sumed that the regenerated *j-tc2/j-tc2* tomato presumably arose by a mutation followed by mitotic recombination, both events occurring before or during shoot organization.

Two dominant mutations (*I-tc1* and *I-tc2*) with the same phenotype were isolated from independently regenerated plants. Tomatoes grown for canning, such as UC82B, are homozygous for the determinate (*sp/sp*) mutant. Mutation *I-tc1* segregated in a 3:1 ratio for indeterminate versus determinate growth in the R_1 progeny grown in the field. Single plant selections from the R_1 plants were analyzed in the R_2 generation. All determinate and some indeterminate plants bred true. Among heterozygous *I-tc1*/+ selections, a 3:1 ratio of indeterminate to determinate was observed (92 indeterminate and 33 determinate; $\chi^2 = 0.067$; 1 d.f.; $0.7 < P < 0.9$).

Thus, various types of morphological mutants were observed. In most cases, the mutations were similar to those that occur spontaneously or after mutagenic treatment of tomato seed. However, seed treatment often produces mosaics, since not all cells in the seed are equally affected by the mutagen. No mosaics were evident in our tissue culture–derived plants. Hence, the observed tissue culture mutations apparently occurred before shoot formation, and the 13 shoots were each derived from a single cell of a callus. As a consequence, 3:1 ratios were observed among R_1 plants in contrast to the distorted ratios often reported for progenies resulting when tomato seeds are treated with mutagens (*15*). Environmental factors and certain chemical and physical treatments can increase the frequency of mitotic recombination (*16*). The recovery of the *j-tc2/j-tc2* regenerated plant suggests that it may be possible to regenerate homozygous R plants after mitotic recombination in mutated heterozygous cells by adding chemicals that promote recombination to the tissue culture medium during regeneration.

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Multiclonal Origin of Polyps in Gardner Syndrome

Abstract. *Electrophoretic analysis of glucose-6-phosphate dehydrogenase was performed on polyp tissue from three black female patients with Gardner syndrome and who are heterozygous for the A and B forms of this enzyme. Polyp tissues from the three patients displayed the AB phenotype. This finding suggests a multiclonal origin of polyps in Gardner syndrome. Studies of tumors originating from such polyps may provide information about the sequence of cellular events leading to malignant transformation.*

During early embryonic development one of the X chromosomes of each mammalian female cell becomes functionally inactive. Whether the paternal or maternal X chromosome is expressed in any given cell is random (*1*). Females heterozygous for X-linked genes are thus mosaic with respect to the expression of the two alleles. Hence, X-linked genes can be used as developmental markers to trace the clonal origin of cells in heterozygous females (*2, 3*). The X-linked enzyme glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49) can be used in humans to determine the clonal composition of tumors. In black females who are heterozygous for the G6PD isozymes A and B (*4, 5*), tumors originating from a single cell express either the A or B allele, whereas tumors arising from more than one cell express both alleles. In most spontaneously arising tumors studied, including acute and chronic myelocytic leukemia (*6, 7*), Burkitt's lymphoma (*4*), carcinoma of the cervix (*8*), and leiomyomas (*9*), G6PD analyses have indicated that the tumor originated from a single cell or clone of cells. In contrast, similar studies of a carcinoma of the colon (*10*) revealed a possible polyclonal origin of this spontaneous tumor. However, the double G6PD-AB phenotype in this particular instance may have been due to stromal cell contamination in the samples (*4, 10*).

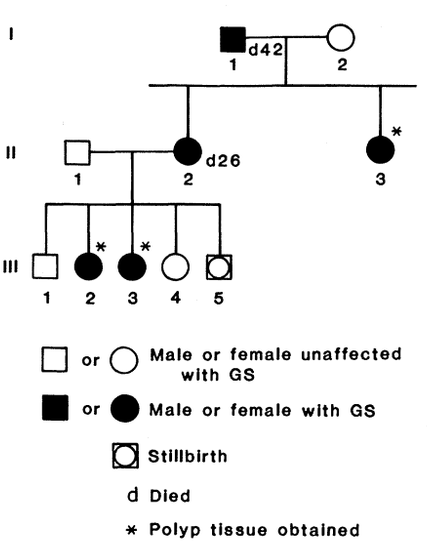
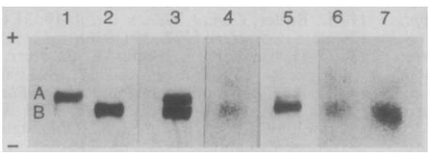


Fig. 1. A partial kindred pedigree identifying three patients with Gardner syndrome (*GS*).

To date, the electrophoretic forms of G6PD have been studied in black female heterozygotes for only three types of inherited tumors. Two of these disorders, inherited neurofibromas (*11*) and trichoepitheliomas (*12*), appeared to be multicellular in origin. Baylin *et al.* (*13*) reported that tumor tissues in black female heterozygotes with inherited medullary carcinoma of the thyroid and pheochromocytoma (Sipple syndrome) were monoclonal; the primary tumors contained either G6PD-A or G6PD-B, but not both. These data suggested that the inherited defect in the patients with familial Sipple syndrome resulted in the production of multiple clones of defective cells and that each tumor then arose as a final mutation in one clone of these cells (*14*).

Gardner syndrome is a precancerous bowel condition in which patients develop multiple polyps of the gastrointestinal mucosa. This syndrome, which has an incidence of 1 per 14,025 births (*15*), involves defects in the derivatives of all three primordial germ layers (*16*). The



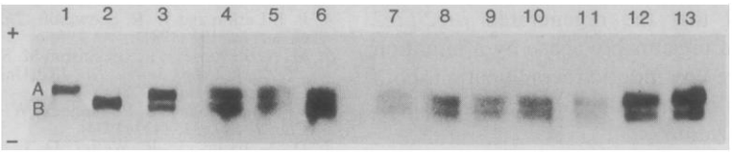


Fig. 2 (left). The electrophoretic patterns of glucose-6-phosphate dehydrogenase in blood and polyp samples from patients III-2 and III-3: (lane 1) A control; (lane 2) B control; (lane 3) red blood sample from III-2; (lane 4) polyp sample from III-2; (lane 5) red blood sample from III-3; and (lanes 6 and 7) separate cuts of polyp sample from III-3. Fig. 3 (right). Electrophoretic patterns of patient II-3: (lane 1) A control; (lane 2) B control; (lane 3) red blood sample from II-3; (lanes 4 to 7) separate cuts of a single polyp; (lanes 8 to 10) separate cuts of another single polyp; and (lanes 11 to 13) three small discrete polyps cultured overnight in medium.

cardinal manifestations are colonic polyposis, with abnormalities of soft and hard tissue (*17*). Its inheritance as an autosomal dominant trait was first recognized by Gardner *et al.* (*18*). About 20 percent of untreated patients develop cancer within 2 years of polyp detection. By the fifth decade the prevalence of carcinoma in Gardner syndrome patients approaches 100 percent (*19*).

We performed G6PD electrophoretic analyses in polyps from three black female Gardner syndrome patients heterozygous for G6PD forms A and B. Because of a history of Gardner syndrome in their grandfather and mother who died of carcinoma of the rectum, the patients, III-2, III-3, and III-4, and their maternal aunt, II-3 (Fig. 1), came to the Johns Hopkins Oncology Center Polyposis Service for medical consultation. Diagnosis of Gardner syndrome was made in III-2, III-3, and II-3. A single polyp each was obtained from III-2 and III-3 for G6PD electrophoretic analysis. Because a large number of small polyps were detected in II-3 by flexible sigmoidoscopy, she underwent a total colectomy with endorectal mucosal stripping, ileal pull-through and ileo-rectal anastomasis 2 months later. From the surgical colon specimen, five distinct polyps, each from a different location, were obtained for G6PD analysis. Three similar adjacent polyps, dissected in the same manner, were sent for histological examination.

The method used for G6PD electrophoresis is our modification (*13*) of the technique of Ellis and Alperin (*20*). Polyps removed by sigmoidoscopic biopsy or from the surgical specimen were dissected under the microscope to remove all mucus and other nonpolyp tissues and then placed in vials containing RPMI 1640. The polyps were either prepared immediately for electrophoresis or cultured overnight in RPMI 1640 with 10 percent human serum. After the polyps were rinsed three times in medium, all remaining adherent mucus was removed with a scalpel. Each polyp measuring more than 0.2 cm in diameter was divided into sections approximately 0.1 cm or smaller. Each piece was homogenized separately in 0.1*M* tris-HCl buffer (*p*H 8) containing 2 m*M* nicotinamide adenine dinucleotide phosphate and centrifuged twice (2000 rev/min, 4°C). The final clear supernatant was used for G6PD electrophoresis.

The red blood cells from patient III-2 had AB bands (lane 3 in Fig. 2), and two different pieces of this patient's polyp also contained AB bands (lane 4 in Fig. 2). The other piece (less than 0.1 cm in size) from the same polyp had faint AB bands that did not show in the photograph. The red blood cell phenotype of patient III-3 was that of G6PD-A^-B heterozygote (lane 5). Both pieces of her polyp had A^-B bands (lanes 6 and 7); however, the A^- band in her polyp tissue was not as prominent as that in her red blood cells. Thus, polyps of both patients appeared to be polyclonal. Mark *et al.* (*21*) reported that the A^- phenotype can be distinguished from A in that the enzyme activity in black males with the G6PD-A^- phenotype is decreased in red blood cells but is normal or only slightly decreased in nucleated tissues. Therefore, black females with the phenotype A^-B in their red blood cells may have normal AB bands in other tissues having normal rates of protein synthesis.

Definitive proof that polyps from Gardner syndrome patients are multiclonal in origin came from studies of five different polyp samples from patient II-3 (Fig. 3). Sample 1 was divided into four small pieces. Sample 2 was cut into three pieces. These multiple pieces of polyps (lanes 4 to 10 in Fig. 3) all had AB bands identical to those found for the patient's red blood cells (lane 3 in Fig. 3). Three discrete polyps each less than 0.1 cm in diameter and cultured overnight also displayed the AB phenotype (lanes 11 to 13). Contamination of our samples with nonpolyp stroma cells is unlikely in view of the homogeneous cell type observed histologically in similarly dissected and washed polyps from these patients. Furthermore, in all three patients multiple sections from single polyps all displayed G6PD-AB phenotypes. These results demonstrated that polyps from patients with Gardner syndrome are multiclonal in origin and suggest possible theories for the development of polyps in Gardner syndrome.

Two-stage and multiple-stage theories were originally proposed by Armitage and Doll (*22*) and Nordling (*23*), respectively, to explain carcinogenesis mathematically. On the basis of a retrospective statistical analysis of neuroblastoma and pheochromocytoma, Knudson (*24*) concluded that a "two-hit" model is compatible with tumorigenesis. In patients with inherited tumors, the first event may occur in germinal cells and be inherited, whereas the second event occurs in somatic cells and is mutational. This model allows for the inherited tumors to be of mono- or multiclonal origin, depending on the number of cells affected during the second mutational event. If only a single cell or a single clone of cells is involved in the second mutational event, the resulting tumor will always be of clonal origin. An example of this type is provided by the finding that individual medullary thyroid carcinomas in patients with Sipple syndrome contained either the A or the B form of G6PD (*14*). The fact that individual patients with Sipple syndrome can have both A and B tumors indicates that the inherited mutation results in the production of multiple clones of cells genetically at risk to neoplastic change. Each tumor subsequently arises from a single or a very small clone of genetically susceptible cells (*14*). On the contrary, if a large number of cells are affected during the second mutational event, the resulting tumors will have multiclonal origin. The G6PD-AB phenotypes observed in trichoepithelioma and neurofibroma suggest that these tumors may originate directly before a second mutational event from a large number of mutated cells or, alternatively, the mutational events subsequent to the initial inherited event might occur simultaneously in large numbers of genetically defective cells (*14*).

Several well-characterized histologic types of colonic polyps have been described, some of which are inherited as dominant traits and others not (*25*). Although these different polyps occur in

distinct clinical syndromes, the polyps all possess varying degrees of potential for malignant degeneration. The study of various polyps will allow a comparison of heritable versus nonheritable tumor lesions at a similar site and also offers an opportunity to observe the phenotypic changes in tissue during malignant transformation. The finding of multiclonal origin of polyps in patients with Gardner syndrome does not permit us to predict whether carcinomas subsequently found in the patients will be of monoclonal or polyclonal origin. A recent report on neurofibromatosis, however, indicates that the benign lesion (neurofibroma) is polyclonal and the malignant lesion (neurofibrosarcoma) is monoclonal (*26*). Studies of tumors originating from known polyps in black females with Gardner syndrome and who are heterozygous for G6PD should provide experimental data to test the hypothesis of a multistage process of human carcinogenesis and the possibility that the malignant neoplasm in the human probably always develops from a single cell (*27*).

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Cell-to-Cell Transfer of Interferon-Induced Antiproliferative Activity

Abstract. *Interferon-treated cells rapidly and efficiently transferred the antiproliferative activity of interferon to untreated cells. This phenomenon was not due to the carry-over of interferon by the interferon-treated cells. Thus, to evoke an antiproliferative state, interferon did not directly contact each cell in a population. The results suggest a novel mechanism by which interferon may indirectly regulate cell growth, and suggests that cells other than those of the immune system may play a role in controlling tumor growth in tissue where cell-to-cell contact occurs.*

Interferons (IFN's) can induce a wide range of activities in cells and the mechanism for these responses is generally attributed to the direct interaction of IFN with membrane receptors on each target cell (*1*). Recently, IFN was shown to have an amplification system in which IFN-treated cells indirectly induce antiviral activity (*2–6*) in other cells. It was hypothesized that a secondary messenger molecule is transferred from the IFN-induced cell to the recipient cells which then express the antiviral activity of IFN (*7*). Here we show that the antiproliferative activity of IFN can also be transferred between cells.

For these experiments we used two cell systems in which the effect of IFN was transferred across the species preference barrier of IFN (to xenogeneic cells) and the recipient cells could be separated from the donor cells prior to the assay of antiproliferative activity. Figure 1A shows that cocultivation of mouse L1210 lymphoblastoid cells with human IFN-treated WISH cells resulted in the transfer of significant antiproliferative activity to the L1210 cells, as measured by reduced incorporation of [^{3}H]thymidine into cellular DNA. Both recombinant IFN-γ (*8, 9*) and partially purified IFN-α were able to induce an effective dose response. Likewise, mouse IFN-treated L cells transferred antiproliferative activity to human lymphoblastoid Daudi cells (Fig. 1B). In all cases, the amount of antiproliferative activity transferred followed asymptotic dose-response curves of similar magnitude. The reduction in growth rate was not due to the direct effect of IFN since donor cells were thoroughly washed before coculturing with recipient cells. Assay of supernatant fluids at the end of the 4-hour coculture period failed to detect carry-over of IFN unless more than 1000 IU had been used to induce the cells, in which case only 1 to 3 U/ml were detected. Also, the phylogenetic barrier of IFN was shown to be effective because direct treatment of L1210 cells with 100 IU of human IFNα or Daudi cells with 100 U of mouse IFN-α and -β resulted in no decrease in [^{3}H]thymidine incorporation (Fig. 1). In our system the incorporation of [^{3}H]thymidine into DNA was shown to be an accurate measure of cell growth, both in lymphoblastoid cells that were cocultured with IFN-treated cells or lymphoblastoid cells cultivated alone in dilutions of IFN (*10*). An assay of specific chromium release (*11*) suggested that nonspecific cellular cytotoxicity was not responsible for the observed reduction in cellular growth rate (*12*).

We also tested the ability of other human IFN's to induce antiproliferative transfer. When human IFN-β (specific activity 10^6 U per milligram of protein) or IFN-γ (specific activity 10^5 U/mg) were used to induce the transfer of antiproliferative activity to L1210 cells, the dose-response curves were similar in shape and magnitude to those in Fig. 1 (data not shown).

Figure 2, A and B, shows the results of two typical experiments in which antiviral activity was transferred to L1210 or Daudi cells concurrently with antiproliferative activity. In both cell systems, transfer of antiproliferative and antiviral activity correlated well with the IFN dose, and the results of many experiments showed that high antiproliferative