

## Formation of the tetraploid intermediate is associated with the development of cells with more than four centrioles in the elastase–simian virus 40 tumor antigen transgenic mouse model of pancreatic cancer

(aneuploidy/cell-division cycle/genomic instability/multipolar mitotic spindles/neoplastic progression)

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Communicated by Leland H. Hartwell, April 25, 1991

**ABSTRACT** The development of pancreatic cancer in transgenic mice expressing the simian virus 40 tumor antigen placed under controlling regions of the elastase I gene is characterized by the sequential appearance of tetraploid and then multiple aneuploid cell populations. Pancreatic tissues from such transgenic mice were studied between 8 and 32 days of age. Virtually 100% of acinar cell nuclei had immunohistochemically detectable tumor antigen by 18 days. Tetraploid cells were demonstrated by DNA content flow cytometry by 20 days and were associated with the appearance of interphase cells that had 5–11 centrioles per cell in single thin sections of pancreatic tissue examined by electron microscopy. Mitotic cells also were observed that had 5 or more centrioles per cell that were incorporated into the poles of bipolar or at least tripolar spindle apparatuses. These observations indicate that formation of the tetraploid intermediate in the diploid → tetraploid → aneuploid sequence of pancreatic tumor formation in elastase–simian virus 40 tumor antigen transgenic mice is accompanied by the development of cells with 5 or more centrioles that can be incorporated into the poles of abnormal mitotic spindles. We speculate that cells with more than 4 centrioles are predisposed to the formation of multipolar mitoses that may yield daughter cells with chromosomal gains and losses, resulting in the subsequent development of aneuploid tumors.

Human solid tissue cancers develop as a result of a multiple-step process involving an acquired genomic instability, loss of proliferative control, and evolution of clones of cells with increasingly aggressive behavior (1). The spontaneous rate of genetic error in normal somatic tissues is low,  $\approx 10^{-10}$  mutations per nucleotide per cell division (2). However, DNA content aneuploidy (3), structural and numerical chromosome abnormalities (4), point mutations (5), loss of heterozygosity (6), and gene amplifications (7) are present much more commonly in human cancers or precancerous states than in normal tissues from the same patients, providing abundant evidence for somatic genomic instability in neoplastic progression. Investigation of the aberrant cellular mechanisms that cause genomic instability is hampered by the rare and asynchronous nature of these events.

Elastase–simian virus 40 (SV40) tumor antigen (TAg) transgenic mice provide a model system in which to investigate genomic instability in a multiple-step pathway of neoplastic progression. Transgenic mice expressing SV40 TAg placed under controlling regions of the elastase I gene reproducibly develop pancreatic tumors (8). Progression to cancer in this model occurs by a diploid → tetraploid → aneuploid

sequence that is associated with biologic progression from phenotypically normal to hyperplasia, to dysplasia, and ultimately to carcinoma. It was suggested that loss of chromosomes from the tetraploid intermediate resulted in the development of aneuploid tumors, but the mechanism of chromosomal instability was not specified (8).

It is often assumed that genomic instability develops only in association with defects in DNA repair pathways, but recent investigations have demonstrated that deficiencies of normal cell cycle gene products (9) or loss of normal cell cycle checkpoints (10, 11) can also lead to genomic instability. In yeast, polyploidy can develop as a consequence of the development of extra spindle pole bodies (12, 13), abnormalities of spindle pole body duplication (12), or extra rounds of DNA synthesis without an intervening mitosis (14, 15). In some cases, such as the *Saccharomyces cerevisiae* cell-division cycle mutant *espl*, which uncouples duplication of spindle pole bodies from replication of DNA (12, 13), formation of a polyploid cell is associated with an increased number of spindle pole bodies that predisposes to multipolar mitoses and aneuploidy. In other cases, formation of the polyploid cells is associated with abnormalities of spindle pole body duplication resulting in a polyploid cell that contains the usual number of spindle pole bodies (12).

In normally proliferating diploid mammalian cells, progress through the cell-division cycle is coupled to duplication of centrioles, mammalian structures that correspond to yeast spindle pole bodies (16, 17). Two centrioles are present in G<sub>1</sub> phase cells, four are present in G<sub>2</sub> phase cells, and two are present in each of two daughter G<sub>1</sub> cells (17). In some experimental systems, cells with five or more centrioles and three or more microtubule organizing centers can be observed under conditions in which DNA synthesis and centriole duplication are uncoupled, including: cultured cells after exposure to x-irradiation (18) or to antimicrotubule agents (19), N115 mouse neuroblastoma cells (20), mitotic mutants of *Drosophila* embryos (21), and embryonal cells exposed to DNA synthesis inhibitors (22) or to protein synthesis inhibitors (23). In some of these circumstances, the dissociation between DNA synthesis and centriole duplication may result from missing cell cycle checkpoints that make one event in cell division dependent on another (11). We therefore investigated the diploid → tetraploid → aneuploid sequence in the elastase–SV40 TAg transgenic mouse pancreas to determine whether the tetraploid intermediate developed in association with the appearance of cells with more than four centrioles or by a mechanism that resulted in cells with the usual number of centrioles.

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Abbreviations: SV40, simian virus 40; TAg, tumor antigen; EM, electron microscopy.

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## MATERIALS AND METHODS

**Transgenic Mice.** Transgenic mice heterozygous for the elastase-SV40 TAg gene were bred by matings of males from the fifth and sixth generations of mice homozygous for the elastase-SV40 construct [transgenic mouse line 264 (8)] with C57 Black females (The Jackson Laboratory). Control mice with the same genetic background, but lacking the elastase-SV40 TAg gene, were derived by matings of SJL males with C57 Black females. [Transgenic mice homozygous for the elastase-SV40 TAg gene and SJL mice were generously provided by R. Brinster and E. Sandgren (University of Pennsylvania, Philadelphia, PA).] Three heterozygous transgenic mice and one or two control mice were sacrificed 8, 14, 18, 20, 24, 27, and 32 days after birth by placing them in an ether/dry-ice chamber for 2–4 min and were promptly dissected in accordance with the policies established by the Animal Care Committee of the University of Washington. Coded samples of pancreatic tissue from each animal were independently evaluated by immunohistochemistry, DNA content flow cytometry, and transmission electron microscopy (EM).

**Immunohistochemistry.** Tissue for immunohistochemistry was frozen in liquid nitrogen, stored at  $-70^{\circ}\text{C}$ , and cryostat-sectioned at a thickness of 6–8  $\mu\text{m}$ . Sections were collected on poly(L-lysine)-coated microscope slides, fixed in 95% ethanol, incubated in 3% (vol/vol) hydrogen peroxide, washed with phosphate-buffered saline (PBS), permeabilized with 0.1% Triton X-100 in 0.1% bovine serum albumin/PBS (PBA), incubated with a primary polyclonal rabbit anti-SV40 TAg antibody [generously provided by A. Messing (University of Wisconsin, Madison, WI)] at a 1:100 or 1:200 dilution in PBS with 5% (vol/vol) normal goat serum. Sections were then washed with PBA between successive applications of solutions of biotinylated secondary goat anti-rabbit antibody at a 1:500 dilution, avidin-biotinylated peroxidase complex, and diaminobenzidine. The procedure for control sections for nonspecific staining omitted the primary antibody. All sections were counterstained with methyl green and evaluated for the location of peroxidase product among tissue sections from transgenic mice of various ages.

**Flow Cytometry.** Tissue for flow cytometry was placed in minimal essential medium with 5% bovine serum and 10% (vol/vol) dimethyl sulfoxide, stored at  $-70^{\circ}\text{C}$ , and processed by mincing in 0.15 M NaCl/0.1 M Tris-HCl, pH 7.4/0.1% Nonidet P-40/2 mM calcium/21 mM magnesium/4,6-diamidino-2-phenylindole (10  $\mu\text{g}/\text{ml}$ ). Aggregates were dissociated by forceful passage through a 25-gauge needle. DNA-content flow cytometry was performed on an ICP-22 flow cytometer as described (24). S-phase and  $G_2$ -phase fractions of diploid and tetraploid cell populations were computed on one or two samples per mouse using the Multicycle program (Phoenix Flow Systems, San Diego, CA) written by one of the authors (P.S.R.) using published methods (24, 25).

**EM.** Tissue for EM was fixed at  $4^{\circ}\text{C}$  for 1–1.5 h in bicarbonate-buffered osmium, dehydrated in graded alcohols, minced into pieces  $<2$  mm in size, separately embedded into resin, thick-sectioned, and examined by light microscopy. Blocks that predominantly contained pancreatic acinar tissue were thin-sectioned at 100–120 nm, placed on grids with areas of  $60 \times 60 \mu\text{m}$  unit bounded by 10- $\mu\text{m}$ -thick grid bars, and stained with uranyl acetate and lead citrate. A single grid from each mouse was randomly selected and examined by transmission EM. Cells in single-section profile with five or more centrioles per cell were counted and normalized for variably sized tissue sections to calculate an index (defined as number of cells with five or more centrioles per EM grid unit area) for a single thin section for each

transgenic ( $n = 21$ ) and control ( $n = 7$ ) mouse. A total of 1224 grid unit areas were evaluated (average 44 per mouse).

## RESULTS

**Immunohistochemistry.** Immunodetectable SV40 TAg was present in  $\approx 20\%$  and  $40\%$  of the nuclei in pancreatic tissue sections of 8- and 14-day-old transgenic mice, respectively. TAg was detected in virtually 100% of acinar cell nuclei from 18- to 32-day-old transgenic mice; these samples also had histologic features of dysplasia. SV40 TAg was not detected in islet, duct epithelial, vascular endothelial, or stromal cells.

**Flow Cytometry and EM Studies.** Pancreatic samples from 8- and 14-day-old transgenic mice were diploid with normal S-phase and 4N fractions (Fig. 1A and Table 1); rare cells with five or more centrioles were observed in one 8- and one 14-day-old transgenic mouse. One 18-day-old transgenic mouse had increased diploid S-phase fractions (23.9% and 21.9%) and marked increases in 4N cells (39.5% and 34.2%) and cells with five or more centrioles. Pancreatic samples from all 20- to 32-day-old transgenic mice had increased

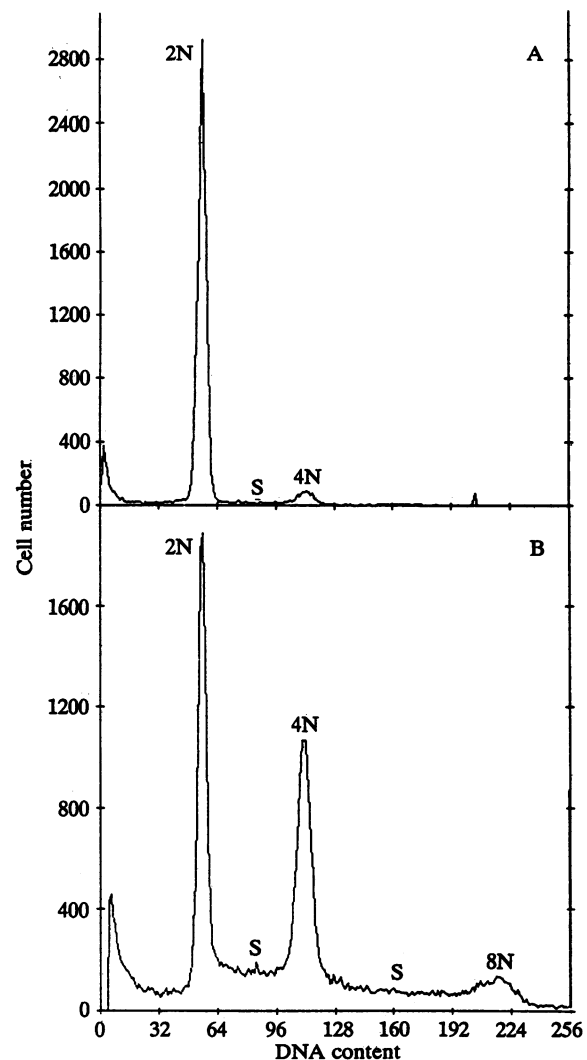


FIG. 1. Flow cytometry histograms of pancreatic samples from elastase-SV40 TAg transgenic mice. (A) Proliferative fractions in a 14-day-old transgenic mouse are S phase (4.8%) and 4N (4.9%). (B) In this sample from a 20-day-old transgenic mouse, the diploid S-phase fraction is 39.1% and a large tetraploid-cell population makes up 55.6% of cells analyzed. The tetraploid population is cycling with an S-phase fraction of 46.5% and a  $G_2$ -phase fraction (with DNA content of 8N) of 8.1%.

Table 1. Proliferative fractions of diploid cell populations measured by DNA content flow cytometry and the index of cells with at least five centrioles detected by EM in pancreatic tissue samples from transgenic mice (heterozygous for elastase-SV40 TAg transgene) and control SJL/C57 Black mice

Mouse age, days	Flow cytometry samples							EM samples			
	No.	% S phase		Confidence interval (95%)	% 4N cells		Confidence interval (95%)	No.	Centriole index		Confidence interval (95%)
		Mean	SEM		Mean	SEM			Mean	SEM	
Transgenic											
8	3	7.6	0.6	5.0–10.2	7.8	1.7	0.6–15.1	3	0.01	0.006	–0.02–0.03
14	3	4.6	1.4	–1.5–10.7	4.3	0.9	0.4–8.2	3	0.01	0.006	–0.02–0.03
18	6	9.5	4.3	–1.5–20.5	13.9	7.3	–4.2–32.8	3	0.01	0.006	–0.05–0.08
20	6	32.0*	4.7	19.6–44.0	46.0*	5.8	31.1–60.9	3	0.14*	0.023	0.03–0.25
24	5	31.6*	3.1	22.9–40.4	55.5*	2.4	48.9–62.1	3	0.13*	0.006	0.10–0.15
27	3	34.2*	3.4	19.6–48.7	54.6*	5.4	31.5–77.8	3	0.24*	0.092	–0.16–0.63
32	5	31.2*	4.9	17.5–45.2	55.6*	6.3	38.1–73.1	3	0.24*	0.023	0.13–0.36
Control											
8–32	12	4.8	1.0	2.6–7.0	3.3	0.8	1.7–5.0	7	0	0	—

Transgenic mice were grouped by age (indicated in days); control mice of all ages were analyzed in one group. \**P* < 0.001 (Student's *t* test) compared with 8- or 14-day-old transgenic mice and all normal control mice.

diploid S-phase fractions and marked increases in 4N cells. The large 4N populations of cells had their own cell cycles with increased tetraploid S-phase fractions (mean 47.9%; range, 34.5–74.9%) and 8N fractions (mean, 9.7%; range, 0–16.2%) (Fig. 1B and Table 1). Mean 8N fractions did not differ significantly among these transgenic mice. No cell populations with a DNA content of 16N or greater were detected.

Pancreatic samples from 20- to 32-day-old transgenic mice also had multiple interphase cells with 5–11 centrioles (Fig. 2 and Table 1). These centrioles often were clustered in one or two centrosomes, one of which was usually situated in the apical region of the cytoplasm (Fig. 3). Abnormal interphase cells in 18- to 24-day-old transgenic mice almost always contained 5–8 centrioles, while 17% of abnormal interphase cells in the 27- and 32-day-old transgenic mice contained 9–11 centrioles (Fig. 3). Mitotic cells with 5 or more centrioles also were observed occasionally. In some cells 3 or more centrioles were present in one centrosome of bipolar mitoses, and in other cells 5 or more centrioles were present in mitotic spindles that were at least tripolar (Fig. 4). Pancreatic sam-

ples from the 8- to 32-day-old control mice had normal S-phase and 4N fractions and never had cells with more than 4 centrioles (Fig. 2 and Table 1).

To evaluate the quantitative significance of cells with five or more centrioles, thin sections from a 27-day-old transgenic mouse and a control mouse were each reexamined for cells with different numbers of centrioles. Cells were scored by EM for centriole number in 31 grid unit areas from each

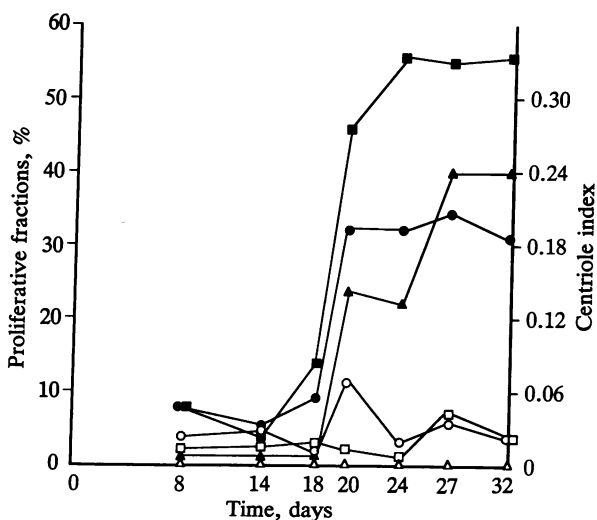


FIG. 2. Diploid S-phase and 4N fractions and the index of interphase cells with at least five centrioles in pancreatic tissue samples from transgenic mice (heterozygous for the elastase-SV40 TAg transgene; solid symbols) and control SJL/C57 Black mice (open symbols) between 8 and 32 days of age (see Table 1). □ and ●, % 4N cells; ○ and ●, % S-phase cells; △ and ▲, index of cells with five or more centrioles.

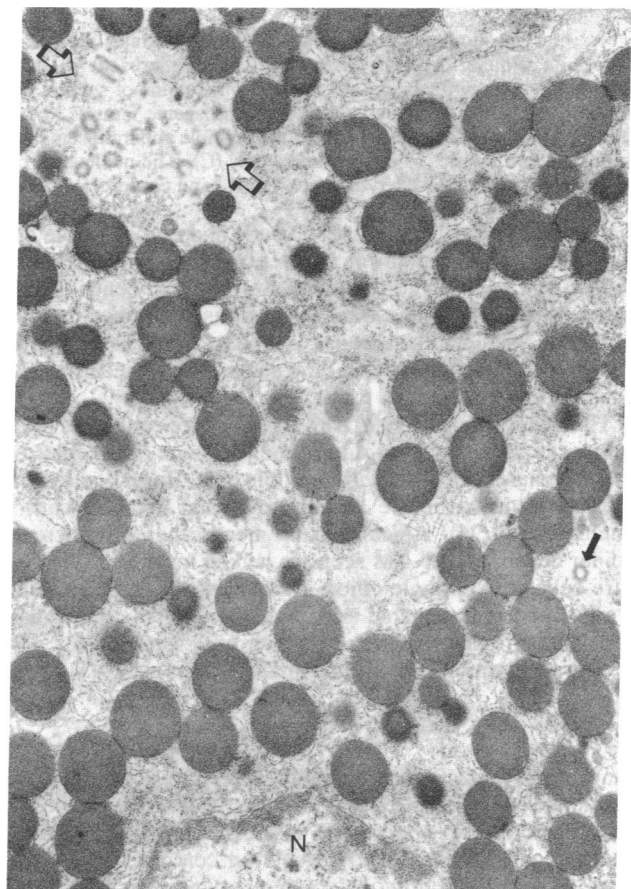


FIG. 3. Interphase pancreatic acinar cell of an elastase-SV40 TAg transgenic mouse. At least 11 centrioles (large open arrows) are present in a cell from a 32-day-old transgenic mouse. Many of the centrioles are in the plane of section and are clustered in the apical region. A single isolated centriole (small closed arrow) also is apparently closer to the nucleus (N). Many secretory granules are present. (×8400.)

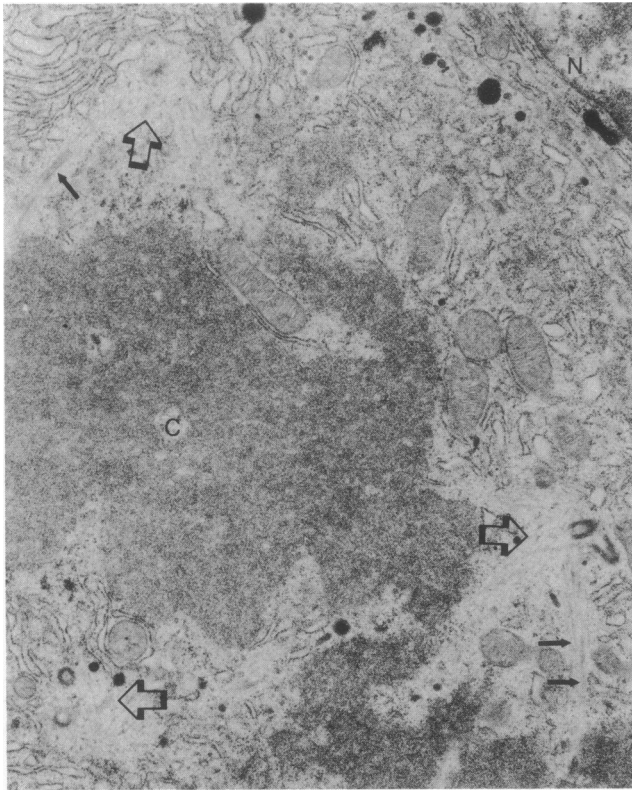


FIG. 4. Mitotic pancreatic acinar cell of an elastase-SV40 TAG transgenic mouse. At least five centrioles are present in three centrosomes (large open arrows) visible in this plane of section of a multipolar mitotic cell from a 20-day-old transgenic mouse. Microtubules (small solid arrows) of the mitotic spindle apparatus emanate from the centrosomes to the chromosomes (C). The nucleus (N) of an adjacent cell is located in the upper right hand corner. ( $\times 11,800$ .)

mouse. In the control mouse, 53 cells had one centriole and 4 cells had two centrioles in the section examined by EM. No cells in the control mouse had more than two centrioles. In contrast, 89 cells in the transgenic mouse pancreas had one centriole, 44 cells had two centrioles, 13 cells had three centrioles, 11 cells had four centrioles, and 14 cells had five or more centrioles.

The observation of cells with five or more centrioles in the transgenic mice was not due to the presence of cytoplasmic basal bodies associated with multiple cilia. Basal bodies have the same ultrastructure as centrioles and are abundant in ciliated cells (26). Apical plasma membranes of pancreatic acinar cells in single EM sections lacked multiple cilia. Only rare cells with a single cilium (one or two cells per section) were found in transgenic and control mice, which is consistent with previous studies in which cilia were occasionally observed in embryonic mouse and chicken pancreas (26).

## DISCUSSION

Our results indicate that the diploid  $\rightarrow$  tetraploid  $\rightarrow$  aneuploid sequence of neoplastic progression in the elastase-SV40 TAG transgenic mouse pancreas is associated with the development of cells that have more than four centrioles. SV40 TAG was universally immunodetectable in pancreatic acinar cells in 18-day-old mice, a result consistent with previous evaluations of mRNA levels (8). TAG expression was followed by the appearance of diploid cells with markedly increased S-phase fractions and cycling tetraploid populations in 18- to 20-day-old transgenic mice. The appearance of the tetraploid population paralleled the development of cells with more than four centrioles and preceded the development of multiple

aneuploid cell populations. It might be argued that an increased 4N population measured by DNA content flow cytometry could represent either G<sub>2</sub> phase or tetraploid cells, but the presence of a 4N cell cycle strongly favors the presence of a tetraploid intermediate in this model (Fig. 1B). Furthermore, cytogenetic and flow cytometric analyses of human neoplastic and preneoplastic tissues have demonstrated a linear relationship between DNA content and chromosome number that supports the existence of a tetraploid intermediate (24, 27-29). The development of tetraploid cells in the transgenic mouse model is consistent with other observations of tetraploid and polyploid cells that express increased amounts of TAG after infection with SV40 (30).

Our methods did not permit direct ultrastructural investigation of cells with a DNA content of 4N or of the total number of centrioles per whole cell. Therefore, additional experiments will be necessary to determine whether cells with more than 4 centrioles are predisposed to the development of the tetraploid intermediate or develop as a consequence of the event that leads to tetraploidy and to assess the relationship between cellular DNA content and quantitative centriole counts. One important question is whether the numbers of centrioles are appropriate for the ploidy and proliferative fractions of the transgenic mouse pancreas. If, for example, the tetraploid intermediate develops as the result of a missed cell cycle checkpoint that normally makes DNA synthesis dependent on completion of the prior mitosis, then the tetraploid G<sub>1</sub> phase cell could have 4 centrioles because the pair that duplicated in the diploid S phase would not have segregated at mitosis. Thus, tetraploid S-phase, G<sub>2</sub>-phase, and mitotic cells might have as many as 8 centrioles. The observation of some cells with 9-11 centrioles suggests that other mechanisms may predispose to even greater numbers than can be explained on the basis of ploidy and proliferation, but this will require additional quantitative data for confirmation.

The mechanisms by which SV40 TAG directly or indirectly leads to formation of the tetraploid intermediate and cells with more than four centrioles in the elastase-SV40 TAG transgenic mouse pancreas are unknown. Although it is theoretically possible that the 4N intermediate arose by cell fusion, we have found no evidence to support this mechanism. Viral or chemical agents that induce cell fusion typically produce heterokaryons that may be transient, and fusion may occur among multiple cells leading to cells with DNA contents of 2N, 4N, 6N, 8N, 10N, and greater (31-33). In spite of extensive histologic and EM evaluation of the elastase-SV40 TAG transgenic mouse pancreas, we have never observed heterokaryons, and flow cytometric evaluation has documented only 2N and 4N populations, both of which have their own cell cycles. It is unlikely that >50% of pancreatic acinar cells would undergo fusion without any supporting histologic, electron microscopic, or flow cytometric evidence.

The relationship between the development of cells with more than four centrioles in the elastase-SV40 TAG transgenic mouse pancreas and the interactions of TAG with known cellular proteins is unknown (34, 35). Our preliminary immunohistochemical experiments suggest that there is an increase in p53 expression by pancreatic acinar cells in elastase-SV40 TAG transgenic mice by 18-20 days of age (unpublished observations). SV40 TAG binds to DNA and the products of the p53 and *Rb* genes (34-37), coprecipitates with p53 and tubulin (38), and localizes with p53 to cytoplasmic, mitotic, and centriolar microtubules *in situ* (39). p53 also interacts with p34<sup>cdc2</sup>, a gene product that has been implicated in cell cycle checkpoints in yeast (40). The role of these and other as yet unknown potential targets of TAG in the development of cells with more than four centrioles and genomic instability will require further investigation.

Our results, therefore, indicate that, in the elastase-SV40 TAg transgenic mouse pancreas, TAg expression predisposes to the development of cells with more than four centrioles by an as yet unidentified mechanism. These centrioles are incorporated into the centrosomes of bipolar and at least tripolar mitotic spindles in at least some cells (Fig. 4), although quantitation of multipolar mitoses will require additional studies using serial EM thin sections. We hypothesize that cells with more than four centrioles may predispose to the formation of multipolar mitotic spindle apparatuses that cause genomically unbalanced cell divisions with resulting chromosomal gains or losses, thereby providing a mechanism for the generation of the multiple aneuploid clones that appear subsequent to the formation of the tetraploid intermediate in this model. Multipolar cell division would usually be lethal, but some abnormal mitoses could predispose to the development of aneuploid clones that have selective proliferative advantages, possibly by the loss or gain of chromosomal regions containing genes that control pancreatic cell division (8). Thus, the consequences of five or more centrioles per cell in the elastase-SV40 TAg transgenic mouse pancreas may be analogous to the multipolar mitoses in the *S. cerevisiae* mutant *espl* that generate aneuploid cell populations (12, 13). These observations further suggest that dysfunction of normal cell-division cycle mechanisms may have an early role in generating genomic instability that can culminate in the development of cancers (41).

Our results have relevance to the development of human cancers such as esophageal adenocarcinoma (24, 42), colon carcinoma (25), bladder cancer (43), and brain tumors (44), in which tetraploid or aneuploid precursors of cancer have been demonstrated (29). Perhaps the best documented parallel between our results in the elastase-SV40 TAg transgenic mouse pancreas and a human premalignant condition is the Barrett esophagus, in which increased 4N fractions are a precursor of carcinoma and in which the transition from increased 4N fractions to single or multiple aneuploidies is associated with progression from dysplasia to carcinoma (24, 42, 45). Our results also have obvious potential relevance to the development of multipolar mitoses that are frequently observed in human cancers (46).

Because our investigation was performed in a mammalian system, it complements studies in cell cultures and microorganisms and may further improve our understanding of neoplastic progression in humans. Our results demonstrate that the development of the tetraploid intermediate in the diploid → tetraploid → aneuploid sequence of the elastase-SV40 TAg transgenic mouse pancreas is associated with the appearance of cells with five or more centrioles. These centrioles can form the poles of mitotic spindles that are at least tripolar, suggesting a mechanism for generating the subsequent development of multiple aneuploid populations in a system in which they can be related to the eventual development of a mammalian cancer. These results suggest potential cellular abnormalities that might contribute to genomic instability that can culminate in the development of human cancers.

We gratefully acknowledge the gifts of transgenic and control mice from R. Palmiter, R. Brinster, and E. Sandgren. We thank K. Neshat, A. Stevens, A. Wass, K. Hudkins, J. Fox, K. Schaefer, R. Mayes, and T. Naujack for providing technical assistance. We also thank B. Byers, L. Hartwell, and R. Palmiter for their helpful discussions and suggestions. This research was supported by National Institutes of Health Grant HIH-RR05432.

- Nowell, P. C. (1976) *Science* **194**, 23–28.
- Loeb, L. A. & Cheng, K. C. (1990) *Mutat. Res.* **238**, 297–304.
- Friedlander, M. L., Hedley, D. W. & Taylor, I. W. (1984) *J. Clin. Pathol.* **37**, 961–974.
- Yunis, J. J. (1983) *Science* **221**, 227–236.
- Vamus, H. E. (1984) *Annu. Rev. Genet.* **18**, 553–612.
- Knudson, A. G. (1985) *Cancer Res.* **45**, 1437–1443.
- Schimke, R. T. (1984) *Cell* **37**, 705–713.
- Ornitz, D. M., Hammer, R. E., Messing, A., Palmiter, R. D. & Brinster, R. L. (1987) *Science* **238**, 188–193.
- Hartwell, L. H. & Smith, D. (1985) *Genetics* **110**, 381–395.
- Weinert, T. A. & Hartwell, L. H. (1988) *Science* **241**, 317–322.
- Hartwell, L. H. & Weinert, T. A. (1989) *Science* **246**, 629–635.
- Baum, P., Goetsch, L. & Byers, B. (1986) in *Yeast Cell Biology*, ed. Hicks, J. (Liss, New York), pp. 151–158.
- Baum, P., Yip, C., Goetsch, L. & Byers, B. (1988) *Mol. Cell. Biol.* **8**, 5386–5397.
- Creanor, J. & Mitchison, J. M. (1990) *J. Cell Sci.* **96**, 435–438.
- Uzawa, S., Samejima, I., Hirano, T., Tanaka, K. & Yanagida, M. (1990) *Cell* **62**, 913–925.
- Byers, B. & Goetsch, L. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **38**, 123–131.
- Hyams, J. S. & Brinkley, B. R., eds. (1989) *Mitosis: Molecules and Mechanisms* (Academic, London).
- Sato, C., Kuriyama, R. & Nishizawa, K. (1983) *J. Cell Biol.* **96**, 776–782.
- Keryer, G., Ris, H. & Borisy, G. G. (1984) *J. Cell Biol.* **98**, 2222–2229.
- Ring, D., Hubble, R. & Kirschner, M. (1982) *J. Cell Biol.* **94**, 549–556.
- Sunkel, C. E. & Glover, D. M. (1988) *J. Cell Sci.* **89**, 25–38.
- Raff, J. W. & Glover, D. M. (1988) *J. Cell Biol.* **107**, 2009–2019.
- Gard, D. L., Hafezi, S., Zhang, T. & Doxsey, S. J. (1990) *J. Cell Biol.* **110**, 2033–2042.
- Reid, B. J., Haggitt, R. C., Rubin, C. E. & Rabinovitch, P. S. (1987) *Gastroenterology* **93**, 1–11.
- Blount, P. L., Rabinovitch, P. S. & Reid, B. J. (1991) in *Premalignant Conditions of the Gastrointestinal Tract*, ed. Eastwood, G. L. (Elsevier, New York), pp. 55–77.
- Ghadially, F. N. (1982) *Ultrastructural Pathology of the Cell and Matrix* (Butterworths, London), 2nd Ed., pp. 856–863.
- Petersen, S. E. & Friedrich, U. (1986) *Cytometry* **7**, 307–312.
- Remvikos, Y., Muleris, M., Vielh, P., Salmon, R. J. & Dutrillaux, B. (1988) *Int. J. Cancer* **42**, 539–543.
- Shackney, S. E., Smith, C. A., Miller, B. W., Burholt, D. R., Murtha, K., Giles, H. R., Detterer, D. M. & Pollice, A. A. (1989) *Cancer Res.* **49**, 3344–3354.
- Laffin, J., Fogleman, D. & Lehman, J. M. (1989) *Cytometry* **10**, 205–213.
- Davidson, R. L. & de la Cruz, F. F., eds. (1974) *Somatic Cell Hybridization* (Raven, New York).
- Ringertz, N. R. & Savage, R. E. (1976) *Cell Hybrids* (Academic, New York).
- Sowers, A. E., ed. (1987) *Cell Fusion* (Plenum, New York).
- Butel, J. S., Jarvis, D. L. & Maxwell, S. A. (1989) *Ann. N.Y. Acad. Sci.* **567**, 104–121.
- Livingston, D. M. & Bradley, M. K. (1987) *Mol. Biol. Med.* **4**, 63–80.
- DeCaprio, J. A., Ludlow, J. W., Figge, J., Shew, J.-Y., Huang, C.-M., Lee, W.-H., Marsilio, E., Paucha, E. & Livingston, D. M. (1988) *Cell* **54**, 275–283.
- Lane, D. P. & Benchimol, S. (1990) *Genes Dev.* **4**, 1–8.
- Maxwell, S. A., Santos, M., Wong, C., Rasmussen, G. & Butel, J. S. (1989) *Mol. Carcinogen.* **2**, 322–335.
- Maxwell, S. A., Ames, S. K., Sawai, E. T., Decker, G. L., Cook, R. G. & Butel, J. S. (1991) *Cell Growth Differ.* **2**, 115–127.
- Sturzbecher, H.-W., Maimets, T., Chumakov, P., Brain, R., Addison, C., Simanis, V., Rudge, K., Philp, R., Grimaldi, M., Court, W. & Jenkins, J. R. (1990) *Oncogene* **5**, 795–801.
- Resnick, M. A. & Vig, B. K., eds. (1989) *Mechanisms of Chromosome Distribution and Aneuploidy* (Liss, New York).
- Reid, B. J. & Rabinovitch, P. S. (1989) *Accomp. Oncol.* **3**, 32–44.
- Tribukait, B., Gustafson, H. & Esposti, P. (1979) *Cancer* **43**, 1742–1751.
- Frederiksen, P. & Bichel, P. (1980) *Flow Cytom.* **4**, 398–402.
- Rabinovitch, P. S., Reid, B. J., Haggitt, R. C., Norwood, T. H. & Rubin, C. E. (1989) *Lab. Invest.* **60**, 65–71.
- Robbins, S. L., Cotran, R. S. & Kumar, V. (1984) *Pathologic Basis of Disease* (Saunders, Philadelphia), 3rd Ed., p. 219.