two different points, the rods themselves and the rod bipolars, and possibly a third (amacrine synapses), it seems likely that they engage in a temporal sequencing of the rod signals, and furthermore could transmit scotopic signals to the brain more rapidly than ordinary types of retinal ganglion cells since they could possibly bypass the usual interneuron circuitry of the retina (*10*).

ANDREW P. MARIANI
Laboratory of Vision Research,
National Eye Institute,
Bethesda, Maryland 20205

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5. Retinas of adult rhesus monkeys were isolated from the choroid and pigment epithelium in oxygenated Eagle's tissue culture medium, placed on wax sheets, and covered with a fixative of 2.5 percent glutaraldehyde in sodium cacodylate for 30 minutes to 1 hour. The fixative was replaced with an aqueous solution of 5 percent glutaraldehyde and 4 percent $K_2Cr_2O_7$ [M. Colonnier, *J. Anat.* **98**, 327 (1964)] or 0.2 percent OsO_4 and 2.4 percent $K_2Cr_2O_7$ [F. Valverde, in *Contemporary Research Methods in Neuroanatomy*, W. J. Nauta and S. O. F. Ebbesson, Eds. (Springer-Verlag, New York, 1970), pp. 12–31] or a mixture of both of these solutions. The retinas were placed between sheets of Whatman No. 50 filter paper, covered by Whatman No. 4 filter paper, sandwiched between glass microscope slides, and lightly bound with rubber bands. This package was immersed in the dichromate solution for 2 to 3 days, then placed in 1 percent $AgNo_3$ for 2 to 3 days, and subsequently processed by routine techniques.
6. After biplexiform cells were found and studied in rhesus retinas, a number of them were also impregnated in another macaque species, *M. fascicularis*; biplexiform cells identical in size and form to those of *M. mulatta* were found in this other species.
7. W. K. Stell, *Anat. Rec.* **153**, 389 (1967). Serial ultrathin sections of three biplexiform cells were placed on slot grids with carbon-coated Formvar films, stained with uranyl acetate and lead citrate, and examined and photographed in an electron microscope. When Golgi-impregnated neurons contact other neurons which contain presynaptic structures, the Golgi-impregnated cells can be identified as postsynaptic at these sites. The converse is not usually possible since the electron-opaque precipitate obscures any presynaptic structures in the Golgi-impregnated cell.
8. Bipolar cell axon terminals in the IPL usually contain a presynaptic ribbon and there are two postsynaptic elements. This arrangement is termed a dyad [J. E. Dowling and B. B. Boycott, *Proc. R. Soc. London Ser. B* **166**, 80 (1966)], but R. A. Allen [in *The Retina: Morphology, Function and Clinical Characteristics*, B. R. Straatsma, M. O. Hall, R. A. Allen, F. Crescitelli, Eds. (Univ. of California Press, Los Angeles, 1969), pp. 101–143] and M. T. T. Wong-Riley [*J. Neurocytol.* **3**, 1 (1974)] report that bipolar cell axon terminals also form nonribbon synapses.
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Isolation of Human Oncogene Sequences (v-*fes* Homolog) from a Cosmid Library

Abstract. *To define the human homolog (or homologs) of transforming sequences (v-*fes *gene) common to Gardner (GA) and Snyder Theilen (ST) isolates of feline sarcoma virus (FeSV), a representative library of human lung carcinoma DNA in a cosmid vector system was constructed. Three cosmid clones were isolated containing GA/ST FeSV v-*fes *homologous cellular sequences, within 32- to 42-kilobase cellular inserts representing 56 kilobases of contiguous human cellular DNA. Sequences both homologous to, and colinear with, GA or ST FeSV v-*fes *are distributed discontinuously over a region of up to 9.5 kilobases and contain a minimum of three regions of nonhomology representing probable introns. A thymidine kinase selection system was used to show that, upon transfection to RAT-2 cells, the human c-*fes *sequence lacked detectable transforming activity.*

That RNA transforming viruses contain acquired cellular genes accounting for their capacity to transform cells in culture and induce tumors of various histological classes in vivo is well established (*1*). Although such cellular derived "oncogenes" have been described only in animal model systems (*1*), their existence makes possible the isolation of related human genomic sequences. Extensive noncoding sequences within the cellular homologs of many such viral transforming genes (*2*, *3*) represent a major difficulty for their cloning in conventional phage and plasmid systems. Because of this problem and the desirability of obtaining such genes with sufficiently extensive flanking sequences for studies of cellular regulatory controls influencing their expression, we used a cosmid cloning vector (*4–6*) in our study. This system involves the use of plasmids containing lambda cos sequences (cohesive ends) permitting insertion of large DNA fragments, in vitro packaging, and transduction to *Escherichia coli*.

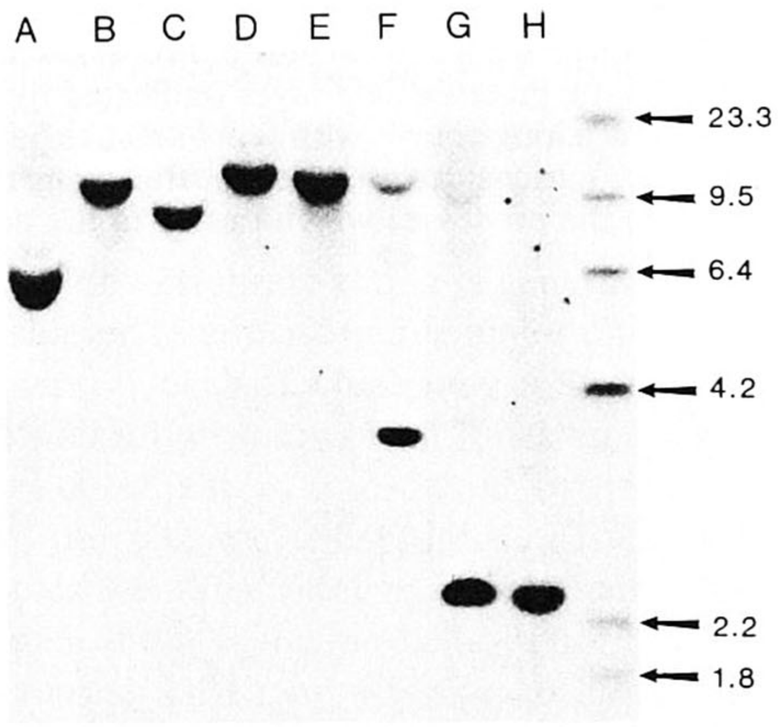

Fig. 1. Restriction patterns of cellular homologs of transformation-specfic DNA sequences common to GA and ST FeSV. (A) High molecular weight DNA's were prepared from kitten lung embryo cells; (B) CCL64 mink lung cells; (C) NIH/3T3 mouse embryo cells; (D and G) normal human lung tissue; and (E, F, and H) human lung carcinoma tissue. DNA samples (20 μg) were digested with Eco RI (A to E), Bam HI (F), or Kpn I (G and H), separated by electrophoresis on 0.75 percent agarose gels, transferred to nitrocellulose, and analyzed by hybridization according to previously described procedures (*19*). ^{32}P-Labeled DNA digested with Hind III is included as a molecular weight standard.

To define the cellular homolog of the GA/ST transforming (v-*fes*) gene, we used a molecular probe corresponding to a 0.5-kb Pst I restriction fragment within the ST FeSV (feline sarcoma virus) acquired sequence (v-*fes* S_L) (*2*, *7*). By Southern blot analysis (*8*), single bands of hybridization were observed at molecular weights of between 7.0 and 12.5 kb in cat, mink, mouse, and human Eco RI restricted DNA's (Fig. 1, A to E). Restriction of DNA's from both normal human lung and human lung carcinoma with Bam HI or Kpn I resulted in generation of single bands hybridizing at 3.8 kb (Fig. 1F) and 2.4 kb (Fig. 1, G and H), respectively.

A cosmid library was constructed and screened for v-*fes* homologous sequences (Fig. 2). Three clones were initially selected on the basis of hybridization to v-*fes* S_L and were propagated for restriction endonuclease analysis. Two of the clones contained a 12.0-kb Eco RI restriction fragment with homology to both v-*fes* S_L and v-*fes* S_R while only the 9.8-kb 5′ region of this fragment was represented in the third clone. Further restriction enzyme analysis indicated that sequences within these clones were overlapping and represented a 58-kb contiguous region of the human genome. The orientation and positioning of the cellular inserts within these clones, both relative to each other and to the cosmid vector, are summarized in Fig. 3. For purposes of fine structure mapping, the above described 12.0-kb Eco RI v-*fes* homologous restriction fragment was subcloned in plasmid pBR328.

As a prerequisite to further analysis of the above described clones it was first necessary to isolate and prepare molecular probes corresponding to the complete

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acquired cellular sequences of both GA and ST FeSV. The ST FeSV genome was cloned in phage Charon 9 as a 6.6-kb fragment of Hind III restricted DNA isolated from the nonproductively transformed mink cell line, ST-FeSV 64 C141. Similarly, GA FeSV was isolated as a 14-kb Eco RI restriction fragment from the mink-transformed clone, G-FeSV 64 F3, in phage λgtWESλB. ^{32}P-Labeled DNA's corresponding to the entire GA and ST FeSV genomes were prepared (Fig. 3).

The results of fine structure mapping of the 12.0-kb Eco RI restriction fragment containing GA and ST FeSV homologous sequences are summarized in Fig. 3. The indicated regions of hybridization are based on identification of restriction fragments hybridizing with each of the four indicated probes. These encompass a total of 4.5 kb of homology distributed discontinuously over a 9.5-kb region. No additional homology with either GA or ST FeSV was observed within the entire 58 kb of human DNA represented within the three cosmid clones. The similarity in arrangement of homologous sequences within the viral and cellular DNA's establishes colinearity between v-*fes* and its human homolog. These findings thus provide a maximum estimate of human DNA sequence homology to the GA and ST FeSV genomes and a minimal estimate of the number of nonhomologous regions representing probable introns.

From a comparison of the results summarized in Fig. 3, to the analysis of total human lung carcinoma DNA (Fig. 1), the major Eco RI, Kpn I, and Bam HI restriction fragments homologous to the v-

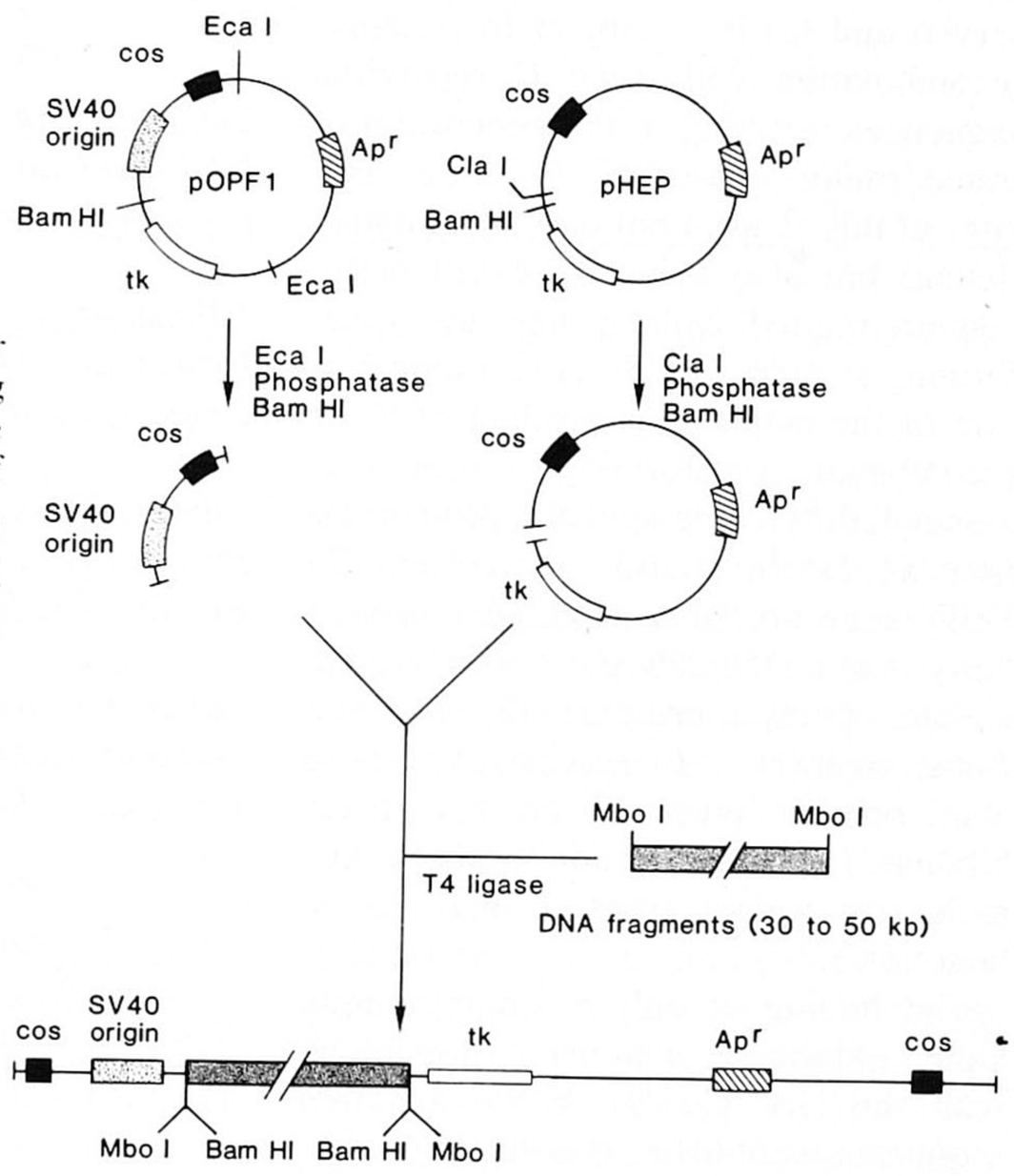

Fig. 2. Construction of a human lung carcinoma DNA library in a cosmid vector system. High molecular weight cellular DNA was purified from human lung carcinoma tissue and partially digested with Mbo I and the fragments were separated by sucrose gradient (5 to 20 percent) fractionation. The DNA's of plasmids pOPF1[19] and pHEP were digested with Eca I and Cla I, respectively, treated with calf intestine phosphatase, and redigested with Bam HI. A 1:1 molar ratio (1.0 μg each) of the restricted plasmid DNA's and restricted cellular DNA fragments (30 to 50 kb) were ligated, packaged in vitro, and transduced into *E. coli* ED8767 (*5*, *6*). Ampicillin-selected bacteria containing recombinant DNA were grown on nitrocellulose (Millipore HAWP) at a density of approximately 15,000 colonies per filter. Approximately 250,000 individual colonies were obtained representing three to four times that required for representation of the entire human genome.

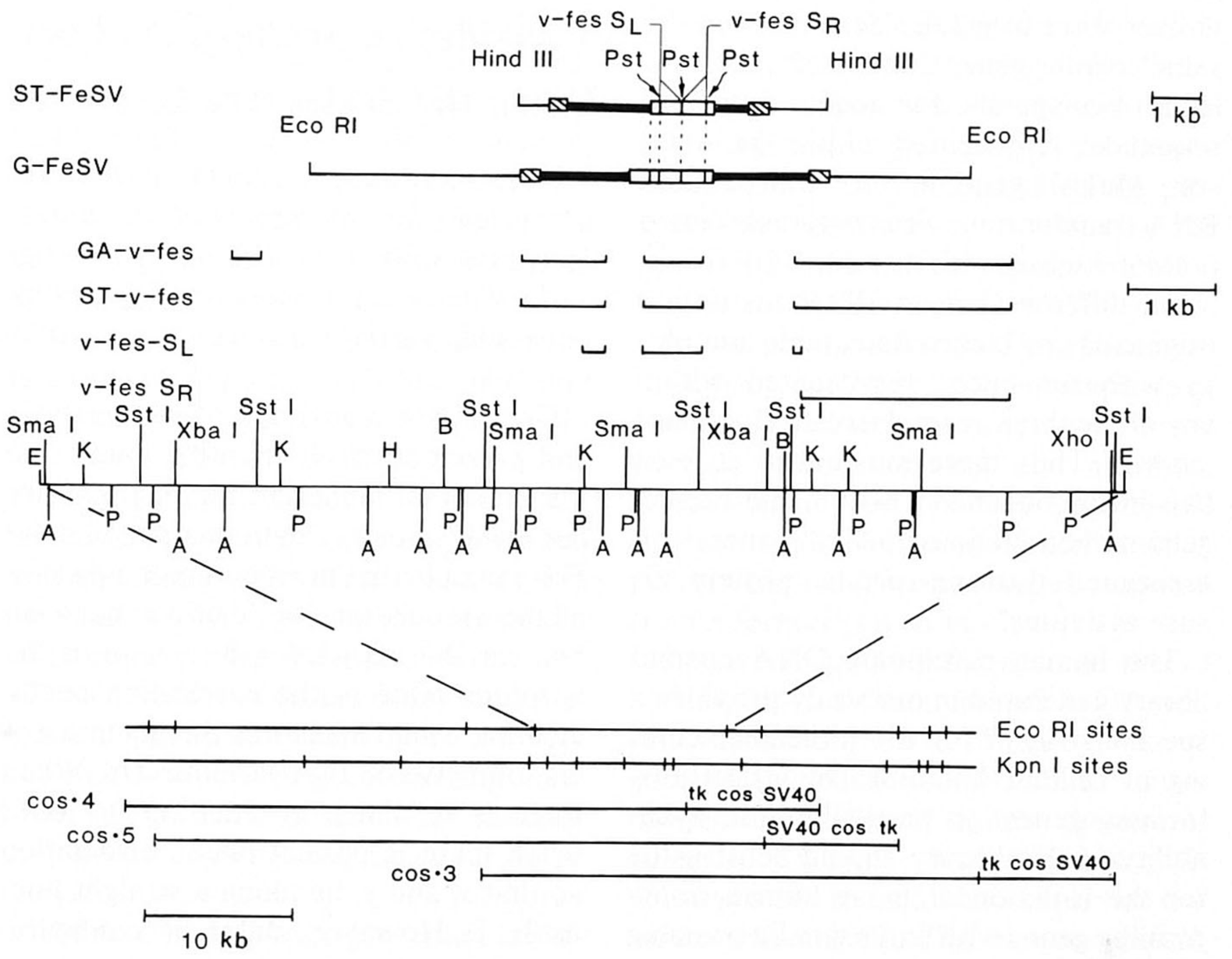

Fig. 3. Restriction map of human cellular sequences homologous to acquired cellular sequences represented within GA and ST FeSV. Open boxes (▭) within the viral genomes represent the relative positions of their respective acquired cellular sequences, while feline leukemia virus cross-reactive sequences are shown as solid lines (▬). The positions of long terminal repeats (▨) and cellular flanking sequences (⎵) are also indicated. The 12.0-kb Eco RI DNA restriction fragment shown in the center of the figure contains the entire human homolog of the GA and ST FeSV acquired cellular sequences. Restriction fragments containing sequences homologous to the complete GA-FeSV genome (GA-v-*fes*), the ST-FeSV genome (ST-v-*fes*), v-*fes* S_L, and v-*fes* S_R are indicated. The lower portion of the figure shows the position of the 12.0-kb Eco RI fragment within a 58-kb contiguous sequence of human cellular DNA; the restriction map of this 58-kb sequence was deduced as a composite of individually mapped overlapping cellular sequences represented within the cosmids shown at the bottom. The relative positions of the thymidine kinase gene (*tk*), cos site, and SV40 restriction fragment within the 9-kb vector are shown for purposes of orientation. Restriction enzymes are abbreviated as follows: Ava I (*A*), Bam HI (*B*), Eco RI (*E*), Kpn I (*K*), Hind III (*H*), and Pst I (*P*).

fes S_L probe are seen to correspond. Similarly, correspondence was observed between v-*fes* S_R homologous restriction fragments as determined by genomic blots of total cellular DNA and the isolated human homolog of the GA/ST v-*fes* gene. The human v-*fes* homolog showed no detectable transforming activity when transfected to RAT-2 cells by means of a thymidine kinase selection system. Molecularly cloned GA/ST FeSV were included as positive controls.

The molecular cloning of human genomic sequences homologous to GA/ST FeSV v-*fes* is of particular interest because (i) the c-*fes* gene is highly conserved and (ii) it is subject to frequent recombination with type C retrovirus sequences resulting in the generation of transforming viruses (*7*, *9*). Virus isolates of this class of not only mammalian (feline) but also avian (chicken) origin contain related cellular derived transforming sequences (*10*). A common feature of the major gene product of these recombinant transforming viruses is an associated tyrosine-specific protein kinase (*11–13*). In addition, the GA and ST FeSV gene products exhibit binding affinity for a 150,000 molecular weight cellular phosphoprotein (*12*, *14*) and transformation by these viruses leads to abolition of epidermal growth factor binding (*15*, *16*) and production of a low molecular weight transforming growth factor (*17*).

The finding of only a single genetic locus exhibiting significant homology with the GA and ST FeSV acquired sequences establishes that the highly related transforming sequences within the genomes of these independently isolated viruses were originally derived from the same cellular gene. Conversely, molecular probes specific for acquired cellular sequences represented within the Abelson MuLV genome, an independent RNA transforming virus with associated protein kinase activity (*11*, *18*), recognized different human DNA restriction fragments and lacked detectable homology with sequences represented within any of the three cosmid isolates (data not shown). Thus there must exist at least two independent loci within the human genome homologous to viral genes with associated tyrosine-specific protein kinase activities.

The human carcinoma DNA cosmid library generated in our study provides a specific reagent for the molecular cloning of cellular homologs of viral transforming genes. In particular, the availability of this library should be of value for the isolation of those human transforming genes with extensive intervening sequences. An important feature of this system is the presence of a functionally active thymidine kinase gene that allows for selection of minority populations of eukaryotic cells containing such cosmids after transfection. In addition, SV40 DNA sequences situated in one of the cosmid arms have been shown to exert a positive influence on transcription in the β-globin system (*6*). If this sequence similarly influences expression of cellular homologs of viral transforming genes, its presence may be important for identification of the translational products of these sequences and a determination of their transforming potential.

JOHN GROFFEN
NORA HEISTERKAMP
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701
FRANK GROSVELD
Laboratory of Gene Structure and Expression, Medical Research, Council, London NW7 1AA
WIM VAN DE VEN
Carcinogenesis and Intramural Program, Frederick Cancer Research Facility, Frederick, Maryland 21701
J. R. STEPHENSON
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701

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Variables on Scatterplots Look More Highly Correlated When the Scales Are Increased

Abstract. *Judged association between two variables represented on scatterplots increased when the scales on the horizontal and vertical axes were simultaneously increased so that the size of the point cloud within the frame of the plot decreased. Judged association was very different from the correlation coefficient,* r, *which is the most widely used measure of association.*

Graphs are mainstays of the analysis and presentation of scientific data. One reason is that numerical summaries cannot always portray data unambiguously. For example, the most common measure of the association, or relation, between two variables $(x_i y_i)$, $i = 1, \ldots, n$, is the absolute value of the correlation coefficient, r, which measures the linear association between two variables (*1*). When there is no linear association, $|r|$ is 0; when there is perfect linear association so that x_i and y_i lie along a straight line, $|r|$ is 1. However, different configurations of points can yield the same value of r, relations can be nonlinear, and a single value of (x_i,y_i) can radically alter r (*2*). A scatterplot can depict the relation between x_i and y_i more reliably than any single numerical measure. But the use of a graph opens the door for perceptual factors to enter into the analysis and interpretation of the data. Although a set of data has only one numerical value for a particular measure of association such as r, the judged association could change according to any one of a number of "display factors" such as the size of the

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