
9. R. Dhar *et al.*, *Science* **217**, 934 (1982).
10. Y. Yuasa *et al.*, *Nature (London)*, in press.
11. D. Pribnow, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 784 (1975).
12. S. L. McKnight and R. Kingsbury, *Science* **217**, 316 (1982).
13. H. Weiher, M. König, P. Gruss, *ibid.* **219**, 626 (1983).
14. D. J. Capon, E. Y Chen, A. D. Levinson, P. H. Seeburg, D. V. Goeddel, *Nature (London)* **302**, 33 (1983).
15. A. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
16. I thank M. Barbacid, S. Tronick, and S. A. Aaronson for providing the DNA clones and R. K. Reynolds and M. J. Smith for technical assistance.

15 February 1983